Review

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Next-Generation Nanocarriers for Skin Cancer Therapy via Transdermal Delivery

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Abstract

The global burden of cancer, a progressive disease with complex etiology, continues to rise. With an estimated 1.5 million new diagnoses each year, including nearly 350 000 cases of highly aggressive melanoma, the need for improved therapeutic strategies is paramount. The World Health Organization (WHO) and the International Labour Organization have established a clear link between occupational exposure to solar ultraviolet radiation (UVR) and increased risk of non-melanoma skin cancer (NMSC). While age, skin tone, and cumulative UVR exposure are recognized risk factors, current treatment modalities, including chemotherapy and surgery, often exhibit limited efficacy and significant side effects. This underscores the urgent need for novel therapeutic approaches that enable targeted drug delivery, enhanced tumor penetration, and reduced systemic toxicity. Transferosomes-based drug delivery systems have emerged as a promising avenue for achieving these goals, with vesicular systems offering a particularly attractive strategy for transdermal skin cancer therapy. This review focuses on transferosomes and transethosomes as such vesicular systems, highlighting recent advances in their application for targeted skin cancer treatment.

Keywords: transdermal drug delivery; transferosomes; transethosomes; skin cancer; ultraviolet radiation (UVR)

Introduction

Skin cancer is a major global health concern, ranking among the most prevalent cancers [1]. According to the International Agency for Research on Cancer's annual status report, an estimated 18.1 million new cancer cases and 9.6 million cancer-related deaths were reported worldwide in 2018 [2]. By 2020, non-melanoma skin cancer (NMSC) (excluding basal cell carcinoma) was diagnosed in 1 198 073 individuals globally (722 348 men and 475 725 women), representing 6.2% of all cancer diagnoses [3]. This resulted in 63 731 deaths (0.6% of all cancer deaths). Melanoma diagnoses in 2020 totaled 324 635 cases (173 844 men and 150 791 women) [4], leading to over 57 000 deaths. While melanoma incidence varies geographically, it is generally higher in men than in women [5]. Projections indicate a continued increase in melanoma diagnoses, with the American Cancer Society estimating that melanoma will account for 6% of new cancer diagnoses in men and 4% in women by 2023, and further increases are expected over the next 20 years [6–9].

Malignant melanoma arises from the malignant

transformation of melanocytes. Non-melanoma skin cancer (NMSC), derived from epidermal keratinocytes, constitutes the most prevalent form of malignancy affecting the skin globally, encompassing squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) [10]. BCC is the most frequently diagnosed cutaneous malignancy, with individuals over 65 years of age exhibiting the highest morbidity, accounting for more than 95% of diagnoses [11]. Established etiological factors for NMSC include solar ultraviolet B (UVB) radiation, chronic actinic exposure, arsenic compounds, human papillomavirus (HPV) infection, and ionizing radiation. Precancerous dermatoses associated with elevated risk of NMSC include actinic keratosis (solar keratosis), xeroderma pigmentosum (a genetic disorder that increases the risk of skin tumors), radiation dermatitis, chronic inflammation, cicatrices, and hypertrophic burn scars [12]. BCC can exhibit local invasion into subcutaneous fatty tissue [13]. Moreover, SCC originates from flat squamous cells (keratinocytes), the skin's outermost layer [14], and can develop *de novo* or within pre-existing lesions such as actinic keratosis, white keratosis (leukoplakia), burn scars, or chronic ulcers. While typically characterized by slow progression, SCC can exhibit local invasion and distant metastasis [15]. The incidence of cutaneous SCC (cSCC) has demonstrated a substantial increase (50%–300%) over the past three decades and is projected to double in European countries by 2030 [16]. Approximately 250 000 individuals are diagnosed with SCC annually in the United States [16].

The incidence of skin cancer has demonstrated a marked increase in recent decades, attributable in part to cumulative exposure to solar radiation, alterations in stratospheric ozone concentrations, and evolving individual and societal behaviors [17]. The interplay between climate change and skin cancer is complex and requires further investigation. Proposed mechanisms by which climate change may influence skin cancer development include elevated ambient temperatures, increased atmospheric pollution, and modifications in human behavior leading to UV radiation exposure. However, the precise contribution of these factors remains to be fully elucidated. Current therapeutic interventions for skin cancer, including chemotherapy and surgical resection, are frequently associated with significant adverse effects and often fail to achieve durable remission [18]. These conventional approaches can also induce cytotoxic effects in non-malignant healthy cells while exhibiting suboptimal efficacy against cancer cells [19, 20].

Although ongoing studies have yielded some therapeutic advances, this disease frequently remains fatal, underscoring the critical need for innovative treatment strategies. Current therapeutic limitations highlight the potential of nanotechnology-based drug delivery systems, such as transferosomes and transethosomes, to overcome these challenges. Transferosomes and transethosomes are both advanced transdermal delivery systems designed to enhance the penetration and efficacy of drugs, particularly through the skin. Transferosomes are composed mainly of phospholipids and edge activators (surfactants) that enhance their flexibility and ability to penetrate the skin. Transethosomes are third-generation vesicles that combine features of both transferosomes and ethosomes, they contain phospholipids, ethanol (typically 30%−40%), and edge activators. These nanocarriers offer the potential for controlled drug release, high delivery efficiency, and targeted delivery to specific sites by their ability to traverse biological barriers. Furthermore, these systems can enhance therapeutic efficacy at reduced doses and minimize adverse effects, presenting a potential advantage in the management of chronic diseases.

Formulation of Transferosomes and Transethosomes

Several techniques are utilized to produce vesicles with controlled size distributions, and these vesicles are subsequently incorporated into topical formulations such as gels or creams to enhance transdermal delivery.

Thin-film hydration using rotary evaporation

The standard vesicle preparation is initiated by dissolving phospholipids and edge activators (the vesicle-forming components) in a round-bottom flask using a suitable (v/v) mixture of volatile organic solvents (e.g., chloroform, methanol, and ethanol). Lipophilic drugs can be incorporated into the lipid mixture at this stage. A thin-film is then formed by evaporating the organic solvent under reduced

pressure using a rotary evaporator above the lipid transition temperature. Residual solvent is removed by further vacuum application. The resulting lipid is hydrated under rotation at a controlled temperature using a buffer solution of appropriate pH (e.g., pH 7.4). For transethosome preparation, hydration is performed using an aqueous ethanol solution or a saline phosphate buffer containing ethanol (Fig.1). After swelling at room temperature, the vesicles are subjected to sonication using either a bath or probe sonicator to achieve size reduction. The resulting vesicles are then homogenized by extrusion through polycarbonate membranes with pore sizes ranging from 100 to 200 nm [21–23]. In contrast to other vesicles, transethosomes are maintained at 4–8 °C during the swelling process at room temperature. Sonbaty et al. [24] investigated the influence of different preparation techniques, including ethanol injection and thin-film hydration, on key vesicle characteristics.

Cold preparation method

Transethosomes are commonly prepared using a cold method, which is advantageous for encapsulating thermolabile drugs and offers facile scalability [25]. In this procedure [26], phospholipids are dissolved in ethanol (organic phase) and heated to 30 °C. Separately, the drug, water, and edge activator are combined (aqueous phase) and heated to 30 °C. The aqueous phase is then added to the organic phase under continuous stirring (700–2 000 r/min) for 5–10 min using a magnetic stirrer, maintaining a constant temperature of 30°C. The resulting mixture is then sonicated [27]. Studies by Gupta et al. [28, 29] have shown that stirring time influences vesicle size depending on the stirring rate. Depending on its physicochemical properties, the drug is incorporated into either the aqueous or organic phase. The final product is stored under refrigeration [30]. This method minimizes heat-induced drug degradation [31].

Hot preparation method

The hot method for transethosome preparation involves heating during vesicle formation to enhance drug entrapment efficiency, promote lipid fusion, and improve drug solubility in the lipid bilayer [26]. In this procedure, phospholipids are dispersed in water at 40 °C to form a colloidal dispersion, while a polyol and ethanol mixture is simultaneously heated to 40 °C in a separate vessel [32]. The organic phase is then added to the aqueous phase under continuous stirring until a homogeneous suspension at 40 °C is achieved [33, 34]. Depending on the drug's lipophilicity or hydrophilicity, it is dissolved in either ethanol or water prior to mixing. To achieve the desired vesicle

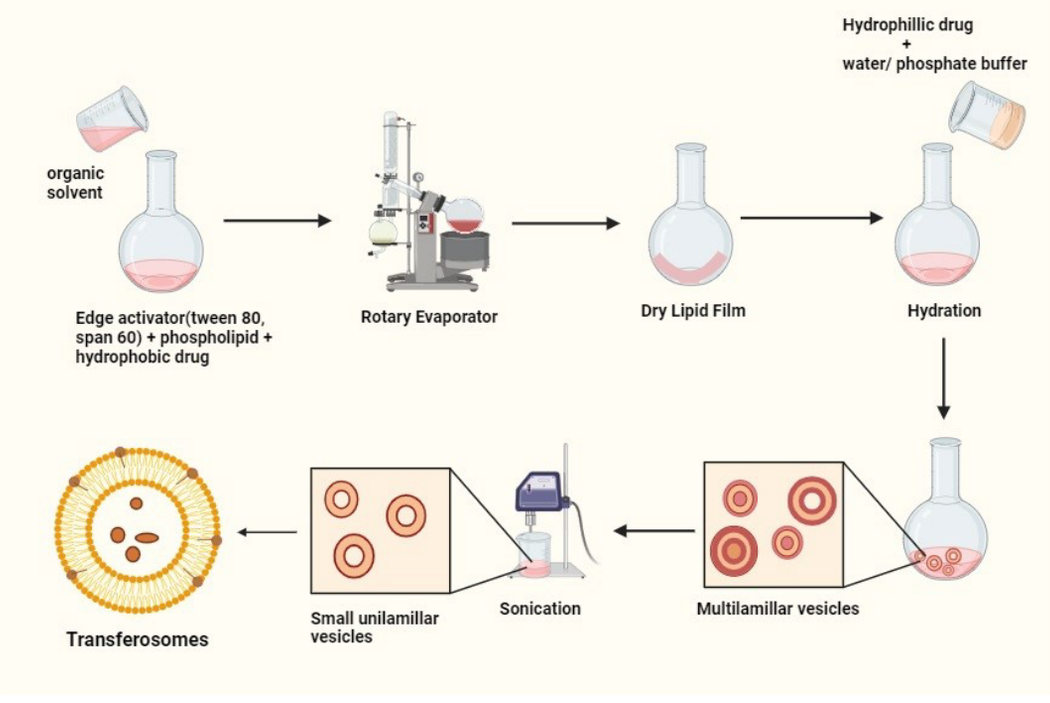

Fig. 1 Schematic illustration of the thin-film hydration method for transferosome preparation using a rotary evaporator.

size, the resulting suspension is subjected to probe sonication or extrusion [35].

Ethanol injection method with sonication

The ethanol injection method is designed to produce small unilamellar vesicles, typically yielding liposomes with sizes ranging from 30 to 170 nm. Liposome size is determined by the lipid concentration and injection rate. In this procedure, phospholipids, edge activators, and active substances are dissolved in ethanol (organic phase), which is then injected into an aqueous phase at a controlled flow rate using a syringe. The resulting mixture is subsequently homogenized using an ultrasonic probe sonicator. To further reduce particle size compared to conventional injection methods, Salem et al. developed an ultrasound-assisted injection technique, where particle size is influenced by ultrasound intensity, injection time, and probe settings [21, 35–37]. Singh et al. prepared transethosomes loaded with a hydrophilic drug using both the ethanol injection method and the hot procedure (with probe sonication). Sonicated vesicles exhibited smaller particle sizes than non-sonicated vesicles [38].

Advancement in Transdermal Drug Delivery Systems

Key distinctions among ethosomes, transferosomes, and transethosomes are summarized in Table 1 [20–44]. Transethosomes have demonstrated comparable or superior entrapment efficiency compared to other vesicular formulations. Their deformability enables penetration of deeper skin layers.

Transferosomes and transethosomes for transdermal drug delivery

Transferosomes are vesicular delivery systems comprising a lipid bilayer enclosing at least one aqueous compartment and incorporating an edge activator. Composed of phospholipids and an edge activator [45], transferosomes exhibit elasticity, enabling them to traverse skin pores smaller than their own size without significant loss [46]. Unlike conventional liposomes, which consist solely of phospholipids (either synthetic or natural), transferosomes incorporate a single-chain surfactant as an edge activator [47]. At optimal ratios, edge activators destabilize the membrane, enhancing deformability and thus increasing skin permeation. This deformability allows transferosomes to penetrate deeper skin layers, overcoming barriers that limit the penetration of conventional liposomes [48]. Furthermore, edge activators can enhance the solubility of hydrophobic drugs, improving encapsulation efficiency [49]. Studies have demonstrated that transferosomes effectively deliver bioactive compounds ranging from low to high molecular weights ($200 \leq M_W \leq 10^6$ Da) including both hydrophilic and lipophilic molecules, achieving skin transport efficiencies exceeding 50%. This targeted delivery to the skin can minimize systemic side effects, offering a significant advantage for treating skin cancers such as BCC, SCC, and melanoma.

Transethosomes are vesicular drug delivery systems composed of phospholipids, ethanol, edge activators, and water. The phospholipid components

Table 1 Comparative characteristics of ethosomes, transferosomes, and transethosomes.

Parameters	Ethosomes	Transferosomes	Transethosomes	Ref.
Composition	Phospholipids (cholesterol, phosphatidylcholine, and phosphatidylethanolamine)	Phospholipids (phosphatidylcholine or lecithin)	Phospholipids (2%–5%, Soya phosphatidyl choline (S75), Lipoid S100, Phospholipon 90G)	[20, 21–39]
	Water	Water	Water	
	Ethanol (20%–50%)	—	Ethanol	
	—	Edge activator (Tween-80, Span-80, or sodium deoxycholate)	Edge activator	
Skin permeation	Lipid perturbation	Vesicle deformation	Vesicle shape alteration	[21,39]
Entrapment efficiency	Higher than liposomes	Higher than ethosomes	Higher than ethosomes and transferosomes[27]	[40–42]
Flux rate	Faster than liposomes	Faster or equal to ethosomes	Faster flux rate	[43,44]
Role	Vesicle-forming component	As a hydration medium, it enhances membrane fluidity	It provides flexibility	—

self-assemble into a bilayer structure, characterized by hydrophobic head groups and hydrophobic acyl chains, providing a matrix for drug encapsulation [50]. Edge activators modulate the bilayer's fluidity and permeability. Ethanol confers deformability to the vesicles, facilitating their passage through the narrow intercellular spaces of the skin. The synergistic interaction of ethanol and edge activators can induce significant bilayer deformation, thereby enhancing permeation into deeper cutaneous layers.

Transferosomes and transethosomes offer several advantages as non-invasive drug delivery systems [51, 52]. Their biocompatible and biodegradable nature allows for the safe and controlled delivery of a diverse range of therapeutic molecules, including proteins, peptides, insulin, corticosteroids, interferons, anaesthetics, nonsteroidal anti-inflammatory drugs (NSAIDs), anticancer, and herbal medicines. These vesicular systems facilitate controlled and sustained drug release and can be formulated as patient-friendly semi-solid dosage forms, such as gel or creams. Importantly, they bypass first-pass metabolism and mitigate potential gastrointestinal side effects, such as gastric irritation and emesis.

While offering numerous advantages, transethosomes and transferosomes also present certain limitations [22, 52]:

(1) The presence of ethanol in transethosomal formulations can potentially cause skin irritation, allergic reactions, or dermatitis.

(2) Inadequate vesicle production can lead to transethosome coalescence.

(3) Transferosomes exhibit susceptibility to oxidative degradation, which can compromise their chemical stability.

(4) The cost of lipid excipients and specialized manufacturing equipment can contribute to the overall expense of transferosomal formulations.

Mechanism of transferosome-mediated drug delivery in skin cancer treatment

The stratum corneum presents a significant barrier to transdermal drug absorption. Drug penetration occurs via three primary pathways: intracellular, intercellular, and follicular. Under optimal conditions, transferosomes can transport approximately 0.1 mg of lipid per hour per square centimeter through intact skin. This enhanced flux is primarily attributed to the transdermal osmotic gradient, which significantly exceeds the driving force provided by the typical transdermal concentration gradient. The transdermal osmotic gradient arises from the moisture differential between the stratum corneum (~15% water content) and the underlying epidermis (~75% water content), which prevents transepidermal water loss. Polar lipids, due to their interaction with hydrophilic lipid remnants and surrounding moisture, attract water. Upon application of a transferosome suspension to partially dehydrated skin, the resulting osmotic gradient drives vesicle migration to prevent complete desiccation. Unlike conventional liposomes, which lack the deformability conferred by surfactants and hydration, transferosomes can traverse narrow skin pores. Liposomes tend to desiccate upon application, exhibiting limited penetration. The high deformability of transferosomes enables them to permeate the stratum corneum, facilitating the delivery of therapeutic agents to deeper skin layers, including the dermis and epidermis, where cancerous tissues are often located, thereby providing sustained therapeutic effects and reducing dosing frequency [38]. These unique characteristics, including enhanced skin penetration, targeted delivery, and controlled release, make transferosomes particularly well-suited for delivering therapeutics directly to skin tumors [53]. Mechanism of action of transethosomes/transferosomes in skin cancer treatment is explained in Fig. 2.

Therapeutic applications

Transferosomes are advanced drug delivery systems specifically designed to enhance transdermal drug delivery, offering significant advantages for skin cancer therapy. These vesicles encapsulate various anticancer agents, including chemotherapeutic drugs, immunotherapeutic agents, and targeted small molecules. Their inherent deformability allows them to efficiently traverse the stratum corneum, the outermost skin barrier, facilitating drug penetration to tumor sites located in deeper skin layers [40, 54]. This controlled release mechanism provides sustained drug exposure at the tumor site, reducing the need for frequent applications and potentially improving therapeutic outcomes. The transdermal application of transferosomes offers a less invasive alternative to traditional procedures or systemic oral medications, minimizing associated side effects. Transferosomes can be employed to deliver a diverse range of anticancer therapies transdermally, including chemotherapeutic agents, gene therapies, and

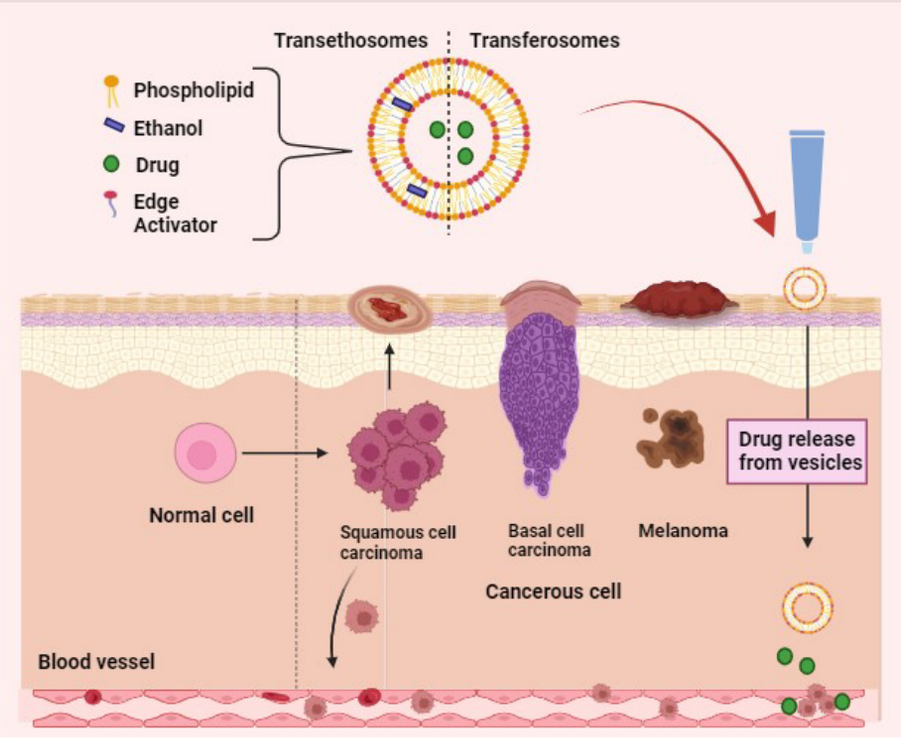

Fig. 2 Mechanism of action of transethosomes/transferosomes in skin cancer treatment.

immunotherapeutic agents. Specific examples of transferosomal drug delivery strategies for skin cancer treatment are presented below [55].

Actinic keratosis

Actinic keratosis (AK), a common skin condition resulting from chronic sun exposure, typically develops on sun-exposed areas such as the face, neck, chest, shoulders, back of arms, and hands. AK is characterized by keratotic macules, papules, or plaques with superficial scales on an erythematous base [55]. Treatment strategies vary depending on lesion presentation and may involve targeting individual lesions, contiguous lesions, or a combination thereof. Therapeutic options include topical treatments (e.g., 5-fluorouracil (5-FU) cream, imiquimod cream, and ingenolmebutate gel), procedures such as curettage or surgical excision, photodynamic therapy (PDT), and cryosurgery. However, topical application of 5-FU exhibits limited percutaneous penetration, reducing its anticancer potency. Encapsulation of 5-FU within transferosomal formulations has been shown to enhance topical delivery and increase its cytotoxic effect *in vivo* [56, 57]. Compared to other formulations, a 5-FU-loaded transferosomal gel demonstrated superior efficacy in treating AK and NMSC, enhancing transdermal release by up to twofold [58].

Basal cell carcinoma

Basal cell carcinoma (BCC) represents the most common form of cutaneous malignancy in the USA, affecting nearly one in five individuals [59]. Among individuals of European descent in North America, BCC incidence is rising by more than 10% annually, resulting in a lifetime risk of approximately 30% [59]. The increasing prevalence of BCC is projected to continue with the expanding older population. Individuals with darkerskin pigmentation experience a significantly lower incidence of BCC. Established risk factors for BCC include phenotypic characteristics such as red or fair hair, blue or green eyes, and skin phototype I (characterized by a tendency to burn and minimal tanning ability) [60]. Additional non-ultraviolet risk factors include exposure to ionizing radiation, elevated dietary fat intake, insufficient vitamin intake, certain environmental pollutants, and arsenic, which is associated with an increased risk of multiple BCCs. Immunosuppression is also a significant risk factor [60], with transplant recipients in the Netherlands exhibiting a 10-fold higher incidence of BCC compared to the general population [50]. Transferosomes have been investigated for targeted delivery of therapeutic agents to BCC, as demonstrated by Fadel et al. [61], who encapsulated indocyanine green for topical PDT.

Kaposi's sarcoma

Kaposi's sarcoma (KS) was first described in 1872 by Moritz Kaposi, a Hungarian dermatologist. Initially a rare tumor, KS became more prevalent with the emergence of AIDS and human immunodeficiency virus. While historically observed predominantly in elderly Jewish or Italian men in Europe and North America, KS also affects other groups, including young adult males of African descent, adolescents, transplant recipients, and individuals undergoing immunosuppressive therapies [62]. The first documented case of immunosuppression-associated KS in a kidney transplant recipient occurred in 1969. Subsequently, KS was observed in several recipients of kidney and other organ transplants shortly after initiating standard immunosuppressive regimens with prednisone and azathioprine [63]. Pathak et al. developed deformable paclitaxel nanovesicles for dermal chemotherapy, specifically targeting deep dermal regions in AIDS-related KS [64]. *In vitro* cytotoxicity assays on KSY-1 cell lines revealed a higher IC_{50} (≤17) for the transferosome formulation compared to the resulting transferosome gel (IC_{50} ≤ 19). Confocal laser scanning microscopy confirmed successful penetration of the dermal skin layers by the nanovesicles, reaching the proposed target site [64].

Squamous cell carcinoma (SCC)

SCC is an epithelial malignancy arising in tissues lined by squamous epithelium, including the lips, mouth, skin, esophagus, prostate, lungs, urinary system, cervix, and vagina. It is a common malignancy globally and in the USA, with the potential for metastasis [65]. While tobacco use and human papillomavirus (HPV) are implicated in the development of certain SCC subtypes, sun exposure is a significant risk factor for cutaneous SCC. Individuals with fair skin, prone to burning and limited tanning ability, have a higher risk of developing cutaneous SCC compared to those with darker skin [66]. Gupta et al. developed protransferosomes for local delivery of cisplatin in the treatment of cutaneous epithelial cancers. These protransferosomes demonstrated enhanced skin penetration using a fluorescent marker. *In vivo* studies indicated improved therapeutic efficacy and reduced systemic toxicity of the drug [67].

Table 2 summarizes the successful incorporation of

Table 2 Transferosome-mediated delivery for skin cancer therapies.

Anticancer agents	Conventional marketed product	Constituents	Study	Outcome
5-Fluorouracil	Fluroplex	Polysorbate 80, Sorbitan oleate, edge activators	Tween-80 and Span-80 were employed as edge activators. A 5-FU-loaded transferosome formulation incorporating 1% Carbopol 940 was developed to enhance penetration into skin tumors and evaluate its anticancer efficacy relative to a commercial skin cancer treatment.	The results indicated that Polysorbate 80 is a more effective edge activator than Span-80. Furthermore, the transferosomal gel demonstrated enhanced skin deposition of 5-FU and superior *in vitro* skin permeability compared to the commercial formulation [68].
Doxorubicin hydrochloride (DOX)	DOXIL	Sodium deoxycholate, lecithin	Hyaluronic acid (HA)-modified transferosomes were developed for transdermal delivery of drugs to the lymphatic system.	DOX-loaded HA-glycerol monostearate-transferosomes (HA-GMS-Ts) effectively penetrated deep into skin tissue, resulting in enhanced lymphatic absorption and reduced systemic toxicity. This study presents a novel approach for treating tumor metastasis via transdermal lymphatic drug delivery using nanomedicine [24].
Celecoxib	Celcoxib topical	Tween-20, ethanol	Three vesicular formulations for celecoxib delivery were investigated: surfactant-modified liposomes, transferosomes, and ethosomes (the latter two incorporating appropriate edge activators).	The results demonstrated the most significant improvement in drug delivery through the skin [25].
5-Fluorouracil (5-FU)	Efudex® Cream	Dimiristoylphosphatidy-choline, dipalmitoylphosphatidylch oline, cholesterol, sodium cholate	5-FU-loaded transferosomes, liposomes, and niosomes were developed for topical treatment of actinic keratosis and non-melanoma skin cancer.	5-FU-loaded transferosomes exhibited the highest cytotoxicity against HaCaT cells compared to liposomes and niosomes. The authors concluded that transferosome-mediated delivery of 5-FU enhances both its topical application and cytotoxic potency [56].

various anticancer drugs into transferosomes for cancer therapy. Transferosomes and transethosomes are highly flexible liposomes that are widely used in the treatment of skin cancer due to their excellent transdermal drug delivery capabilities. Additionally, they have successfully encapsulated various drugs , demonstrating their potential for the treatment of different diseases, such as diabetes and liver cancer. Transferosomes as delivery systems for biomedical applications are well summarized in Table 3.

Table 3 Transferosomes as delivery systems for biomedical applications.

Sr. No.	Drug	The category	Method of preparation	Result
1	Eulophiamacrobulbon	phosphodiesterase-5 (PDE-5) enzyme inhibitors	—	Significantly enhanced skin permeation [69]
2	Tolnaftate	Antifungal	Thin-film hydration	Enhanced drug penetration [70]
3	Cephalexin	Anti-bacterial	Thin-film hydration method optimized using a Quality by Design Box-Behnken approach.	Enhanced drug penetration and prolonged drug release allowed for reduced dosage and administration frequency [71]
4	Famciclovir	Anti-viral	Thin-film hydration	Enhanced penetration and deposition of the drug within the dermis [72]
5	Concanavalin-A	Anticancer	Rotary evaporation-sonication	Enhanced both *in vitro* and *in vivo* permeation and skin deposition of apigenin compared to the marketed formulations [73]
6	Resveratrol	Antioxidant and anticancer	High-pressure homogenization	The coating did not affect antioxidant activity but improved stability, bioavailability, solubility, and safety [74]
7	Apigenin	Anticancer	Rotary evaporation-sonication using Tween-80	The formulation exhibited more prolonged action than both pure drug suspension and the commercial product [71]
8	Insulin	Anti-diabetic	Thin-film hydration	Transdermal administration resulted in a prolonged hypoglycemic effect in diabetic rats over 24 h [75, 76]
9	Lidocaine	Local anesthetic	Thin-film hydration method. SPC, CH, SC, SP80, and Brij 35 were dissolved in a methanol: chloroform mixture (2:2:1, v/v/v), followed by hydration using an isotonic phosphate buffer (pH 5.8) and sonication.	Improved skin absorption resulted in greater efficacy of the local anesthetic [77,78]
10	Repaglinide	Anti-hypoglycemia drug	Thin-film hydration. Soy lecithin, SP80, SP60, SP40, and TW80 were dissolved in a methanol: chloroform mixture (3:1, v/v). The resulting thin-film was hydrated with phosphate-buffered saline (PBS, pH 6.8), followed by sonication using a bath sonicator.	Enhanced targeting to the specific site and extended drug release [79]
11	Mefenamic acid	NSAID	Modified handshaking and thin-film hydration. SPC and SP60 were dissolved in a chloroform solution (2:1, v/v), hydrated with PBS (pH 7.4), and stirred using an orbital shaker.	The thin-film hydration technique yielded superior results, demonstrating the highest drug content, optimal spreadability, and sustained drug release over 12 hours [80]
12	Sildenafil citrate	Phosphodiesterase inhibitors	Thin-film hydration and sonication. Sodium lauryl sulfate, P90 G, TW80, SP80, and cetrimide were dissolved in a chloroform and methanol mixture (1:1), combined with PBS (pH 5.8), and then sonicated using a probe sonicator.	Enhanced transdermal penetration and bioavailability allowed for a reduced dosing frequency [81]
13.	Itracanazole	Antifungal drug	Thin-film hydration. SPC, TW80, and SP80 were dissolved in a chloroform mixture (3:1), mixed with phosphate buffer (pH 6.8), and sonicated for 30 min.	Extended drug release [31]
14	Curcumin	Anti-inflammatory	Modified handshaking method. Lecithin, TW80, and SP80were dissolved in a 1:1 (v/v) mixture of ethanol and chloroform at lecithin ratios of 95:05 and 85:15. The solvents (ethanol and isopropyl alcohol) were removed and the resulting film was hydrated with phosphate buffer (pH 7.4).	Strong anti-inflammatory effects, enhanced absorption, and bioavailability [82]
15	Brucine - strychnine	Anticancer	Thin-film hydration	The transdermal brucine-strychnine transethosomal formulation inhibited the growth of human HepG2 liver cancer cells [83]
16	Rolapitant	Anticancer	Thin-film hydration	The formulation demonstrated promise as a treatment for lung cancer [84]

Evaluation of Transferosomes and Transethosomes in Gel Formulations

Characterization of transferosomes/transethosomes

Vesicle morphology, size, and zeta potential

Transmission electron microscopy (TEM) revealed the vesicular morphology of these systems [85]. Kaur et al. observed atypically spherical transethosomes using TEM. Vesicle diameter can be determined using photon correction spectroscopy or dynamic light scattering (DLS) [86]. Zeta potential measurements quantify the electrostatic interactions within the colloidal dispersion and provide insights into surface chemistry and colloidal stability [87].

Entrapment efficiency

The percentage entrapment efficiency (%EE) quantifies the amount of drug encapsulated within the formulation. Mini-column centrifugation, using both direct and indirect methods, separates free drugs from vesicles, enabling the determination of EE. In the direct method, following ultracentrifugation, the supernatant is removed, and the vesicles are disrupted with n-propanol. The resulting solution is then diluted and filtered through a syringe filter to remove any particulate matter. Drug content is subsequently quantified using spectrophotometric analysis or high-performance liquid chromatography (HPLC) [39]. The %EE is calculated as entrapment efficiency (EE) = (quantity of drug encapsulated/total amount of drug) × 100.

In vitro drug release

In vitro drug release can be assessed using Franz diffusion cells. The donor chamber is affixed to the receptor chamber using adhesive tape. The receptor chamber is continually stirred using a magnetic stir bar. At predetermined time intervals (e.g., 0, 0.5, 1, 2, 3, 4, and 6 h), 1 ml aliquots of the receptor medium are withdrawn and replaced with an equal volume of fresh phosphate buffer to maintain sink conditions. Collected samples are then analyzed using UV spectroscopy or HPLC [30].

In vivo skin deposition

Skin disposition studies in animal models, such as rats and mice, were employed to assess drug distribution across skin layers following transferosomal or transethosomal delivery. Drug deposition in the stratum corneum was quantified 24 h post-administration using fluorescence spectrophotometry [87]. Confocal laser scanning microscopy was used to visualize drug distribution through the various skin layers. Albash et al. demonstrated the ability of transethosomes to penetrate the stratum corneum and reach deeper skin layers using this technique [88].

Turbidity measurements

Nephelometry is used to determine the turbidity of a medication in aqueous solution [89].

Deformability degree

Deformability was assessed using pure water as a control. The preparation was serially filtered through microporous filters with pore sizes ranging from 50 to 400 nm. After each filtration step, particle size and size distribution were determined using DLS. The degree of deformability (D) was calculated using the following equation:

$$D = J \times (r_v / r_p) \quad (1)$$

where D means deformability degree, J means suspension volume extruded over 5 min, r_v means vesicle size, and r_p means barrier pore size [89, 90].

Stability

Formulations are stored in sealed amber vials under various temperature and humidity conditions. In accordance with International Council on Harmonization guidelines, standard storage conditions are as follows: long-term stability testing at (25 ± 2) °C/(60% ± 5%) RH or (30 ± 2) °C/(65% ± 5%) RH for 12 months; accelerated stability testing at (40 ± 2) °C/(75% ± 5%) RH for 6 months. For refrigerated products, long-term storage and accelerated testing are conducted at (5 ± 3) °C for 12 months and (25 ± 2) °C/(60% ± 5%) RH for 6 months, respectively. Deviation from these specifications is considered a significant change in the drug product. Samples from each vial are assessed for leakage after 30 days. Drug loss is determined by comparing the measured drug content to the initial (100%) entrapment [31, 41].

Drug content

Drug content is determined using pharmacopoeial analytical methods employing instrumental techniques. This typically involves a modified HPLC method equipped with a UV detector, column oven,

autosampler, pump, and computerized data acquisition software [90].

Gel assessment

Rheological properties of the transferosomal gel

Rheological studies on the transferosomal gel require real-time assessment of its elasticity and flow properties. This analysis provides insights into gel behavior and stability.

Viscosity measurements

Gel viscosity is measured using an Anton Paar MCR 52 Rheometer with a 50 mm diameter parallel plate geometry. The gap between the plate and the fixed platform is maintained at 1 mm. The excess sample is carefully removed after placement and spindle adjustment. Viscosity and shear stress are measured as a function of shear rate, with dynamic shear rates ranging from 0.1 and 100 s^{-1}. A2-minequilibration period is implemented after sample loading to ensure system relaxation and accurate measurements [91].

Amplitude sweep tests

Amplitude sweep tests are essential for characterizing the viscoelastic properties of materials, such as gels, by determining the stress response to oscillatory strain. These tests are primarily used to identify the linear viscoelastic (LVE) region and assess material behavior at various strain amplitudes. The formulations are analyzed within the LVE region using increasing shear stress amplitudes ranging from 0.01% to 1000% at a constant frequency of 10 rad/s [92].

Hysteresis area

A controlled shear rate test is performed using a rheometer to determine the hysteresis area. This involves increasing the shear rate to a predefined maximum value and subsequently decreasing it. Shear stress (Pa) is then plotted against the shear rate (s^{-1}) for both the ascending and descending shear rate ramps, resulting in two distinct curves [93].Rheological measurements are performed using an Anton Paar rheometer equipped with a CP60-1geometry (60 mm in diameter, 1° cone angle, serial No. SN44803) [94].

Transferosomes in clinical trials

Transferosomes are being actively researched, and some formulations have entered clinical trials for various applications. However, the specific status of transferosomes in clinical trials may vary depending on the drug being delivered and the targeted condition. Here are some key points regarding their clinical trial status. The current clinical trials of transferosomes are shown in Table 4 [50].

Marketed Formulations and Future Directions

Details of the marketed transferosomal preparation and the patented formulation are provided in Tables 5 and 6 [95−98], respectively.

Transferosomes for non-invasive vaccine delivery

Transferosomes have potential applications in

Table 4 Transferosomes in clinical trials.

Title of the study	Drug	Clinical trial phase
A double-blind, placebo-controlled study comparing the safety and efficacy of epicutaneously applied IDEA-033 (ketoprofen in transferosomes) with oral celecoxib for managing pain associated with knee osteoarthritis.	Ketoprofen	Phase II
A multi-dose, randomized, double-blind, placebo-controlled study assessing the efficacy of topically applied ketoprofen Transferosome® Gel, with and without concomitant oral celecoxib, for treating muscle pain induced by eccentric exercise.	Ketoprofen	Phase I
A multicenter, randomized, double-blind, placebo-controlled study assessing the safety and effectiveness of topically applied Diractin® (ketoprofen in Transferosome® Gel) for treating knee osteoarthritis.	Ketoprofen	Phase III
A multicenter, randomized, double-blind study, placebo- and active-controlled study assessing the safety and efficacy of two doses of topically administered Diractin® (ketoprofen in Transferosome® Gel) for treating knee osteoarthritis.	Ketoprofen	Phase III

Table 5 Marketed transferosome formulations.

Sr. No.	Brand name	drug	licensor	conditions	Effect observed
1.	FLEXISEQ	Plant-based phospholipids	Pro Bono Bio (UK, 2011)	Pain associated with osteoarthritis	By coating the cartilage and acting as a lubricant, FLEXISEQreduces friction between cartilage surfaces, minimizing further inflammation

Table 6 Patents on transferosome drug delivery systems.

Sr. No.	Patent No.	Patent title	Inventor	Assignee	Patent details	Year
1	US7867480B1	Non-invasive vaccination through the skin	Gregor Cevc and amla chopra	Idea AG	The inventor has developed innovative vaccines for transcutaneous, non-invasive antigen delivery using ultradeformable carriers for therapeutic and prophylactic applications [95].	2011
2.	US20070042030A1	Preparation for the application of agents in mini droplets	Gregor Cevc	Idea AG	The invention discloses a method for preparing agents as tiny fluid droplets exhibiting membrane-like characteristics [96].	2007
3.	US7175850B2	Formulation for topical non-invasive application *in vivo*	Gregor Cevc	Idea AG	The invention relates to formulations containing molecular arrangements capable of penetrating barrier pores smaller than their average diameter due to their adaptive penetrant properties [97].	2007
4.	US6165500A	Preparation for the application of agents in the mini droplets	Gregor Cevc	Idea AG	The invention relates to a preparation of tiny fluid droplets with a membrane-like structure composed of one or more layers of an amphiphilic carrier substance for transdermal delivery [98].	2000

transcutaneous vaccine delivery, controlled drug release, and targeted delivery to the peripheral submucosa. Their deformability makes them a promising vehicle for transcutaneous immunization [99]. Compared to liposomes, transferosomes exhibit increased skin permeability to antigens and enhance antigen delivery to antigen-presenting cells (APCs) [100]. However, studies on transferosome-mediated skin permeation have yielded variable results. For instance, ultra-deformable cationic liposomes delivering hepatitis B surface antigen (HBsAg) plasmid DNA–cationic complexes elicited more robust cellular and humoral immune responses than conventional liposomes [101]. Chopra et al. successfully formulated transferosomes containing 180 nm vesicles loaded with tetanus toxoid antigen using thin-film hydration [102]. *In vivo* studies demonstrated complete immunization of mice against tetanus toxoid using these transferosomes, supporting their potential as antigen delivery vehicles for non-invasive vaccination strategies. Further research is needed to explore the potential for controlled cytokine release and its implications for studying immune responses in intact organs [103].

AI-enhanced transferosome drug delivery

Artificial intelligence (AI) and machine learning are increasingly recognized as powerful tools for advancing nanoscale drug assessment and formulation, including the development of transferosomes. While optimizing formulation ratios can be a time-consuming process, it is essential for achieving the desired physicochemical properties [104]. AI-driven approaches offer the potential to accelerate the high-throughput and systematic design of effective nanoformulations and smart materials with specific functionalities. For instance, recent research has shown that artificial neural networks can significantly improve the prediction of nanoparticle properties, reducing prediction error for particle size and drug release to 2.93% and 2.99%, respectively, from initial errors of 28.0% and 19.4%. This methodology could be similarly applied to transferosome formulation development. Reker et al. demonstrated the potential of a random forest machine learning model for rapidly identifying optimal drug-excipient combinations for nanocarriers with enhanced loading capacity [105]. Collaborative efforts between pharmaceutical scientists and AI experts are crucial for developing sophisticated AI systems integrated with automated robotic particle synthesis to further enhance outcomes in this field [106].

Regulatory considerations

Pharmaceutical regulatory affairs encompass laws and regulations designed to ensure the safety, quality, and efficacy of drug products. Several regulatory bodies, including the US Food and Drug Administration, the World Health Organization (WHO), the Japanese Ministry of Health and Welfare, the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, and the International Pharmaceutical Excipients Council, maintain lists of excipients considered Generally Recognized as Safe (GRAS) or deemed non-toxic

based on clinical assessments. Phospholipids, crucial components of transferosome-based drug delivery systems, frequently utilize unsaturated phosphatidyl choline (PC), such as egg PC or soybean PC (SPC). SPC is GRAS-listed and conforms to Food Chemicals Codex requirements [106]. Edge activators, typically surfactants, enhance elasticity and destabilize the lipid bilayer of elastic liposomes. Commonly used edge activators include sodium cholate, sodium deoxycholate, Span-80, Tween-80, and Tween-20 [107]. While sodium cholate is generally considered non-toxic as an edge activator, it can cause skin, eye, and respiratory irritation. Surfactant concentrations exceeding 25% can induce significant gastrointestinal distress; safe concentrations typically range from 10% to 25%. Ethanol also enhances skin penetration by interacting with lipid polar head groups, reducing the melting point of stratum corneum lipids and increasing membrane fluidity and permeability. Maximum approved potencies of inactive ingredients are detailed in Table 7 [108]. All excipients used in the transethosomal formulations were within these established limits.

These vesicular systems offer a range of therapeutic possibilities due to their demonstrated efficacy and safety profiles. For instance, Swiss Medic, the Swiss regulatory authority, approved a transferosome formulation of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen for commercial use (marketed under the Diractin trademark). IDEA AG reports ongoing clinical development of additional transferosome-based therapeutic products. Transethosomes offer an improved carrier system for stabilizing various proteins and medications and can be used to enhance drug efficacy through combination therapies. Although commercially available transethosome-based products are limited, preclinical evidence suggests their significant potential for topical and transdermal drug delivery [109, 110].

Table 7 FDA-approved inactive pharmaceutical ingredients.

Inactive ingredient	Maximum potency (highest unit dose amount)
Propylene glycol	20% (w/w)
Oleic acid	9.6 mg
Ethanol	74.1% (w/w)
Isopropyl alcohol	20% (w/w)

Conclusions

Next-generation nanocarriers integrated into transdermal drug delivery systems hold promise for revolutionizing skin cancer care. By harnessing the unique properties of nanomaterials, these advanced carriers offer the potential to address current limitations in skin cancer treatment, including poor bioavailability, systemic toxicity, and patient compliance. Nanocarriers such as transferosomes and transethosomes facilitate more efficient and targeted drug delivery, enhancing penetration and controlled release of therapeutic agents directly through the skin. The convergence of nanotechnology and transdermal delivery offers the potential for personalized, non-invasive treatment modalities, potentially improving both efficacy and patient quality of life. However, successful clinical translation requires addressing challenges related to manufacturing scalability, regulatory approval, and long-term safety. Continued multidisciplinary research and collaboration are essential for optimizing these technologies and overcoming these barriers. The potential of nanocarriers for transdermal drug delivery may usher in a new era of more efficient, less invasive, and personalized cancer care, with the prospect of improved patient outcomes.

CRediT Author Statement

Bhushan Rane, Nandini Mhatre: data curation, writing–review, and editing. **Bhushan Rane and Nandini Mhatre:** visualization, writing original draft, writing–review, and editing. **Bhushan Rane, Nandini Mhatre, and Ashish Jain**: data curation, formal analysis, and investigation. **Bhushan Rane**: conceptualization, methodology, project administration, and supervision.

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Conflict of Interests

The authors declare no conflict of interests.

References

[1] S. An, K. Kim, S. Moon, et al. Indoor tanning and the risk of overall and early-onset melanoma and non-melanoma skin cancer: Systematic review and meta-analysis. *Cancers*, 2021, 13(23): 5940. https://doi.org/10.3390/cancers13235940

[2] T. Hanawa. IARC monographs on the evaluation of carcinogenic risks to humans. *Journal of Clinical Pathology*, 2019, 48: 691. https://doi.org/10.1136/jcp.48.7.691-a

[3] H. Sung, J. Ferlay, R.L. Siegel, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 2021, 71(3): 209−249. https://doi.org/10.3322/caac.21660

[4] J. Chandra, N. Hasan, N. Nasir, et al. Nanotechnology-empowered strategies in treatment of skin cancer. *Environmental Research*, 2023, 235: 116649. https://doi.org/10.1016/j.envres.2023.116649

[5] N. Hasan, M. Imran, A. Sheikh, N. Tiwari, A. Jaimini, P. Kesharwani, G. K. Jain, F. J. Ahmad. Advanced multifunctional nano-lipid carrier loaded gel for targeted delivery of 5-flurouracil and cannabidiol against non-melanoma skin cancer. *Environmental Research*. 2023, 233: 116454. https://doi.org/10.1016/j.envres.2023.116454

[6] *Skin cancer statistics | World Cancer Research Fund International*. WCRF International. https://www.wcrf.org/cancertrends/skin-cancer-statistics/ (accessed 2024-06-06).

[7] World Health Organization. Radiation: Ultraviolet (UV) radiation and skin cancer. World Health Organization, Geneva, Switzerland. 2017 Oct. https://www.who.int/news-room/questions-and-answers/item/radiation-ultraviolet-(uv)-radiation-and-skin-cancer.

[8] *Annual Cancer Facts & Figures | American Cancer Society*. www.cancer.org. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/.

[9] World Health Organization. Compendium of WHO and other UN guidance on health and environment. World Health Organization; 2022.

[10] S. Dasari, C.G. Yedjou, R.T. Brodell, et al. Therapeutic strategies and potential implications of silver nanoparticles in the management of skin cancer. *Nanotechnology Reviews*, 2020, 9(1): 1500−1521. https://doi.org/10.1515/ntrev-2020-0117

[11] P. Aggarwal, P. Knabel, A.B. Fleischer. United States burden of melanoma and non-melanoma skin cancer from 1990 to 2019. *Journal of the American Academy of Dermatology*, 2021, 85(2): 388−395. https://doi.org/10.1016/j.jaad.2021.03.109

[12] X. Bonilla, L. Parmentier, B. King, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nature Genetics*, 2016, 48(4): 398−406. https://doi.org/10.1038/ng.3525

[13] V. Lai, W. Cranwell, R. Sinclair. Epidemiology of skin cancer in the mature patient. *Clinics in Dermatology*, 2018, 36(2): 167−176. https://doi.org/10.1016/j.clindermatol.2017.10.008

[14] M. Ciążyńska, G. Kamińska-Winciorek, D. Lange, et al. The incidence and clinical analysis of non-melanoma skin cancer. *Scientific Reports*, 2021, 11(1): 4337. https://doi.org/10.1038/s41598-021-83502-8

[15] C.R. Pickering, J.H. Zhou, J.J. Lee, et al. Mutational landscape of aggressive cutaneous squamous cell carcinoma. *Clinical Cancer Research*, 2014, 20(24): 6582−6592. https://doi.org/10.1158/1078-0432.ccr-14-1768

[16] L. Fania, D. Didona, F.R. Di Pietro, et al. Cutaneous squamous cell carcinoma: From pathophysiology to novel therapeutic approaches. *Biomedicines*, 2021, 9(2): 171. https://doi.org/10.3390/biomedicines9020171

[17] J. D'Orazio, S. Jarrett, A. Amaro-Ortiz, et al. UV radiation and the skin. *International Journal of Molecular Sciences*, 2013, 14(6): 12222−12248. https://doi.org/10.3390/ijms140612222

[18] A. Costello, M. Abbas, A. Allen, et al. Managing the health effects of climate change. *The lancet*, 2009, 373(9676): 1693−1733. https://doi.org/10.1016/s0140-6736(09)60935-1

[19] A.F. Bais, R.L. McKenzie, G. Bernhard, et al. Ozone depletion and climate change: Impacts on UV radiation. *Photochemical & Photobiological Sciences*, 2015, 14(1): 19−52. https://doi.org/10.1039/c4pp90032d

[20] E.R. Parker. The influence of climate change on skin cancer incidence - A review of the evidence. *International Journal of Women's Dermatology*, 2021, 7(1): 17−27. https://doi.org/10.1016/j.ijwd.2020.07.003

[21] I.M. Abdulbaqi, Y. Darwis, N.A. Khan, et al. Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, *in vivo* studies, and clinical trials. *International Journal of Nanomedicine*, 2016, 11: 2279−2304. https://doi.org/10.2147/IJN.S105016

[22] M.Z., D. Mahapatra, T. Joseph. Transethosomes: Novel technology for skin delivery of drugs. *International Journal of Medical & Pharmaceutical Sciences*, 2021, 11(08). https://doi.org/10.31782/ijmps.2021.11801

[23] S.R. Kodi, M.S. Reddy. Transferosomes: A novel topical approach. *Journal of Drug Delivery and Therapeutics*, 2023, 13(2): 126−131. https://doi.org/10.22270/jddt.v13i2.5952

[24] M.M. El-Sonbaty, M.A. Akl, M. Khalid, et al. Does the technical methodology influence the quality attributes and the potential of skin permeation of Luliconazole loaded transethosomes. *Journal of Drug Delivery Science and Technology*, 2022, 68: 103096. https://doi.org/10.1016/j.jddst.2022.103096

[25] P. Chowdary, A. Padmakumar, A.K. Rengan. Exploring the potential of transethosomes in therapeutic delivery: A comprehensive review. *MedComm–Biomaterials and Applications*, 2023, 2(4): e59. https://doi.org/10.1002/mba2.59

[26] M. Adnan, M.F. Haider, N. Naseem, et al. Transethosomes: a promising challenge for topical delivery short title: transethosomes for topical delivery. *Drug Research*, 2023, 73(04): 200−212. https://doi.org/10.1055/a-1974-9078

[27] S. Shah, V. Dhawan, R. Holm, et al. Liposomes: Advancements and innovation in the manufacturing process. *Advanced Drug Delivery Reviews*, 2020, 154: 102−122. https://doi.org/10.1016/j.addr.2020.07.002

[28] V. Garg, H. Singh, A. Bhatia, et al. Systematic development of transethosomal gel system of piroxicam: formulation optimization, *in vitro* evaluation, and *ex vivo* assessment. *AAPS pharmscitech*, 2017, 18: 58−71. https://doi.org/10.1208/s12249-016-0489-z

[29] V. Gupta, N. K. Joshi. Formulation, development and evaluation of ketoprofen loaded transethosomes gel. *Journal of Drug Delivery and Therapeutics*, 2022, 12(1): 86−90. https://doi.org/10.22270/jddt.v12i1.5177

[30] C.K. Song, P. Balakrishnan, C.K. Shim, et al. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: characterization and in vitro/in vivo evaluation. *Colloids and Surfaces B: Biointerfaces*, 2012, 92: 299−304. https://doi.org/10.1016/j.colsurfb.2011.12.004

[31] S.S. Hallan, M. Sguizzato, P. Mariani, et al. Design and

characterization of ethosomes for transdermal delivery of caffeic acid. *Pharmaceutics*, 2020, 12(8): E740. https://doi.org/10.3390/pharmaceutics12080740

[32] M. Costanzo, E. Esposito, M. Sguizzato, et al. Formulative study and intracellular fate evaluation of ethosomes and transethosomes for vitamin D3 delivery. *International Journal of Molecular Sciences*, 2021, 22(10): 5341. https://doi.org/10.3390/ijms22105341

[33] A. Youssef, N. Dudhipala, S. Majumdar. Ciprofloxacin loaded nanostructured lipid carriers incorporated into in-situ gels to improve management of bacterial endophthalmitis. *Pharmaceutics*, 2020, 12(6): 572. https://doi.org/10.3390/pharmaceutics12060572

[34] A.H. Aodah, S. Hashmi, N. Akhtar, et al. Formulation development, optimization by box–behnken design, and *in vitro* and *ex vivo* characterization of hexatriacontane-loaded transethosomal gel for antimicrobial treatment for skin infections. *Gels*, 2023, 9(4): 322. https://doi.org/10.3390/gels9040322

[35] K.K. Mishra, C.D. Kaur, A. Gupta. Development of itraconazole loaded ultra-deformable transethosomes containing oleic-acid for effective treatment of dermatophytosis: Box-Behnken design, *ex-vivo* and *in-vivo* studies. *Journal of Drug Delivery Science and Technology*, 2022, 67: 102998. https://doi.org/10.1016/j.jddst.2021.102998

[36] H.F. Salem, M.M. Nafady, R.M. Kharshoum, et al. Mitigation of rheumatic arthritis in a rat model via transdermal delivery of dapoxetine HCl amalgamated as a nanoplatform: in vitro and in vivo assessment. *International Journal of Nanomedicine*, 2020, 15: 1517−1535. https://doi.org/10.2147/ijn.s238709

[37] M. Singh, K.E. Lee, R. Vinayagam, et al. Synthesis and assessment of lipid nanovesicles for efficient transdermal delivery of hydrophilic molecules. *Nano*, 2022, 17(04): 2250031. https://doi.org/10.1142/s179329202250031x

[38] P. Chaurasiya, E. Ganju, N. Upmanyu, et al. Transfersomes: a novel technique for transdermal drug delivery. *Journal of Drug Delivery and Therapeutics*, 2019, 9(1): 279−285. https://doi.org/10.22270/jddt.v9i1.2198

[39] K.K. Mishra, C.D. Kaur, S. Verma, et al. Transethosomes and nanoethosomes: Recent approach on transdermal drug delivery system. In: Nanomedicines. London: IntechOpen, 2019. https://doi.org/10.5772/intechopen.81152

[40] S. Rai, V. Pandey, G. Rai. Transfersomes as versatile and flexible nano-vesicular carriers in skin cancer therapy: The state of the art. *Nano Reviews & Experiments*, 2017, 8(1): 1325708. https://doi.org/10.1080/20022727.2017.1325708

[41] M. Bragagni, N. Mennini, F. Maestrelli, et al. Comparative study of liposomes, transfersomes and ethosomes as carriers for improving topical delivery of celecoxib. *Drug Delivery*, 2012, 19(7): 354−361. https://doi.org/10.3109/10717544.2012.724472

[42] A. Kumar, K. Pathak, V. Bali. Ultra-adaptable nanovesicular systems: A carrier for systemic delivery of therapeutic agents. *Drug Discovery Today*, 2012, 17(21-22): 1233−1241. https://doi.org/10.1016/j.drudis.2012.06.013

[43] E. Anwar, D. Ramadon, G.D. Ardi. Novel transethosome containing green tea (Camellia sinensis L. Kuntze) leaf extract for enhanced skin delivery of epigallocatechin gallate: Formulation and in vitro penetration test. *International Journal of Applied Pharmaceutics*, 2018, 10(1): 299. https://doi.org/10.22159/ijap.2018.v10s1.66

[44] S.R. Zahid, N. Upmanyu, S. Dangi, et al. Ethosome: a novel vesicular carrier for transdermal drug delivery. *Journal of Drug Delivery and Therapeutics*, 2018, 8(6): 318−326. https://doi.org/10.22270/jddt.v8i6.2028

[45] B. Bhasin, V.Y. Londhe. An overview of transfersomal drug delivery. *International Journal of Pharmaceutical Sciences and Research*, 2018, 9(6): 2175−2184. https://doi.org/10.13040/ijpsr.0975-8232.9(6).2175-84

[46] S.A.T. Opatha, V. Titapiwatanakun, R. Chutoprapat. Transfersomes: A promising nanoencapsulation technique for transdermal drug delivery. *Pharmaceutics*, 2020, 12(9): 855. https://doi.org/10.3390/pharmaceutics12090855

[47] N. Matharoo, H. Mohd, B. Michniak-Kohn. Transferosomes as a transdermal drug delivery system: Dermal kinetics and recent developments. *WIREs Nanomedicine and Nanobiotechnology*, 2024, 16(1): e1918. https://doi.org/10.1002/wnan.1918

[48] W. Lei, C.Q. Yu, H.Q. Lin, et al. Development of tacrolimus-loaded transfersomes for deeper skin penetration enhancement and therapeutic effect improvement *in vivo*. *Asian Journal of Pharmaceutical Sciences*, 2013, 8(6): 336–345. https://doi.org/10.1016/j.ajps.2013.09.005

[49] A. Pandey. Role of surfactants as penetration enhancer in transdermal drug delivery system. *Journal of Molecular Pharmaceutics & Organic Process Research*, 2014, 2: 2. https://doi.org/10.4172/2329-9053.1000113

[50] G. Cevc, A. Schätzlein, H. Richardsen. Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 2002, 1564(1): 21−30. https://doi.org/10.1016/s0005-2736(02)00401-7

[51] J. Shaji, R. Bajaj. Transethosomes: A new prospect for enhanced transdermal delivery. *International Journal of Pharmaceutical Sciences and Research*, 2018, 9(7): 2681−2685. https://doi.org/10.13040/ijpsr.0975-8232.9(7).2681-85

[52] P. Verma, K. Pathak. Therapeutic and cosmeceutical potential of ethosomes: An overview. *Journal of Advanced Pharmaceutical Technology & Research*, 2010, 1(3): 274−282. https://doi.org/10.4103/0110-5558.72415

[53] B. Dixena, R. Madhariya, A. Panday, et al. Overcoming skin barrier with transfersomes: Opportunities, challenges, and applications. *Current Drug Delivery*, 2025, 22(2): 160−180. https://doi.org/10.2174/0115672018272012231213100535

[54] H. Gayathri, S. Sangeetha. Design and development of tofacitinib citrate loaded transferosomal gel for skin cancer by box-Behnken design- doe approach. *International Journal of Health Sciences*, 2022: 3119–3140. https://doi.org/10.53730/ijhs.v6ns6.10118

[55] J.V. Schmitt, H.A. Miot. Actinic keratosis: A clinical and epidemiological revision. *Anais Brasileiros de Dermatologia*, 2012, 87(3): 425−434. https://doi.org/10.1590/s0365-05962012000300012

[56] I.A. Alvi, J. Madan, D. Kaushik, et al. Comparative study of transfersomes, liposomes, and niosomes for topical delivery of 5-fluorouracil to skin cancer cells: preparation, characterization, in-vitro release, and cytotoxicity analysis. *Anti-Cancer Drugs*, 2011, 22(8): 774−782. https://doi.org/10.1097/cad.0b013e328346c7d6

[57] M.A. Khan, J. Pandit, Y. Sultana, et al. Novel carbopol-based transfersomal gel of 5-fluorouracil for skin cancer treatment: in vitro characterization and in vivo study. *Drug delivery*, 2015, 22(6): 795−802. https://doi.org/10.3109/10717544.2014.902146

[58] R. Marks, M. Staples, G.G. Giles. Trends in non‐melanocytic skin cancer treated in Australia: the second national survey. *International Journal of Cancer*, 1993, 53(4): 585−590. https://doi.org/10.1002/ijc.2910530410

[59] R. Corona, E. Dogliotti, M. D'Errico, et al. Risk factors for basal cell carcinoma in a Mediterranean population: role of recreational sun exposure early in life. *Archives of Dermatology*, 2001, 137(9): 1162−1168. https://doi.org/10.1001/archderm.137.9.1162

[60] M.M. Hartevelt, J.N. Bavinck, A.M. Kootte, et al. Incidence of skin cancer after renal transplantation in The Netherlands. *Transplantation*, 1990, 49(3): 506−509. https://doi.org/10.1097/00007890-199003000-00006

[61] M. Fadel, N. Samy, M. Nasr, et al. Topical colloidal indocyanine green-mediated photodynamic therapy for treatment of basal cell carcinoma. *Pharmaceutical Development and Technology*, 2017, 22(4): 545−550. https://doi.org/10.3109/10837450.2016.1146294

[62] A. Friedman-Kien, B. Saltzman, Y.Z. Cao, et al. Kaposi's sarcoma in HIV-negative homosexual men. *The Lancet*, 1990, 335(8682): 168−169. https://doi.org/10.1016/0140-6736(90)90041-3

[63] I. Penn. Kaposi's sarcoma in organ transplant recipients: Report of 20 cases. *Transplantation*, 1979, 27(1): 8−11. https://doi.org/10.1097/00007890-197901000-00003

[64] K. Pathak, V. Sharma, M. Sharma. Optimization, *in vitro* cytotoxicity and penetration capability of deformable nanovesicles of paclitaxel for dermal chemotherapy in Kaposi sarcoma. *Artificial Cells, Nanomedicine, and Biotechnology*, 2016, 44(7): 1671−1683. https://doi.org/10.3109/21691401.2015.1080169

[65] M.P. Marinkovich. Laminin 332 in squamous-cell carcinoma. *Nature Reviews Cancer*, 2007, 7: 370−380. https://doi.org/10.1038/nrc2089

[66] B. Akgül, J.C. Cooke, A. Storey. HPV‐associated skin disease. *The Journal of the Pathology*, 2005, 208(2): 165−175. https://doi.org/10.1002/path.1893

[67] V. Gupta, C. Karthikeyan, P. Trivedi. Localized delivery of cisplatin for the effective management of squamous cell carcinoma from protransfersome formulation. *Archives of Pharmacal Research*, 2012, 35: 851−859. https://doi.org/10.1007/s12272-012-0510-3

[68] M.L. González-Rodríguez, C.M. Arroyo, M.J. Cózar-Bernal, et al. Deformability properties of timolol-loaded transfersomes based on the extrusion mechanism. Statistical optimization of the process. *Drug Development and Industrial Pharmacy*, 2016, 42(10): 1683−1694. https://doi.org/10.3109/03639045.2016.1165691

[69] S. Potisuwan, N. Apichatwatana, S. Rujivipat. Improved skin permeation of transferosomes containing Eulophia macrobulbon extract. *Colloids and Surfaces B: Biointerfaces*, 2023, 229: 113474. https://doi.org/10.1016/j.colsurfb.2023.113474

[70] K. Geetha, K.G. Nandini, A. Harshika, et al. Formulation and evaluation for enhancing tolnaftate permeation with transferosomal gel. *Latin American Journal of Pharmacy: A Life Science Journal*, 2023, 42(6): 167−177.

[71] N.R. Rarokar, S.D. Saoji, N.V. Deole, et al. Preparation and formula optimization of cephalexin loaded transferosomal gel by QbD to enhance the transdermal delivery: *in vitro, ex vivo* and *in vivo* study. *Journal of Drug Delivery Science and Technology*, 2023, 89: 104968. https://doi.org/10.1016/j.jddst.2023.104968

[72] S. Bhattacharyya, L. Kalai Tamilselvi, A. Muthukumar. Formulation, and evaluation of transferosomal gel of famciclovir for transdermal use. *Turkish Journal of Pharmaceutical Sciences*, 2024, 21(4): 303−312. https://doi.org/10.4274/tjps.galenos.2023.46735

[73] M.S. Jangdey, C.D. Kaur, S. Saraf. Efficacy of Concanavalin-a conjugated nanotransfersomal gel of apigenin for enhanced targeted delivery of UV induced skin malignant melanoma. *Artificial Cells, Nanomedicine, and Biotechnology*, 2019, 47(1): 904−916. https://doi.org/10.1080/21691401.2019.1578784

[74] P.S. Wu, Y.S. Li, Y.C. Kuo, et al. Preparation and evaluation of novel transfersomes combined with the natural antioxidant resveratrol. *Molecules*, 2019, 24(3): 600. https://doi.org/10.3390/molecules24030600

[75] G. Cevc. Transdermal drug delivery of insulin with ultradeformable carriers. *Clinical Pharmacokinetics*, 2003, 42: 461−474. https://doi.org/10.2165/00003088-200342050-00004

[76] G. Cevc, D. Gebauer, J. Stieber, et al. Ultraflexible vesicles, Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1998, 1368(2): 201−215. https://doi.org/10.1016/s0005-2736(97)00177-6

[77] R. Bnyan, I. Khan, T. Ehtezazi, et al. Roberts. Formulation and optimisation of novel transfersomes for sustained release of local anaesthetic. *Journal of Pharmacy and Pharmacology*, 2019, 71(10): 1508−1519. https://doi.org/10.1111/jphp.13149

[78] M.M. Omar, O.A. Hasan, A.M. El Sisi. Preparation and optimization of lidocaine transferosomal gel containing permeation enhancers: a promising approach for enhancement of skin permeation. *International Journal of Nanomedicine*, 2019, 14: 1551−1562. https://doi.org/10.2147/ijn.s201356

[79] G. Hussain, K. Kohli, A. Umar, et al. Nanovesicular delivery of repaglinide through skin. *Science of Advanced Materials*, 2013, 5(7): 810−821. https://doi.org/10.1166/sam.2013.1522

[80] K. Sailaja, R. Supraja. Formulation of mefenamic acid loaded transfersomal gel by thin film hydration technique and hand shaking method. *Nanomedicine Journal*, 2017, 4(2): 126−134. https://doi.org/10.22038/nmj.2017.8414

[81] S.M. Badr-Eldin, O.A. Ahmed. Optimized nano-transfersomal films for enhanced sildenafil citrate transdermal delivery: ex vivo and in vivo evaluation. *Drug Design, Development and Therapy*, 2016, 10: 1323−1333. https://doi.org/10.2147/dddt.s103122

[82] R. Agrawal, S.K. Sandhu, I. Sharma, et al. Development and evaluation of curcumin-loaded elastic vesicles as an effective topical anti-inflammatory formulation. *AAPS PharmSciTech*, 2015, 16: 364−374. https://doi.org/10.1208/s12249-014-0232-6

[83] Y. Wang, R. Wang, X. Qi, et al. Novel transethosomes for the delivery of brucine and strychnine: Formulation optimization, characterization and in vitro evaluation in hepatoma cells. *Journal of Drug Delivery Science and Technology*, 2021, 64: 102425. https://doi.org/10.1016/j.jddst.2021.102425

[84] M.F. Kabil, M. Nasr, I.T. Ibrahim, et al. New repurposed rolapitant in nanovesicular systems for lung cancer treatment: Development, *in-vitro* assessment and *in-vivo* biodistribution study. *European Journal of Pharmaceutical Sciences*, 2022, 171: 106119. https://doi.org/10.1016/j.ejps.2022.106119

[85] A. Samad, Y. Sultana, M. Aqil. Liposomal drug delivery systems: an update review. *Current Drug Delivery*, 2007, 4(4): 297−305. https://doi.org/10.2174/156720107782151269

[86] P.R. Kaur, V.A. Garg, P.A. Bawa, et al. Formulation, systematic optimization, in vitro, ex vivo and stability assessment of transethosome based gel of curcumin. *Asian Journal of Pharmaceutical and Clinical Research*, 2018, 11(2): 41−47. https://doi.org/10.22159/ajpcr.2018.v11s2.28563

[87] A. Radomska-Soukharev. Stability of lipid excipients in solid lipid nanoparticles. *Advanced Drug Delivery Reviews*, 2007, 59(6): 411−418. https://doi.org/10.1016/j.addr.2007.04.004

[88] R. Albash, A.A. Abdelbary, H. Refai, et al. Use of transethosomes for enhancing the transdermal delivery of olmesartan medoxomil: in vitro, ex vivo, and in vivo evaluation. *International Journal of Nanomedicine*, 2019, 14: 1953−1968. https://doi.org/10.2147/ijn.s196771

[89] N. Chauhan, K. Kumar, N.C. Pant. An updated review on transfersomes: A novel vesicular system for transdermal drug delivery. *Universal Journal of Pharmaceutical Research*, 2017, 2(4): 42−52. https://doi.org/10.22270/ujpr.v2i4.rw2

[90] G. Cevc, D. Gebauer. Hydration-driven transport of deformable lipid vesicles through fine pores and the skin barrier. *Biophysical Journal*, 2003, 84(2): 1010−-024. https://doi.org/10.1016/s0006-3495(03)74917-0

[91] J. Aho, V.H. Rolón-Garrido, S. Syrjälä, et al. Measurement technique and data analysis of extensional viscosity for polymer melts by Sentmanat extensional rheometer (SER). *Rheologica Acta*, 2010, 49(4): 359−70. https://doi.org/10.1007/s00397-010-0439-8

[92] A. Öhrlund. Evaluation of rheometry amplitude sweep cross-over point as an index of flexibility for HA fillers. *Journal of Cosmetics, Dermatological Sciences and Applications*, 2018, 8(2): 47−54. https://doi.org/10.4236/jcdsa.2018.82008

[93] M. Schenker, J. Schoelkopf, P. Gane, et al. Quantification of flow curve hysteresis data–a novel tool for characterising microfibrillated cellulose (MFC) suspensions. *Applied Rheology*, 2018, 28(2): 201822945. https://doi.org/10.3933/applrheol-28-22945

[94] C.S. Maurya, C. Sarkar. Characterization of highly stable water-based magnetorheological gel using OPTIGEL-WX as an additive: The study of magneto-induced rheological and viscoelastic properties. *Journal of Industrial and Engineering Chemistry*, 2022, 110: 137−149. https://doi.org/10.1016/j.jiec.2022.02.043

[95] G. Cevc. A. Chopra, Non-invasive vaccination through the skin. US patent 7,867,480, Jan 11, 2011.

[96] G. Cevc. Preparation for the application of agents in mini-droplets. US patent 11/481,804, Feb 22, 2007.

[97] G. Cevc. Formulation for topical non-invasive application in vivo. US patent 7,175,850. Feb 13, 2007.

[98] G. Cevc. Preparation for the application of agents in mini-droplets. US patent 6,165,500. Dec 26, 2000.

[99] K.B. Ita, J. Du Preez, J. du Plessis, et al. Dermal delivery of selected hydrophilic drugs from elastic liposomes: effect of phospholipid formulation and surfactants. *Journal of Pharmacy and Pharmacology*, 2007, 59(9): 1215−1222. https://doi.org/10.1211/jpp.59.9.0005

[100] V. Garg, H. Singh, S. Bimbrawh, et al. Ethosomes and transfersomes: Principles, perspectives and practices. *Current Drug Delivery*, 2017, 14(5): 613−633. https://doi.org/10.2174/1567201813666160520114436

[101] J. Wang, J.H. Hu, F.Q. Li, et al. Strong cellular and humoral immune responses induced by transcutaneous immunization with HBsAg DNA–cationic deformable liposome complex. *Experimental Dermatology*, 2007, 16(9): 724−729. https://doi.org/10.1111/j.1600-0625.2007.00584.x

[102] A. Chopra, G. Cevc. Non-invasive, epicutaneous immunisation with toxoid in deformable vesicles protects mice against tetanus, chiefly owing to a Th2 response. *European Journal of Pharmaceutical Sciences*, 2014, 56: 55−64. https://doi.org/10.1016/j.ejps.2014.01.006

[103] R. Damoiseaux, S. George, M. Li, et al. No time to lose—high throughput screening to assess nanomaterial safety. *Nanoscale*, 2011, 3(4): 1345−1360. https://doi.org/10.1039/c0nr00618a

[104] X. Liu, Y. Jia, Z. Han, et al. Integrating a concentration gradient generator and a single‐cell trapper array for high‐throughput screening the bioeffects of nanomaterials. *Angewandte Chemie International Edition*, 2021, 60(22): 12319−12322. https://doi.org/10.1002/anie.202101293

[105] D. Reker, Y. Rybakova, A.R. Kirtane, et al. Computationally guided high-throughput design of self-assembling drug nanoparticles. *Nature Nanotechnology*, 2021, 16(6): 725−733. https://doi.org/10.1038/s41565-021-00870-y

[106] R.S. Tade, S.N. Jain, J.T. Satyavijay, et al. Artificial intelligence in the paradigm shift of pharmaceutical sciences: A review. *Nano Biomedicine and Engineering*, 2024, 16(1): 64−77. https://doi.org/10.26599/NBE.2023.9290043

[107] G. Cevc, U. Vierl. Nanotechnology and the transdermal route: A state of the art review and critical appraisal. *Journal of Controlled Release*, 2010, 141(3): 277−299. https://doi.org/10.1016/j.jconrel.2009.10.016

[108] R. Kumar, A. Philip. Modified transdermal technologies: Breaking the barriers of drug permeation via the skin. *Tropical Journal of Pharmaceutical Research*, 2007, 6(1): 633−644. https://doi.org/10.4314/tjpr.v6i1.14641

[109] US Food and Drug Administration. Guidance for Industry: Using the Inactive Ingredient Database. 2019; 13.

[110] N.P. Mane, B.R. Rane, A.S. Jain. Advances in nanosuspension technology: Current trends and future horizons. *Nano Biomedicine and Engineering*, 2024, 16(4): 574−587. https://doi.org/10.26599/NBE.2024.9290092