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Topical delivery of nanoemulsions for skin cancer treatment

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ABSTRACT

Skin cancer chemotherapeutics often lead to the development of severe cytotoxicity, compelling the development of novel delivery systems to not only enhance therapeutic efficacy but also minimize side effects and improve patient compliance. In recent years, topical nanoemulsions have emerged as powerful tools in the field of skin cancer therapeutic management. This review delves into the potential of these innovative formulations to revolutionize the treatment of skin malignancies, due to their unique properties, having relevant advantages, such as allowing high drug strength, skin drug permeation and retention enhancement, biocompatibility, and controlled release capacity. Despite the skin's formidable permeability challenges, it remains an accessible interface for the delivery of therapeutic carriers such as nanoemulsions both locally (topical and dermal) and systemically (transdermal). Nanoemulsions, once associated primarily with cosmetic applications, are now gaining prominence as essential components of skin cancer treatment strategies. This review explores the potential of topical nanoemulsions, shedding light on their ability to efficiently deliver a wide range of molecules,

Abbreviations: 5-FU, 5-Fluorouracil; AG-NE, Andrographolide nanoemulsion; AG, Andrographolide; Ahr, aryl hydrocarbon receptor; AOC, amphiphilic oligochitosan; BA, betulinic acid; BCC, basal cell carcinoma; BCS, Bio-pharmaceutical classification system; CAF-NE, Caffeine nanoemulsion; CAM, chorioallantoic membrane; Capryol® 90, propylene glycol monocaprylate type II; CEs, carotenoid extracts; CNE, carotenoid nanoemulsion; Cremophor® EL, macrogol glycerol ricinoleate; CTLA-4, T-lymphocyte-associated protein-4; CUR-NEM, curcumin-loaded nanoemulsion; DAC-NE, dacarbazine nanoemulsion; DAC, dacarbazine; DHODH, dihydroorotate dehydrogenase enzyme; DMSO, dimethyl sulfoxide; DOTAP, N-1,2-dioleoyloxy-3-trimethylammonium propane chloride; DZ-NE, Daidzein nanoemulsion; DZ-NEG, Daidzein nanoemulsion-based gel; DZ, Daidzein; EIP, emulsion inversion point; FDA, Food and Drug Administration; HLB, hydrophilic-lipophilic balance; IFN-α, interferon-alpha; IL-2, interleukin-2; IM, intramuscular; irAEs, autoimmune-related adverse events; IV, intravenous; LDC, lipid drug conjugates; LFD, leflunomide; Lipoid E80, egg phospholipids with 80 % phosphatidylcholine; MCT, medium-chain triglycerides; MDR, multidrug resistance; MSCs, melanoma skin cancers; NCTD, norcantharidin; NE, nanoemulsion; NLC, nanostructured lipid carriers; NMSCs, non-melanoma skin cancers; O/W, oil-in-water; OA, oleic acid; Op-AG-NE, optimized AG-NE; PD-1, programmed cell death protein-1; PDI, polydispersity index; PIT, phase inversion temperature; Pluronic® F-127, Poloxamer 407; PNP, polymeric nanoparticles; PS, phosphatidylserine; PTCH1, patched homologue 1 gene; ROS, reactive oxygen species; SC, *Stratum corneum*; SCC, cutaneous squamous cell carcinoma; SEM, scanning electron microscope; SLN, solid lipid nanoparticles; Span® 80, sorbitan monooleate; Span® 85, sorbitan trioleate; TDS, topical delivery system; TEO-NE, thyme essential oil nanoemulsion; TEO, thyme essential oil; THC-NEs, tetrahydrocurcumin nanoemulsions; TP53, tumor protein 53 gene; TPL-NE, triptolide nanoemulsion; TPL, triptolide; Transcutol® HP, diethylene glycol monoethyl ether; TRP-1, tyrosinase-related protein 1; TRP-2, tyrosinase-related protein 2; Tween® 80, polyethylene sorbitol ester; TYR, Tyrosinase; W/O, water-in-oil; ZnPc, Zinc Phthalocyanine; ZnPc, Zinc Phthalocyanine nanoemulsion; ZP, zeta potential; α-MSH, alpha-melanocyte-stimulating hormone.

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overcoming lipophilic barriers inherent to skin. In this comprehensive analysis of several distinct studies investigating NEs for skin cancer treatment, a diverse array of formulations and components were explored, revealing a spectrum of characteristics. The PDI spans from a minimum of 0.105 nm to a maximum of 0.421 nm, reflecting variations in droplet size distribution. Droplet sizes exhibit considerable diversity, ranging from a small 16 nm to a larger 200 nm, signifying varied potential for skin penetration. ZP values further contribute to this diversity, ranging from highly favorable (-66.6 mV) to less advantageous or near zero values, indicative of distinct surface charge characteristics. As healthcare costs continue to escalate, this nuanced overview of nanoemulsion characteristics provides valuable insights into their potential applications in the targeted treatment of melanoma and, to a lesser extent, non-melanoma skin cancers. The value of such innovative and safer drug delivery systems becomes increasingly evident. Here, we focus exclusively on the role of topical nanoemulsions in advancing skin cancer therapy.

1. Introduction

Skin cancers also referred to as cutaneous malignancies, are continuously positioned year after year among the most prevalent forms of cancer, occupying leading positions on this list and showing no tendency to reach a *plateau*. According to the World Health Organization, for one out of every three cancer diagnoses and surpassing all the other cancer forms as the most common in Caucasian populations [1–3]. These pathologies burden unequivocally the health care systems as a preeminent global public health problem that scopes all life spans, geographic regions, ethnicities, socioeconomic status, and demographic cohorts, coming obviously at the cost of tremendous economic obligations [2,3]. Over the last decades, the general incidence of skin cancers has ramped up drastically, requiring treatment costs estimated in billions of dollars [4]. This increasing incidence, as well as mortality tendency, over the next few years, can alarmingly be extrapolated almost worldwide, both for melanoma and non-melanoma skin cancers [5]. Prompted by all this, a disease area that has experienced significant investment and growth in terms of potential therapeutical approaches is skin cancer [1]. The available therapeutical management of this type of pathology has evolved from surgery and chemotherapy although the secret weapon is still missing. Despite the existence of numerous effective drugs, achieving optimal therapeutic outcomes remains a significant challenge. This challenge arises not only from the formidable barrier properties of the skin but also from the unfavorable physicochemical characteristics inherent in many chemotherapeutic agents. These factors often lead to premature limitations in treatment efficacy [6]. The conjugation of three main layers (epidermis, dermis, and hypodermis), different cell types, and different skin annexes hinder the delivery of drugs through this organ. In particular, the most external layer of the human skin and final product of differentiation, constituted of dead keratinized cells, the *Stratum corneum (SC)* forms a barrier particularly resistant to permeation. Its hyperkeratinization, induced also by exposure to the sun armors it from drug penetration, creating major challenges for novel formulations [7]. More so, due to the physicochemical properties of chemotherapeutic agents used in the treatment of skin cancer, topical administration poses a significant challenge, necessitating the exploration of novel technological strategies to effectively surmount the barrier imposed by intact skin [8]. Beside, ineffective targeted drug delivery systems, cancer cell non-specificity, the toxicity of traditional chemotherapeutics (*e.g.* nausea, vomiting, hair loss, fatigue, and even death), and an incomplete understanding of cancer development and progression mechanisms remain additional significant challenges. Skin cancers are characterized by the uncontrolled proliferation of aberrant skin cells, that give rise to the development of tumors, which are categorized based on several factors, including the precursor cell type, histopathologic pattern, and clinical behavior. The clinical persistence of cutaneous melanoma, basal cell carcinoma, and squamous cell carcinoma poses a significant challenge, primarily stemming from the intricate interplay of various factors. These factors include the heterogeneous nature of tumor biology, the limited success of systemic therapies, and the complexities in achieving both complete and lasting responses, particularly in advanced-stage disease. The melanoma cancer scenario is also notorious for a particularly high multidrug resistance (MDR), low survival rate, and ease of relapse. For that reason, a significant percentage of melanoma patients are non-responsive or suffer from harsh drug-related toxicity. Melanoma represents therefore a great challenge since, in advanced stages, it does not respond to conventional chemotherapy and tends to develop resistance, especially following targeted therapy [9, 10]. As a global health issue, combined efforts are being made to develop novel carrier systems capable of tackling skin cancers. In this context, topical drug delivery has emerged as an ideal approach for self-administered localized management of skin cancers, offering several advantages. These benefits encompass minimal systemic absorption, thus bypassing issues associated with the non-specificity of traditional chemotherapeutic drug delivery systems. Associated with this theme, nanocarriers have emerged and have been explored as a new oncology treatment tool, aiming to reduce numerous enclosures of conventional drug delivery systems [11]. The crescent investment in the development and medical application of nanocarriers, which display promising possibilities for encapsulating a wide array of drugs with great delivery characteristics and preferential target specificity have been switching the topical treatment paradigms [3,12,13]. The advent of nanomedicine has ushered in a new era of versatile and advanced targeting strategies. These systems offer a promising avenue for the development of innovative therapeutic products, that improve the therapeutic effectiveness and reduce unwanted side effects [14]. These systems seek continuous, direct, and controlled drug release towards malignant cells in a selective manner, with improved drug targetability and cellular uptake. Nanosystems can also be designed for automated identification of malignant cells, enabling precise drug delivery while avoiding contact with normal cells. Furthermore, advancements in drug delivery systems have successfully addressed several drawbacks, including systemic toxicity, low oral bioavailability, reduced solubility, narrow therapeutic indices, and multidrug resistance (MDR) [15–17]. Many published studies further attest to the role of nanotechnologies-based technics as an important part in the enhancement of skin penetration even to poor soluble drugs, namely chemotherapeutics [18–20]. Moreover, these innovative carrier systems sustain controlled drug release, shield the encapsulated drug from degradation, and raise the intracellular concentration of drugs lessening consequently the cytotoxicity associated with skin chemotherapy [21]. All of which leaves unequivocal the tremendous potential of overcoming the hurdles encountered by the available conventional therapies. As skin cancer remains a major public health concern, there is a growing need for innovative and effective treatment strategies. Numerous studies have demonstrated the potential of nanotechnology-based drug delivery systems, distinguished by their unique compositions, characteristics, and properties. These systems, including nanoemulsions (NEs), liposomes, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), lipid drug conjugates (LDC), polymeric nanoparticles (PNP), quantum dots, among others, offer promising solutions to address these challenges in various administration routes and applications, including to enhance the penetration of drugs into the skin [7,11,22–24]. Nanoemulsions, which are at the core of this work, exhibit physicochemical properties appropriate for surpassing these drug delivery drawbacks and delivering the drug of

interest through the skin [21]. Exhibiting also the potential to enhance drug solubility, stability, and bioavailability, nanoemulsions offer a promising avenue for improving the efficacy and safety of skin cancer treatment [25]. Therefore, this review article aims to provide a comprehensive and up-to-date overview of the most recent progress, discoveries, and applications of nanoemulsions in skin cancer management. Specifically, it focuses on the potential of nanoemulsions, administered topically, as highly promising novel drug delivery systems for skin cancer treatment. In conclusion, by synthesizing and analyzing the latest research findings and trends, this article aims to provide a valuable resource for researchers, practitioners, and students interested in the exciting and constantly evolving field of nanotechnology.

2. Skin cancers: cutaneous melanoma, basal cell carcinoma, and squamous cell carcinoma

While the complex mechanisms driving cancer progression are not fully understood, certain factors such as the interplay between carcinoma cells, genotype, phenotype, and environmental conditions are recognized as significant contributors to the risk of developing skin cancer. In particular, UV radiation is indicated as the principal environmental risk factor. This noxious stimulus conduces to DNA damage, immunosuppression, gene mutations, oxidative stress, and inflammatory responses, which are all phenomena in a straight line related to cutaneous malignancies genesis [2,26]. UV radiation is for this reason a carcinogen, that not only initiates tumorigenesis by mutating tumor suppressor genes but also supports tumor development. More specifically, UVA radiation acts indirectly through the abnormal generation of reactive oxygen species (ROS), which in turn promotes the activation of transcriptional factors causative of mitochondrial DNA mutations [21,26]. On the other hand, UVB radiation directly damages the DNA through tumorigenesis and inflammatory response. Despite the mechanisms of UV radiation-induced damage leading to skin cancer being complex, it is also acknowledged that this radiation causes mutations to *p53* tumor suppressor genes, impeding their ability to repair the DNA. In turn, the resulting deregulation of the apoptotic mechanisms conducts in deregulated mitosis and initial cancer growth [2]. Beside, genetic predisposition, exposure to viral infections (such as human papillomavirus), and/or some chemicals (namely arsenic and aromatic hydrocarbons), and even a compromised immune system are also held accountable as potential risk factors for skin cancer development [27]. Skin cancer is a highly heterogeneous and complex disease characterized by the unrestrained proliferation of abnormal skin cells, whose classification depends on multiple factors. Classification of skin cancers depends essentially on the precursor cell type and clinical behavior, but also on histological subtype, genetic and molecular

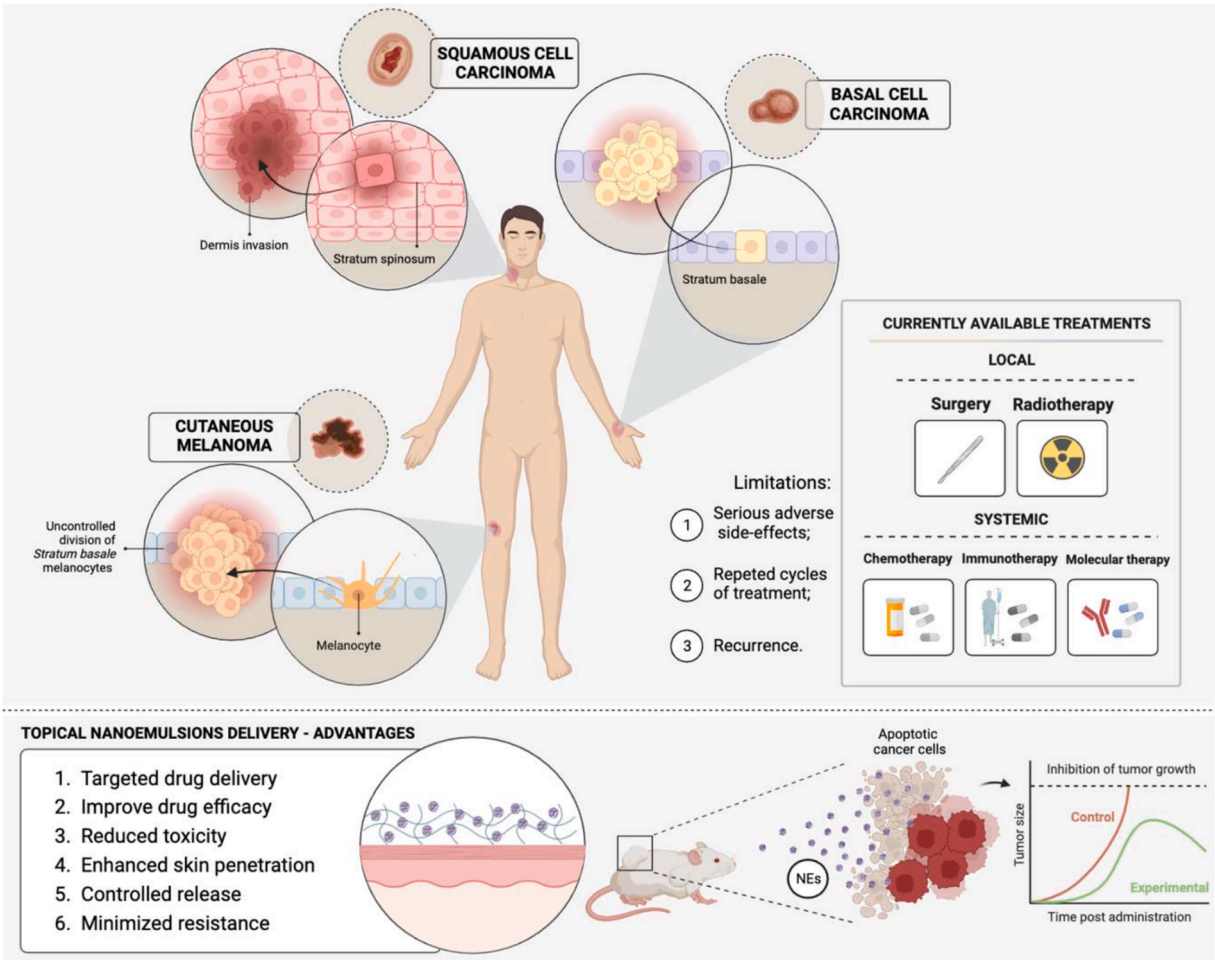

Fig. 1. Schematic representation of the different types of skin cancer (cutaneous melanoma, basal cell carcinoma, and squamous cell carcinoma), the currently available therapeutic strategies, and their principal limitations. Wide surgical excision persists as the first-line therapeutic option. Other modalities such as chemotherapy, radiotherapy, immunotherapy, and molecular targeted therapy can be used as monotherapy or adjuvant therapies depending on the tumor type, region, size, and development stage. More so it also highlights the limitations of conventional topical formulations for skin cancer treatment and the advantages of using nanoemulsions for improved drug delivery efficacy. Nanoemulsions can penetrate deeper into the skin layers, minimize systemic toxicity, and restrict drug delivery to the affected area, making them a promising alternative to conventional formulations. The use of nanoemulsions represents a well-tolerable, promising, and exciting area of ongoing research in the field of skin cancer treatment.

alterations, and anatomical location of the tumor [1,7,26]. Generically, skin cancers are usually divided into two major groups: (1) melanoma skin cancers and (2) non-melanoma skin cancers (NMSCs). Basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (SCC) constitute the main NMSC subtypes [26]. Melanoma generally refers to a type of skin cancer that develops from the uncontrolled division of the skin-darkening pigment-producer cells, the melanocytes, primarily found in the basal layer of the epidermis [28] (Fig. 1). Nevertheless, the presence of melanocytes is not restricted to the skin, and for this reason, although less frequent, extracutaneous melanoma cases, such as ocular and mucosal melanoma, can occur [29,30]. Therefore for convenience, from now on, melanoma will be used to describe cutaneous melanoma, the most common presentation of melanoma skin cancers (MSCs). Overall, MSCs constitute approximately 5 % of all skin cancer cases. However, their mortality rate is notably higher, accounting for the majority of skin-cancer-related deaths. This is primarily due to the intricate pathophysiology associated with melanoma [31]. Even though this malignancy may initially develop slowly from an existing skin dark spot or mole, later lesions tend to thicken and penetrate the skin, expanding aggressively and metastasizing through the bloodstream or lymph nodes to distant organs, such as the liver, bones, lungs, and brain [7]. Malignant melanoma is therefore one of the most aggressive, lethal, heterogenous, highly mutable, and unpredictable types of cancer, especially if detected at advanced stages [2]. Its long-term treatment efficacy is limited by significant adverse effects, treatment-refractory, insufficient clinical effects, and emerging resistance. In comparison, BCC and SCC account together for over 95 % of all skin carcinomas, with BCC alone being responsible for 80 % of all cutaneous malignancies, representing therefore the most common form of all malignant skin cancers [2,21]. But as opposed to MSC, most of these non-melanoma malignancies are highly curable, particularly if diagnosed in the early stages, have a better prognosis, and show higher survival rates [32]. Yet, alarmingly it is predicted that the NMSC incidence may double in the next few years (a situation already noticeable in the increasing prevalence of BCC over the past two decades), a circumstance that will detonate skyrocketing healthcare costs. [2,27]. BCC is a malignant tumor, with origin in the follicular germinative cells of the epidermis, that can grow rapidly causing damage to a large region of tissue, representing, for this reason, significant morbidity. However, the mortality rate is low and the incidence of metastases is rare [1,21,27]. The back of the hands, head, and face, specifically of middle-aged or elderly patients, show a higher predisposition for the occurrence of this pathology, due to chronic sun exposure (Fig. 1) [1]. However, BCC growth is not dependent on precursor lesions, as it happens with SCC [2]. It has also been found that BCC holds the highest mutational burden among all cancers, with mutations in patched homologue 1 (PTCH1) or tumor protein 53 (TP53) genes being the more frequent. The subtypes of BBC, namely superficial, nodular, or morphea forms, have different treatment options, as the rate of recurrence varies. In any case, surgical excision is perfectly suitable to cure the majority of BCCs. Nevertheless, to treat advanced, relapsed, or metastatic BBC not responsive to surgery and/or radiotherapy, FDA registered the chemotherapeutic Vismodegib, in 2012 [1,33]. SCC results from an uncontrolled proliferation of defective keratinocytes cells that invade the dermis and spread [1,2]. Its resulting lesions account for significant morbidity and hold a malignant potential capable of metastasizing. The head and neck regions are particularly susceptible to sun-induced damage due to their high sun exposure (Fig. 1). Moreover, the progression from precancerous lesions, known as actinic keratoses, has been proposed as a contributing factor [1,21]. The clinical manifestations include papules, nodules, or plaques, and hyperkeratotic, smooth, and even erosive lesions [2]. Except for high-risk factors, most SCCs are curable using surgery and radiotherapy [1,33]. Malignant melanoma has its genesis from epidermal melanocytes, having therefore the capacity to occur in any tissue where these cells are present. Most of the lesions appear on the skin surface, enabling the detection by visual examination (Fig. 1) [2]. As age increases the chance of developing melanoma grows, yet it afflicts all age groups and is one of the most common forms of cancer among young adults [34]. The described malignant melanoma-inducing mechanisms are multiple, however not entirely clear, and include melanocyte DNA damage, immune system suppression, melanocyte cell division, and free radical production [35]. The identification of the *p16 gene,* a melanoma susceptibility gene, also corroborates the connection between genetics and malignant melanoma [2]. Most of the mutations occur in the aberrantly operating BRAF/NRAS/MEK/MAPK pathways, justifying the fact that these pathways are among the recurrent aimed therapy targets to approach melanoma treatment [32]. The chosen treatment for MSC includes both conventional therapies, such as surgical excision and radiotherapy, but also innovative treatments, such as targeted therapy, immunotherapy, and combinations of topical, transdermal, and systemic therapies, depending on the disease stage. In the cases where the metastatic ability does not exist, the cure rate is about 90 %. Contrarily, the survival rate for patients with metastasis drops to around 10 % [32,36]. The lack of effectiveness of existing melanoma therapies correlates firstly, with not an entire understanding of the molecular mechanisms underlying the disease. Secondly, identifying the protein targets responsible for the disease has proven rather challenging. Thirdly, combination therapies have not been successful in improving treatment outcomes. Finally, defective targeted delivery systems with limited activity in tumor cells also contributed to the lack of effectiveness of the currently available melanoma therapies [7].

2.1. Clinical management of skin cancers

Early diagnosis and prompt treatment are crucial for improving the prognosis of any type of skin cancer. With skin cancer being one of the most common types of cancer worldwide, accounting for over one-third of all cancer cases, the importance of timely detection and intervention cannot be overstated. Delays in diagnosis and treatment can lead to the progression of the disease, increasing the risk of metastasis and reducing the chances of successful treatment [37]. Therefore, raising awareness of the importance of regular skin checks and early intervention is vital for improving outcomes and reducing the burden of skin cancer [38]. Surgical excision has been, for decades, the treatment of choice for patients with skin carcinomas, although some non-surgical procedures are also available [39] (Fig. 1). Surgical resection is very effective in most cases, but the decision to perform surgery relies on several considerations, such as the patient's comorbidity, the affected tissue, and the patient's potential resistance to repeated courses of treatment. Patient preference is also considered regarding convenience, costs, and tolerance [40]. Nonetheless, excision or Moh's micrographic surgery should be, when possible, employed for lesion removal to treat high-risk tumors [37]. Photodynamic therapy, laser therapy, dermabrasion, cryosurgery, curettage, desiccation, and other procedures, are some other NMSC treatment options that despite being effective for single and visible lesions come with several inconveniences. Specifically, due to pain, scarring, disfigurement, edema, hair loss, secondary infection, hypopigmentation, and blister formation, among others. In some cases, topical treatment may be more appropriate, especially if surgical intervention is not possible or if rejected by the patient [40]. Topical therapy is often recommended for patients with field cancerization or multiple lesions over an extensive anatomical area. Treatment with topical drugs also allows for higher drug levels specifically at the tumor site and may be potentially less toxic than systemic drugs [1,37]. In this respect, the conjugation of both effective drugs but also efficient drug delivery systems is key to achieving effective topical chemotherapy [41–44]. Fluoropyrimidine 5-Fluorouracil (5-FU) is the most frequently topical agent used in modalities of NMSC treatment, while imiquimod is suitable for the management of superficial BCCs modalities. 5-FU, a hydrophilic drug was the first topical therapy to be registered by the Food and Drug Administration (FDA) to treat these conditions. This pyrimidine analog is an antineoplastic antimetabolite that binds to

thymidylate synthase through the 5,10-methylene tetrahydrofolate co-factor, resulting in the inhibition of thymidine synthesis, DNA replication defection, and subsequent apoptosis induction. The use of this therapeutic is commonly limited to patients with small lesions located in low-risk areas, not suitable for surgical intervention [2,37,45,46]. Topical (cream or solution) and injectable 5-FU products are the formulations currently available in the market for NMSC treatment. Oral formulations are impractical given the serious adverse side effects and extremely low bioavailability [47,48]. Radical surgical excision is also considered the standard of care for primary melanoma. However, in some situations, concerning for example inoperable anatomical areas, large tumor extension, advanced age, or underlying patient comorbidities, surgery may be contraindicated [49]. Additionally, the surgical approach is not suitable in the cases of advanced melanoma, which often occurs due to the spread of cancer stem cells through the lymphatic and hematogenous systems [50]. In such circumstances, non-surgical therapies, such as chemotherapy, radiotherapy, immunotherapy, and targeted molecular therapy, may be pondered on an individual basis, taking into account the disease stage. In chemotherapy regimens, the effect of the antineoplastic agents, conventionally dacarbazine and temozolomide, are systemic and affect indiscriminately both tumor and healthy cells. While chemoprevention holds promise as a strategy to prevent the development of invasive carcinoma, conventional chemotherapeutic approaches for treating carcinogenic skin lesions often fall short of expectations. These approaches, characterized by their non-specific targeting, frequently result in significant and undeniable toxicity. Such toxicity impairs the use of adequate doses for treatment periods sufficient to accomplish the desired results. The lack of specificity leads to poor chemotherapy outcomes that manifest through severe adverse side effects, including nausea, vomiting, pain, and inflammation [51–53]. Likewise, the utilization of radiotherapy practices also entails side effects such as inflammatory skin reactions [53]. Considered the first-generation immunotherapy, the utilization of exogenous cytokines such as interleukin-2 (IL-2) and interferon-alpha (IFN-α) has seen a gradual decline in use. This decline is attributed to not only the low response rates but also the significant adverse effects, including cardiotoxicity, nephrotoxicity, hepatotoxicity, gastrointestinal complications, and hypotension [50,52,53]. Conversely, the use of immunotherapy strategies involving immune checkpoint inhibitors, which inhibit cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) receptors or programmed cell death protein-1 (PD-1) expression (*e.g.*, ipilimumab and nivolumab, respectively), has demonstrated superior responses compared to chemotherapy and, in some cases, even potential cures. However, the occurrence of autoimmune-related adverse events (irAEs), encompassing symptoms such as fatigue, rash, nausea, vomiting, diarrhea, dermatitis, neuropathies, endocrinopathies, colitis, hepatitis, and hepatotoxicity, raises concerns about their application [51–53]. As for melanoma molecular targeted therapy, it most commonly uses either BRAF (*e.g.* sorafenib, vemurafenib, dabrafenib) or MAPK/ERK kinase inhibitors (*e.g.* trametinib). Nevertheless, while demonstrating response rates and overall survival benefits superior to conventional chemotherapy, the utility of BRAF and MEK inhibitors is hampered by associated toxicities. Furthermore, the efficacy of BRAF inhibitor treatments, in particular, is constrained by the emergence of adaptive drug-resistant phenotypes. Furthermore, these treatments can suppress anticancer immune responses, ultimately reducing the therapeutic response of molecular-targeted therapies [52–54]. The combination of these different treatment modalities is also a common practice, however, the amount of available evidence supporting the efficacy of such a strategy is so far limited [52]. While substantial efforts have been dedicated to addressing skin cancers, the persistence of significant limitations, including severe toxicity, primary or acquired therapy resistance, limited efficacy, poor response rates, and the risk of recurrence, underscores the critical need to invest in the development of novel therapeutic alternatives along with efficient routes of administration. Topical delivery should for that reason be considered the preferential route of administration as it ensures targeted drug delivery to the site of action, minimizing the potential occurrence of undesired toxic side effects while assuring the intended therapeutic efficacy. More so, the use of nanoemulsions as versatile systems, extensively studied for the transport of various synthetic and natural compounds across the lipophilic skin barrier, is also expected to improve the bioavailability and stability of drugs, ultimately leading to better clinical outcomes for skin cancer-afflicted patients.

2.1.1. Topical delivery

The skin is a multifunctional and multilayered organ that comprises, briefly, three main layers: epidermis, dermis, and hypodermis. One of its most important functions is to act as a protective barrier against the incursion of external aggressors, but also to prevent the loss of essential endogenous materials like water. This fundamental attribute is maintained precisely by the skin's stratified structure. The epidermis, the outermost layer of the skin, comprehends four major cell types and subdivides itself into four basic layers (*Stratum basale, Stratum spinosum, Stratum granulosum, and Stratum corneum*, from the deepest to most the superficial layer, respectively), each with different stages of cellular differentiation of the actively dividing cells, the keratinocytes. Further elaborating in terms of skin composition, and also present in the epidermis, the melanocytes produce the melanin pigment, that shields the skin from ultraviolet radiation, Merkel cells respond to light touch, and Langerhans cells participate in the immune system activation. While the dermis underlays the epidermis providing support and nourishment. Within the ground substance and fibers that compose this layer, there are also some specialized cells including hair follicles, sebaceous, apocrine, and eccrine glands, as well as blood vessels and nerves responsive to touch, pain, and temperature. The fibroblasts produce elastin and collagen. The hypodermis, located below the dermis, contains connective tissue, nerves, and some larger blood vessels, storing fat, regulating the body temperature, and providing shock absorption capacities [2,3,26,55]. Some important difficulties arise when crossing the SC, a hyper-keratinized lipophilic loosely attached epithelium, and other epidermal skin barriers [3].

When a substance is applied to the skin, it can take one of three possible routes to surpass this barrier: the transcellular route, which involves passage through cellular structures; the intercellular route, where the substance navigates through the ECM without traversing the cells; and the appendageal route, which includes pathways through hair follicles, associated sebaceous glands, and sweat ducts. The fractional appendage area available for transport is quite limited and for that reason, this route typically makes only a minor contribution to the overall steady-state drug flux. However, it may be particularly relevant for the transport of ions and large polar molecules that encounter challenges in crossing an intact SC. In the transcellular route, the substance undergoes a sequence of partitioning and diffusion, alternating between hydrophilic and lipophilic domains within cells and the ECM. In contrast, the intercellular route involves the substance traversing a more direct path within the ECM, bypassing the cellular structures. Generally, small hydrophilic molecules exhibit a preference for the transcellular route, while lipophilic molecules tend to favor the intercellular route [56,57]. However, the majority of the topically applied compounds follow the tortuous intercellular route [58]. The transcellular and intercellular routes together constitute the transepidermal pathway. It is commonly acknowledged that the transepidermal pathway governs skin permeation and that the diffusion through the SC typically serves as the rate-limiting step for the permeant, under sink conditions [56,57].

Nevertheless, topical delivery earns its crescent pharmaceutical, nutraceutical, and cosmeceutical interest specifically because, from a medical perspective, it offers an excellent alternative to treat skin diseases, since its use can be restricted to the affected area, minimizing undesirable conditions and systemic toxicity (Fig. 1). Particularly when treating skin cancers, given the cytotoxicity of the standard drugs used,

this significant advantage may improve patient's compliance to the therapeutic, while assuring a much-needed greater quality of life for cancer-afflicted people. On the other hand, topical formulations also offer advantageous characteristics when treating lesions over a large body surface area. When confronted with the formidable barrier honed by thousands of years of evolution that governs this challenging environment, conventional formulations, due to their typically larger dimensions, primarily target the outermost layers of the epidermis. This limitation constrains their potential performance and effectiveness. This contrast arises from a set of distinctive characteristics and mechanisms inherent to nanoemulsions (NEs). These properties enable NEs to improve skin penetration, thereby facilitating a wider range of potential activities and granting access to deeper layers of skin tissue (Fig. 1) [59,60]. Firstly, NEs exhibit a notable solubilization capability for both lipophilic and hydrophilic active substances (Table 1), consequently elevating the formulation's loading capacity and dosage application. Additionally, NEs extensive surface area and effective skin contact, combined with their occlusive properties, guarantee robust interaction with the surface of the SC. Furthermore, the constituents of NEs, including oils and surfactants, may directly enhance the permeation by affecting the lipid structure of the SC (Table 1) [61].

3. Nanoemulsions for topical skin cancer treatment

Nanotechnology bridges the biological and physical sciences barriers through the application of nanomaterials, offering a combination of diagnostic and therapeutical nanoparticle solutions with unique structural, biological, chemical, mechanical, and electrical features. Nanomedicine is a rapidly emerging field where the application of these tools is of particular interest and orientated to the combination of diagnosis, targeted, and controlled drug delivery modalities [16,60]. The employment of such techniques is convenient for chronic human diseases management but also in innovative scenarios such as immunotherapy, imaging, and chemotherapy. So far, most of the nanotechnology drug-delivery systems approaches are centered mainly toward cancer cure [11]. In the skin cancer scenario, the application of nanomaterials to the skin reflects precisely an increasing interest in topical targeting, justifiable because of the differentiated characteristics of these technologies (Fig. 1). The increased permeation and retention, the reduced side effects, as these carriers address questions related to skin irritation and drug toxicity, and the shielding of the encapsulated drug from degradation are some of the valuable attributes [3,21,33,62,63]. Additionally, these formulations hold sustained and controlled drug release not restricted to topical but also to, transdermal, oral, nasal, intravenous, and ocular routes. For all these reasons, nanotechnology finds its niche as a very promising way of drug delivery through several administration routes, being the topical and transdermal routes at the core of this work [63]. In comparison to the oral administration route, and taking advantage of using the biggest organ of the body, the systemic delivery of drugs topically demonstrates several advantages. These include but are not limited to, circumventing first-pass metabolism, reducing the likelihood of drug degradation—both chemically and enzymatically—by the gastrointestinal system, and even the potential to mitigate gastric injury [63].

There are a considerable number of differentiated nanodelivery systems that have been exploited and successfully applied to date. Anticipating that in the future this number may be even higher. The extensive drug designing of nanostructures studies is explainable by its remarkable advantages, such as the opportunity to manipulate properties like solubility, bioavailability, drug release profiles, diffusivity, and immunogenicity [64]. Liposomes are concentric sphere-shaped vesicles, with dimensions ranging from 50 to 450 nm, composed of one or more phospholipids (natural nontoxic phospholipids or cholesterol) bilayers that enclose an aqueous core entirely. These systems are extensively used by the pharmaceutical and cosmetic industries, as carriers for several molecules, because of their aptitude to enclose both hydro and lipophilic drugs, low toxicity profile, simplify site-specific drug delivery, biocompatibility, and biodegradability. The principal drawbacks concerning liposome use correlate with high production costs, low solubility, short half-life, susceptibility of the phospholipids to hydrolysis and oxidation, and fusion or leakage of the encapsulated molecules [65,66]. Solid lipid nanoparticles (SLN) are first-generation lipid-based nanocarriers formulated using lipids (such as triglycerides, steroids, fatty acids, and waxes), solid at body temperature, stabilized by the incorporation of emulsifiers. The biocompatible and biodegradable characteristics of the used materials, the low toxicity potential, and the ease of scale-up production are among their principal advantages. However, the rigid crystalline structure causes the drug loading efficiency to be low and favors drug expulsion, a phenomenon caused by lipid crystallization during manufacturing and storage. The initial burst release, common in these formulations, accounts for another significant disadvantage. NLC and LDC result from modifications of lipid-based nanoparticles that intend to surpass the SLN limitations. The mixture of solid and liquid lipids in NLC allows for matrix flexibility, augments payload, and prevents drug expulsion. On the other hand, LDC allows extending these systems' applicability to lipophobic molecules [67–70]. Quantum dots are nanoparticle fluorescent probes constituted of semiconducting material, with unique and superior photochemical and photophysical properties, most frequently applied to biological detecting and tumor imaging, essential for cancer diagnosis. Its principal limitation correlates with the toxicity profile of the used materials. For example, the toxicity assessment, concerning the use of semiconductors containing cadmium, the main QD component with noble optical characteristics, on human living cells is not entirely elucidated yet [71]. Unlike liposomes and quantum dots, which have limited stability and may induce toxicity, nanoemulsions have demonstrated excellent stability and biocompatibility, reducing the risk of adverse effects. Solid lipid nanoparticles, on the other hand, are limited in their drug-loading capacity due to their crystalline structure, whereas nanoemulsions can accommodate a higher drug payload, improving their efficacy in skin cancer treatment. Moreover, nanoemulsions have shown to be efficient in minimizing systemic toxicity and undesirable conditions, providing targeted drug delivery to the affected area while preserving healthy skin. The ease of preparation, low cost, and scalability of nanoemulsion production further support their potential for clinical translation. Overall, the unique properties of

Table 1
Properties of nanoemulsion systems.

	Principal characteristics	Advantages	Disadvantages
Nanoemulsions	Small particle size (typically <200 nm) Isotropic High kinetical stability Transparent and aesthetically pleasing physical appearance	Enhanced solubility for both lipophilic & hydrophilic drugs Improved penetration, due to the constituents that reduced resistance to drug transport Flexibility in formulation design to accommodate various drugs, compounds, and therapeutic goals Minimized systemic toxicity Controlled and sustained release Preservation of the stability and integrity of sensitive active ingredients	Formulation complexity that requires careful selection of components and optimization of formulation parameters Limited payload Potential instability Regulatory challenges

nanoemulsions, such as their small size, stability, biocompatibility, and drug-loading capacity, position them as promising candidates for topical drug delivery in skin cancer treatment, outperforming other nanocarriers currently available [72].

4. Nanoemulsions

NEs emerged as advanced drug delivery systems promising to surpass some of the major difficulties associated with conventional formulations [73]. As drug delivery systems, NEs can improve the active ingredient(s) therapeutic efficacy while minimizing adverse effects and toxic reactions. Also referred to in the literature as mini, submicron, or ultrafine emulsions, NEs are examples of newer specialized emulsion systems proven to be advantageous for topical, dermal, and inclusively transdermal delivery, due to their controlled droplet size, both hydrophilic and lipophilic drug delivering capacity, enhanced skin permeation to drugs, and extended-release characteristics (Table 1). Via topical route particularly, NEs vehicles offer reduced side effects and toxicity, apart from the ability to stabilize some drugs in comparison to conventional formulations [63,74,75]. Class II and Class IV drugs, as per the Bio-pharmaceutical classification system (BCS), which present a hydrophobic profile, poor aqueous solubility, and challenging oral administration, are included in the long list of drugs that could benefit from the incorporation in NEs [63,76]. It has also been repetitively emphasized that the drug encapsulation and penetration rates when using NEs are, in comparison, much higher than those observed with suspensions, macroemulsions, micellar solutions, gels, and other carriers [75]. The drug nanoencapsulation allows bypassing the skin irritation forced by the use of high dosages necessary to achieve the desired therapeutic effect, which is ultimately caused by boundaries imposed by the skin barrier and by the insufficient penetration of the drug of interest per se [77]. These versatile biphasic systems consist of dispersions either of water-in-oil (W/O) or oil-in-water (O/W) (Fig. 2). The generated droplets detain particularly small dimensions, ranging from 20 to 200 nm (even though this value is not consensual in the scientific community), and result from a mixture of two immiscible liquids stabilized by the incorporation of an emulsifying agent, *i.e.*, surfactant and co-surfactant. Its obtention requires less emulsifier, but higher length co-surfactant than macroemulsions and microemulsions, respectively [78]. The terms external phase, continuous phase, or dispersion medium refer to the outer phase. Meanwhile, the internal phase, which is dispersed in the outer phase, is referred to as the discontinuous or internal phase. In the same manner, interphase or intermediate is the term used for surfactants acting as emulsifiers [73]. Surfactants are typically categorized into non-ionic, anionic, cationic, and zwitterionic. These compounds possess the capability to improve skin permeation, a function thought to be linked to their ability to bind reversibly to keratin filaments. This binding process, in turn, leads to the alteration of corneocytes, ultimately affecting the diffusion coefficient within the SC. Non-ionic surfactants are the most commonly utilized in transdermal NEs. They are favored for their low toxicity and minimal interference with NE formulations. On the other hand, anionic surfactants are known to enhance the skin penetration of target molecules more effectively, as they exhibit a stronger interaction with keratin and lipids. Additionally, cationic surfactants also influence cornified cells by interacting with keratin fibrils and disrupting the cell-lipid matrix [57,79].

Overall, exist three main categories based on the continuous and dispersed phases' composition: water-in-oil in which the aqueous droplets are dispersed within the continuous oily phase; oil-in-water where the oily droplets are dispersed within the continuous aqueous

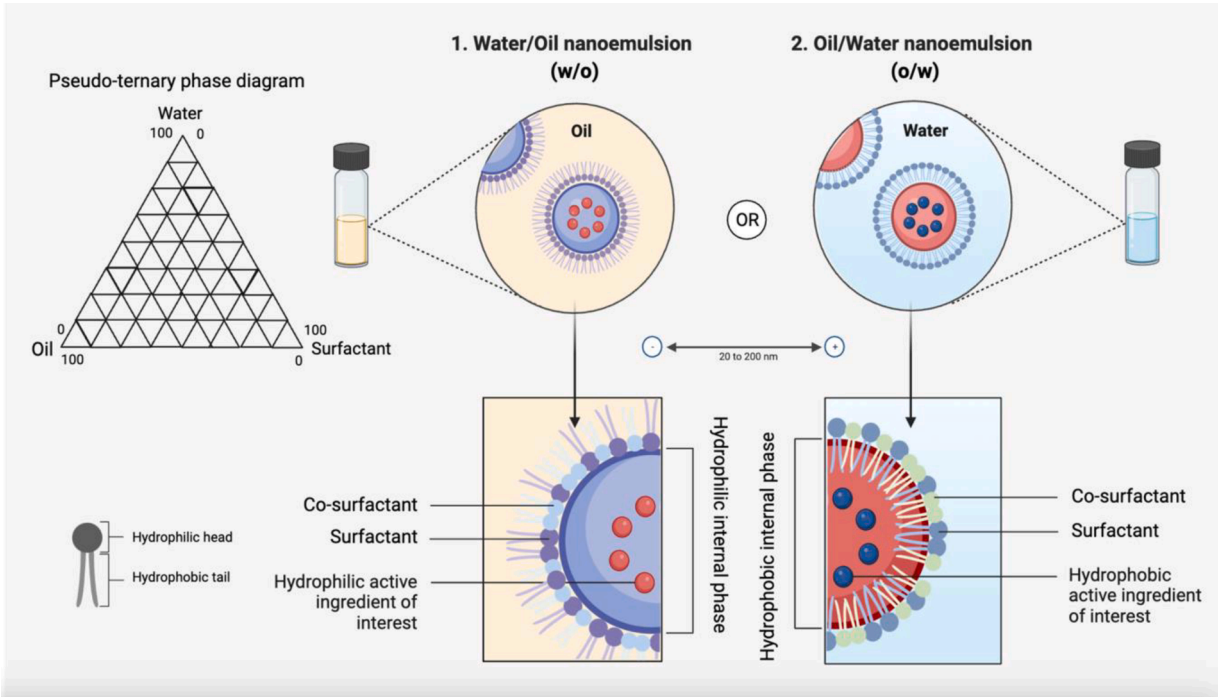

Fig. 2. Schematic representation of the structure of W/O and O/W nanoemulsions, highlighting the differences between these systems. In a W/O emulsion, water droplets are dispersed in a continuous oil phase and the hydrophilic active ingredient of interest is located within the aqueous core of the nanoemulsion droplets. Conversely, in an O/W nanoemulsion, oil droplets are dispersed in a continuous aqueous phase and the hydrophobic active ingredient of interest is located within the oily core of the nanoemulsion droplets. The type of nanoemulsion formed depends on the relative hydrophobicity/hydrophilicity of the active ingredient and the oil/surfactant used. Understanding the composition of O/W and W/O nanoemulsions is important as these impact the final product properties and performance. The pseudo-ternary phase diagram is used to identify the optimal water/oil/surfactant nanoemulsion composition by showing the different phases that can exist under different combinations of the three components. This diagram helps to identify the region where a stable nanoemulsion can be formed, which is typically constructed by varying the ratios of the components and observing the resulting phases that form at different compositions and temperatures. O/W – oil-in-water; W/O – water-in-oil.

phase (Fig. 2); and bi-continuous NEs where the aqueous and oily droplets occur interdispersed (W/O/W or O/W/O) [27,63,73,80]. Their dimensions profit from distinguishable kinetical stability and even though these systems are not devoid of thermodynamic limitations, NEs can be functionalized with a large and diverse number of additives [2, 27]. These colloidal formulations typically exhibit long-term kinetic stability thanks to their nanoscale droplet dimensions, thereby avoiding phenomena such as creaming and sedimentation during storage. Flocculation and coalescence are likewise prevented for the same reason. On the other hand, NEs are particularly sensitive to the Ostwald ripening effect [75,81]. Meaning that, with time, NEs tend to revert to their original phases [78]. Their advantages over other more traditional and unstable oil-based carriers, such as suspensions and emulsions, include enhanced solubilization, permeation, bioavailability, thermodynamic stability, and the delivering capacity of many lipophilic drugs, *in vitro* as well as *in vivo*. In terms of appearance, it may vary between transparent or translucent (Table 1) [82,83] depending on its droplet size, which in turn is impacted by the component's characteristics and ratio, as well as by the applied mechanical shearing forces [63,74]. More so, the use of safe-grade reagents in its formulation confers non-irritant and nontoxic properties both to mucous membranes and skin tissue. Because they lack thickeners in their composition and possess fluidity, they exhibit a transparent and aesthetically pleasing physical appearance (Table 1) [84,85]. Furthermore, when applied to the skin these lipidic nanoparticles promote adhesiveness and increase skin hydration, owing to the ability of the nanosized particles to form a film on the skin surface that promotes occlusiveness and reduces water evaporation [21]. This thigh occlusive contact is also justifiable by its large surface area, which contributes to the uniform distribution of the formulation on the skin surface, facilitates permeation, and promotes deeper drug delivery inside the skin. The capacity of tissue targeting is another valuable characteristic offered. Additionally, the presence of some oils and surfactants may change the lipidic structure of the SC also promoting skin permeation [27,75]. Oleic acid (OA) for instance is frequently used as an oil phase component in the formulation of NEs. OA possesses a natural ability to enhance penetration by promoting increased water absorption and swelling within the SC. Additionally, it affects certain structural components of the SC, thereby diminishing the integrity of this protective barrier. Indeed, there is evidence suggesting that NEs have the potential to disrupt the natural structural organization of the SC, leading to a loss of its barrier properties. This disruption can create openings or breaches through which nanoparticulated systems can penetrate more easily [74]. The utilization of modern excipients, such as ceramides, is noteworthy in the literature as they share a structural resemblance with the lipids found in the SC. These excipients are often cited as effective agents for promoting drug delivery into the skin compartments [7]. NEs can additionally take the form of a variety of pharmaceutical formulations including creams, foams, liquids, and sprays for topical use, making them even more appealing since it has been proven to be simple to scale up, avoiding intricated processing steps and justifying their viability in an industrial scale [63,74]. The sum of all these characteristics provokes the constant interest of investigators in the development of these formulations, which are consistently pointed out as effective novel vehicles for drug targeting inclusively in cancer treatment [63, 73]. While their effectiveness is typically restricted by the skin's protective barrier, there are instances where this barrier may be compromised, such as in the case of diseased or aged skin. In the context of skin cancer, where this scenario often applies, the use of NEs emerges as an exceptional treatment approach, offering the potential to enhance particle penetration [12]. Beside, the efforts of several research teams focused on studying different dermal and transdermal applications of NEs allow the association of a plethora of activities to these formulations. Especially in the treatment of various dermatological conditions such as acne, psoriasis, atopic dermatitis, vitiligo, wound healing, scabies, frostbites, onychomycosis management, and topical delivery against skin carcinomas, the use of NEs has been frequently implicated, pointing out the valence of the use of these formulations topically. NEs have also been employed as vehicles for anti-inflammatory and antioxidant agents. Drug-loaded NEs with UV-B radiation photoprotective purposes, local anesthetics, against UVA-mediated oxidative stress, for thrombophlebitis, vaginitis, *Candida albicans*, and other fungal infections treatment are also sustained by the literature (Table 1) [74, 86–89].

4.1. Nanoemulsions preparation methods

According to the theory of emulsification, emulsions are considered to be unstable dispersions of two liquids that are typically immiscible. This instability is due to the positive Gibbs free energy of formation (ΔG), which tends to become positive as the energy term is not sufficiently compensated by the entropy of the system [90]. As a result, the formation of an emulsion is considered to be a non-spontaneous process that requires the input of energy, as well as the use of surfactants, to disperse one phase into another [91]. NEs formulation in particular requires a much higher amount of energy, when in comparison with macro- or microemulsions [85]. Hence, during its preparation, external energy must be applied to the manufacturing process. The reduced nanoscale size and enhanced stability of nanoemulsions come at the expense of the high energy input required to overcome the energy barriers associated with their production (Fig. 3). The nanoemulsification preparation methods are divided into two major categories: high-energy and low-energy or spontaneous preparation methods. The combination of both approaches is sometimes a common strategy [2,74,75,78].

4.1.1. High-energy preparation methods

The employed methods known as high-energy emulsification methods comprehend techniques such as high-pressure homogenization, high-shear homogenization, membrane emulsification, microfluidization, and ultrasonic emulsification [2,73,74] (Fig. 3). Such mechanical devices force the dispersed phase to split into nanometric-size droplets [80]. These emulsification processes applied to the biphasic mixture must be carefully evaluated since the obtention of a monodisperse system, more predictable and stable, varies depending on the applied shear stress and shear rate, the viscosity of both continuous and dispersed phases and chosen surfactant properties. The desirable minimum polydispersity of droplets results typically from the conjugation of high energy input, convenient surfactant concentration, and a low dispersed-phase fraction [2]. The high energy consumption necessary for dispersion to occur, especially in viscous systems, the average droplet size increment, and the coalescence phenomena due to the insufficient amount of surfactant necessary for the complete adsorption of the formed droplets are some of the inconveniences inherent to these methodologies. The complex design of these apparatuses is also inextricably linked to high-energy preparation methods [75].

4.1.2. Low energy or spontaneous preparation methods

As for the low energy or spontaneous methods, and in contrast with the high energy-preparation methods, the droplet formation is dissociated from significant external energy support. The necessary energetic input relies merely on the intrinsic physicochemical properties and potential of the non-equilibrium mixture to produce nanoemulsions. Therefore, the required internal energy is generated by the dilution of a monophasic mixture onto the desired continuous or dispersed phase, by changes in the temperature, or by surfactant concentration alterations. The emulsion inversion point (EIP), phase inversion temperature (PIT) method and spontaneous emulsification in nonequilibrium systems are the most widely used. Emulsions prepared via "phase-inversion" tend to hold lower polydispersity and size. The interest in the cited methods has also grown significantly because these methodologies are energy-saving, non-destructive, and conservative of the encapsulated molecules, moreover being attractive in terms of large production scale-up and associated costs [2,73,75,80].

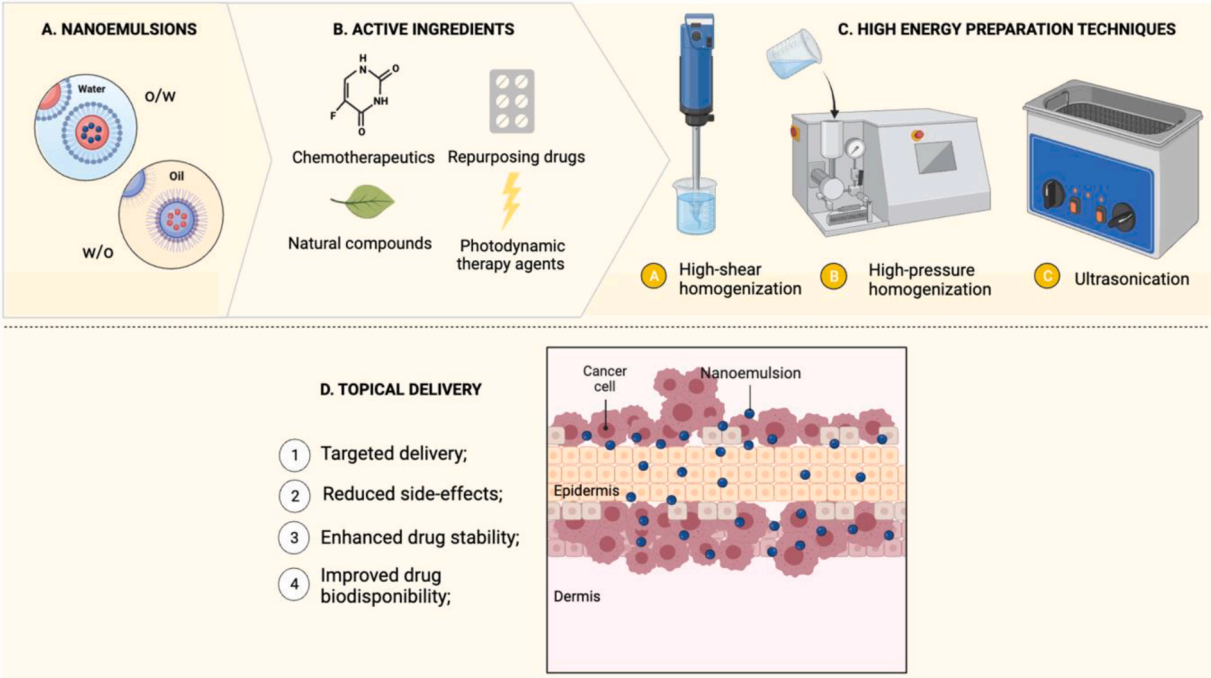

Fig. 3. Schematic representation of O/W and W/O nanoemulsions commonly used for skin cancer treatment, along with the active ingredients most frequently incorporated in these formulations and commonly used high-energy preparation techniques. As well as a highlight of the major advantages of using the topical route to administer these nanoemulsions, including targeted delivery, improved skin penetration and bioavailability, and reduced risk of systemic side effects. O/W – oil-in-water; W/O – water-in-oil.

Beside a convenient preparation method selection, to prepare nanoemulsions, a combination of oil and aqueous phases, surfactants and/or cosurfactants, active ingredient(s), and additives are required. The chemical nature and physical properties of these components play a pivotal role in the formulation process, making their accurate selection crucial. Such choices can significantly influence the stability and performance of the nanoemulsion, both *in vitro* and *in vivo* [85]. The oily phase of a nanoemulsion can consist of individual oils or their combination. Usually, long and medium-chain triglyceride oils are the preferred choices for safety reasons. Among these options, those with varying degrees of saturation are utilized, although the preference leans towards the ones with higher saturation levels due to their enhanced safety profile. More still, a mixture of oils and triglycerides may be used to facilitate the emulsification of the drug. Semisynthetic medium-chain derivatives with surfactant-like properties are also in the scope of materials utilized in the oil phase. A variety of oils are commonly employed in the formulation of NEs, such as arachis oil, Capmul® MCM, Capryol® 90, Captex® 200, Captex® 355, Captex® 8000, castor oil, coconut oil, corn oil, ethyl oleate, isopropyl myristate, isopropyl palmitate, jojoba oil, Labrafil® MM44CS, Labrafac ™ Lipophile WL 1349, lanolin, Maisine® CC, Miglyol® 812N, mineral oil, Myritol® 318, olive oil, oleic acid, palm oil esters, sesame oil, sefsol 218, soybean oil, triacetin, and others. The oil and water mixture results in a temporary emulsion which, after some time, due to the coalescence of the dispersed globules, segregates in its distinct phases. Surfactants are essential to the stability of these systems avoiding such phenomena by contributing to the NEs preparation by lowering the interfacial tension between the two immiscible liquids. The hydrophilic-lipophilic balance (HLB) is an important aspect to take into consideration when selecting a surfactant. Surfactants with high HLB values (8–18) are more hydrophilic and are preferred for preparing O/W NEs, while surfactants with low HLB values (3–6) are more lipophilic and preferred for preparing W/O NEs [85]. Furthermore, to prepare NEs, surfactants must have nontoxic properties, as well as taste, odor, and chemical stability compatible with the final product. They must also rapidly adsorb around dispersed phase globules to form a complete and cohesive film. This film serves the dual purpose of preventing coalescence and facilitating the attainment of appropriate zeta potential and viscosity within the system, thereby ensuring optimal stability. Finally, the concentration required for its effectiveness should be relatively low [73]. Some examples of commonly used surfactants in the preparation of O/W and W/O type nanoemulsions include Capryol® 90, Cremophor® RH 40, Imwitor® 780 K, Lauroglycol™ 90, PEG 400 monostearate, Poloxamer 188, Poloxamer 407, Polyoxyethylene lauryl ether, Span™ 20, Span™ 60, Span™ 80, Tween 20, and Tween 80. Butanol, ethanol, isopropyl alcohol, and propylene glycol are frequently employed as co-surfactants in emulsion and other formulation preparations. They are chosen for their capacity to enhance the solubilization of lipophilic compounds and improve the stability of emulsions. Carbitol and PEG 400 are also used as cosurfactants in some formulations because of their tolerability and ability to increase the permeation of drugs or other compounds through biological membranes [25,85]. Overall, nanoemulsions are complex systems whose properties are highly dependent on the constituent components employed during their formulation. Thus, a meticulous selection of ingredients, along with an appropriate determination of the quantity and weight ratio of oil and surfactants, can yield nanoemulsions with desired properties and attributes. By tailoring the composition of these systems, it is possible to optimize their stability, solubility, and bioavailability, making them promising candidates for various applications, including drug delivery in skin cancer formulations.

4.2. Selected nanoemulsions-based topical formulations for skin cancer treatment

The selected nanoemulsion application examples elucidate the extensive capacity of nanoemulsions to convey and improve the efficacy of synthetic, natural, novel, or old drugs and even for drug repurposing (Fig. 3). All the work put into these types of studies, given especially its variety and the crescent number represents a step forward towards the discovery of a much-needed efficacious strategy to finally tackle skin

cancers.

4.2.1. Nanoemulsion-based gel containing leflunomide

Leflunomide (LFD) is a disease-modifying anti-rheumatic drug, also considered valuable for treating melanoma [92]. Leflunomide has been demonstrated to be an aryl hydrocarbon receptor (Ahr) agonist and to hold inhibitory activity over the dihydroorotate dehydrogenase enzyme (DHODH). Both pathways are involved in tumor progression and carcinogenic cells' potential migratory capacity [92–95]. While there are high hopes for its therapeutic potential, administering leflunomide orally is inconvenient due to the unwanted gastrointestinal side effects it causes. Even attempting to deliver it directly to the affected skin, bypassing the oral route and preventing systemic effects, is hindered by its low permeability and limited aqueous solubility, less than 40 μg/mL. Notably, leflunomide falls under the category of BCS Class II drugs [92, 96]. As a form to surpass these obstacles, an innovative nanoemulsion-based gel of LFD, for transcutaneous delivery, was formulated using a self-nanoemulsifying technique. The developed carrier system used Transcutol® HP, Capryol® 90, and Cremophor® EL as co-solvent, oil, and non-ionic hydrophilic surfactant, respectively (Table 2). The choice of components reflects a keen understanding of the need for stability and efficient drug delivery. Pluronic® F-127 was the selected gelling agent. Given its thermoreversible properties have promoting, and at the same time, retarding drug permeation through the skin capacities, which are convenient for topical delivery with minimum systemic permeation. For this reason, its use in this formulation, given these attributes, is desirable and advantageous for the intended topical administration route. The resulting O/W nanoemulsion-based gel presented a 123.7 nm mean globule size, convenient for skin penetration and consistent within the NE droplet size range; a zeta potential (ZP) less than −7.8 mV, that despite being suggestive of poor physical stability is not, in this case, very significative, since the NE is intended to be immediately gelled [97]; and a 0.278 polydispersity index (PDI), indicative of a narrow globule size distribution. The evaluation of the nanoemulsion-based gel viscosity, at room temperature, showed a viscosity value inversely proportional to the shear rate (9620 ± 93 cps at a shear rate of 50 rpm and 7523 ± 82 cps at a shear rate of 100 rpm), which indicates that this formulation has satisfactory spreadability on the skin and easy removal from the container. To study the anti-proliferative potential and to confirm the LFD anti-melanoma activity, Pund. et al. conducted *in vitro* cytotoxicity assays, using A375 and MEL-2 melanoma cancer cell lines. The obtained results showed a significant cell viability decrease and a faster mortality rate on the cell lines treated with the developed LFD nanoemulsion-based gel when compared to the pristine LFD-treated ones, showing that this strategy selectively targets melanoma cells. Moreover, the *ex vivo* drug permeation assays, performed through Wistar rats' abdominal skin, showed improved dermakinetic parameters when compared to a simple LFD conventional gel, which proves that the nanoemulsification process favors the transcutaneous penetration and LFD deposition in the skin [92, 98].

4.2.2. Nanoemulsions and nanoemulsion-based gels containing daidzein

Daidzein (DZ) is a water-insoluble isoflavone with evident anticarcinogenic activity against melanoma, involving cell cycle arrest, autophagy, and deactivation of PI3K/AKT signaling pathways [99,100]. However, its use is limited given its low solubility (0.0849 mg/mL) and low partition coefficient of oil/water. To overcome its limitations a DZ-NE and a nanoemulsion-based gel (DZ-NEG) for topical application, containing the phytosteroid DZ were prepared to attest to its treatment potential against melanoma. Kaplan et al., formulated these nanosystems using the following ingredients: ethyl oleate, as oil, Lipoid S100, as an emulsifier, Tween® 80 (polyethylene sorbitol ester), as an emulsifier, DMSO (dimethyl sulfoxide), as co-solvent, and ultrapure water (Table 2). A high-energy homogenization technique was applied. The resultant DZ-NE droplet size, PDI, and ZP values were 149.80 ± 3.52 nm, 0.222 ± 0.013, and −19.32 ± 1.06 mV respectively. The analysis of these values shows a narrow droplet size distribution in the nanometer range, of a stable nanosystem, due to the relatively high zeta potential. Even after the addition of Protasan ™ UP G 213, a natural, nontoxic, bioadhesive polymer, intended for gelation and production of a DZ-NEG (200.25 ± 11.09 nm droplet size; 0.311 ± 0.007 PDI; 19.35 ± 0.66 zeta potential), the same is observed, despite the slight droplet size increase. The gelation process is a valuable strategy, for topical administration, as due to the viscosity increment, it favors the ease of application and augments spreadability onto the skin. The release studies performed using Franz diffusion cells, for both conventional emulsion, DZ-NE and DZ-NEG, suggest that despite the higher DZ release rate from DZ-NE, understandable given the smaller droplet size and larger droplet size area, the DZ-NEG slower drug release profile is preferable, especially when in combination with prolonged effect and attractive rheological characteristics. As for the conductivity evaluation, it was possible to classify both DZ-NE and DZ-NEG as O/W emulsions (high conductivity). The macroscopic analysis of the performed centrifuge test (15 min, 3500 rpm) showed no phase separation or creaming both for DZ-NE and DZ-NEG formulations, however for the conventional emulsion a slight creaming was observed. As for the pH, the obtained values, in the 4.53–5.54 range, suggest these formulations' suitability for topical delivery. The viscosity, another important physicochemical parameter for topical formulations, was also evaluated. The results suggest an almost Newtonian flow behavior for the NE formulations. Moreover, the DZ addition showed no significant difference ($p > 0.05$) in the viscosity values. The cytotoxic effects of freshly prepared DZ-NEs and DZ-NEG were evaluated by comparison of the cell viability in SK-MEL30 melanoma and normal fibroblasts PCS-201–012 cell lines. All the formulations containing DZ (DZ-NE and DZ-NEG) preserved their cytotoxic activity against the melanoma cell line and did not affect the normal cell lines [100]. The analysis of the obtained results highlights the use of these formulations, in particular DZ-NEG, as a promising approach to melanoma treatment.

4.2.3. Nanoemulsions containing 5-FU

5-FU is a well-established and efficient treatment option for various cancer types, such as skin cancer, breast cancer, colon cancer, and lung cancer. Unfortunately, 5-FU use entails extremely poor and erratic oral bioavailability, low tumor affinity, and high toxicity, making its oral and topical utilization inefficient and at risk of serious secondary effects [48, 101]. In fact, despite the market availability of injectable and topical 5-FU formulations, given its hydrophilic nature, direct permeation across the SC, of lipophilic characteristics, is likewise not possible. Furthermore, oral administration is also invalidated by its severe side effects [102]. With that in mind, Shakeel et al., proceed, employing a spontaneous emulsification method, with the development of a low HLB surfactant 5-FU-NE as a tool to overcome these limitations (Table 2). The optimized W/O NE was intended for a transdermal chemoprevention approach to skin cancer. A series of formulations, with quantitative variations of compounds, were fabricated using Lauroglycol-90 as the oil phase, deionized water, as the aqueous phase, Transcutol® HP as the surfactant, and isopropyl alcohol as the selected co-surfactant. A low HLB surfactant was used to prepare these NEs since they favor the formation of W/O NEs, while higher HLB surfactants favor the formation of O/W. As an initial screening, all preparations were subjected to thermodynamic stability tests and observed for phase separation, creaming, and coalescence, to exclude all metastable and unstable forms. After this process, the formulation that was found stable at all stress conditions proceeded to further characterization. This NE had an average ($n = 3$) droplet size of 68.20 ± 2.65 nm, a −25.92 mV ZP (with values in the ±25–30 mV range being associated with a stable formulation [103]), a 0.219 PDI, indicative of droplet size uniformity, and a viscosity mean of 50.15 ± 3.16 cps, convenient for topical application. This same formulation went under a further series of thermodynamic stability tests (centrifugation, cooling, and heating cycles) to assure its long-term

Table 2
Characteristics of the nanoemulsions utilized in melanoma and non-melanoma skin cancers management.

NE	Materials	Skin cancer	Preparation technique	Droplet size (nm)	PDI	ZP (mV)	Key findings
Pund et. al (2015) – **LFD-NEG**	Transcutol® HP Capryol® 90 Cremophor® EL Pluronic® F127	Melanoma	Self-emulsification	123.7	0.278	-7.8	Halts tumor growth and suppresses cancer cell migration Specific and accelerated mortality rate in cell lines treated with the LFD-NE
Ugur Kaplan et al. (2019) – **DZ-NEG**	Ethyl oleate Lipoid S100 Tween® 80 DMSO Ultrapure water Protasan ™ UP G 213	Melanoma	High energy homogenization	149.8	0.222	-19.32	Enhanced viscosity for easier application and improved skin spreadability Maintained cytotoxic effectiveness against the melanoma cell line
Shakeel et al. (2015) **5-FU-NE**	Lauroglycol-90 Deionized water Transcutol® HP Isopropyl alcohol	Melanoma	Spontaneous emulsification	68.20 ± 2.65	0.219	-25.92	Transdermal chemoprevention attributes Significant drug permeation improvement
Kumar et al. (2015) **5-FU-NE**	Span® 80 Span® 85 Tween® 80 Isopropyl alcohol Oleic acid Isopropyl myristate Triacetin	Non-melanoma	Oil phase titration	100	n.r.	n.r.	Higher skin retention and better control over the drug release in comparison to a topical 5-FU marketed cream
Tagne et al. (2008) **DAC-NE**	Ethanol Soybean oil Polysorbate 80 Ultrapure water	Melanoma	Microfluidization	131	0.421	−5.49	10-fold greater tumor reduction Promising alternative to the conventional IV route
Dehelean et al. (2011) **BA-NE**	Flax-seed oil Egg phosphatidylcholine Deionized water	Melanoma	Microfluidization	145 ± 1.5	0.4	-39.1 ± 1.2	Duality as prophylactic and therapeutic agent Interference with the angiogenic process
Chen et al. (2004) **TPL-NE**	Oleic acid Propylene glycol Tween® 80 1 % (v/v) menthol	n.r.	Magnetic stirring	Between 10 and 150	Narrow	n.r.	Controlled, sustained, and prolonged delivery of TPL transdermally
Asasutjarit et al. (2021) **AG-NE**	Coconut, sesame, and jojoba oils Tween® 80 Lecithin Propylene glycol Ethanol Paraben concentrate	Melanoma and non-melanoma	Micro fluidization	176.6 ± 1.8	0.332 ± 0.004	−11.78 ± 0.11	Selective inhibitory activity against melanoma and non-melanoma cell lines Convenient reduced dosing frequency
Liu et al. (2021) **CNE**	Soybean oil Vitamin E, Tween® 80 Lecithin Deionized water	Melanoma	Homogenization and ultrasonication	16	0.132	−66.6	Dose-dependent decrease in cell viability
Nasr et al. (2022) **TEO-NE**	TEO Lecithin Tween®80 Amphiphilic oligochitosan	Melanoma	Low-energy emulsification	184.74 ± 1.27	0.19	+23.82 ± 0.55	Bilayered emulsion modified with an oppositely charged biopolymer Strong attachment to negatively charged PS expressed on the melanoma cells surface
Nagaraja et al. (2021) **Chrysin-NE**	Caproyl® 90 Tween® 80 Transcutol® HP	Melanoma	Self-emulsification	156.9 ± 3.4	0.26	−15	Transforming chrysin into a nanoemulgel formulation augmented its therapeutic efficacy
Shakeel et al. (2010) **CAF-NE**	Lauroglycol-90 Transcutol® HP Isopropyl acid	Skin cancer caused by sun exposure	Oil phase titration	20.14–105.25	0.105–0.177	n.r.	NE components functioned as permeation enhancers, obviating the need for additional chemicals Safe and effective transdermal delivery
Tang et al. (2021) **THC-NE**	Transcutol® Medium-chain triglycerides	Melanoma	High-speed and high-pressure homogenization	n.r.	n.r.	n.r.	Protective effects from H2O2-induced cell death Effective inhibition of α-MSH induced melanin production
Guerrero et al. (2018) **CUR-NE**	Miglyol 812 Epikuron 145V Ethanol Acetone Ultrapure water	Melanoma post-surgery reincidence and metastasis	Spontaneous-emulsification	200	≤ 0.2	-30	Specific cytotoxicity against cancer cell lines, including melanoma B16F10 cells Prevention of tumor recurrence and spontaneous lung metastasis
Dalmolin et al. (2018) **ZnPc-NE**	MCTs DOTAP Lipoid E80 Water	Melanoma	Ultrasonication	200	0.20 ± 0.02	43 ± 7	Enhanced ZnPc skin permeation through synergistic nanocarrier and iontophoresis Improved ZnPc penetration and

(*continued on next page*)

Table 2 (*continued*)

NE	Materials	Skin cancer	Preparation technique	Droplet size (nm)	PDI	ZP (mV)	Key findings
	Polysorbate 80 Poloxamer 188						uniform drug distribution within the tumor
Martínez-Razo et al. (2023) **NCTD-NE**	Almond oil Urea Glyceryl monostearate Glycerin Cetyl alcohol Stearic acid Polysorbate 80 Eumulgin B1®	Melanoma	Ultrasonication	117 to 120	0.26 to 0.28	0	Consistent prolonged NCTD release Approximately 10-fold solubility augment

Abbreviations; 5-FU-NE – 5-Fluorouracil nanoemulsion; **AG-NE** – Andrographolide nanoemulsion; **BA-NE** – Betulinic acid; **CAF-NE –** Caffeine nanoemulsion; **CNE –** Carotenoid extract nanoemulsion; **CUR-NE** – Curcumin nanoemulsion; **DAC-NE** – Dacarbazine nanoemulsion; **DZ-NEG** – Daidzein nanoemulsion-based gel; **LFD-NEG** – Leflunomide nanoemulsion-based gel; **n.r. –** Not reported; **NCTD-NE** – Norcantharidin nanoemulsion; **TEO-NE** – *Thymus vulgaris L.* essential oil nanoemulsion; **THC-NE –** Tetrahydrocurcumin nanoemulsion; **TPL-NE** – Triptolide nanoemulsion.

stability, this having been verified. Based on the results obtained by *in vitro* permeation studies, performed on Franz diffusion cells, using rat abdominal skin, a highly significant enhanced drug permeation was found when comparing this new NE formulation both to control (saturated aqueous solution of 5-FU) and other formulations (NEs formulated using different oil-phase, aqueous-phase, surfactant, and co-surfactant proportions). Was also found by i*n vivo* cytotoxicity studies, performed on SK-MEL5 melanoma cell lines, that the investigated NE was much more potent and efficacious than the 5-FU aqueous solution. Therefore, the optimized NE might be considered promising for the chemoprevention and treatment of skin cancers. However, further pre-clinical and clinical studies are necessary to confirm these results [3,48]. Kumar and colleagues invested in a different development approach of a novel and efficient W/O NE of FU-5 containing Span® 80, Span® 85 (sorbitan trioleate), Tween® 80, and isopropyl alcohol, combined with different oils such as oleic acid, isopropyl myristate, and triacetin (Table 2). The oil phase titration method was the selected preparation method. In comparison with a marketed 5-FU cream product, the synthesized NE showed higher skin retention and improved control over the drug release rate. Better control of drug release through the skin further decreased the occurrence of systemic and topical toxicity, a very relevant aspect. Additionally, the conducted skin irritation and histopathology studies prove the safe use of this formulation topically, even in a chronic use scheme [101]. Goindi and his colleagues conducted the manufacturing of another variant of this delivery model whose goal was also the transdermal delivery of 5-FU but employing an Ionic Liquid-Based microemulsion [104].

4.2.4. Nanoemulsion containing dacarbazine

Dacarbazine (DAC) is a commonly antineoplastic chemotherapeutic used in the treatment of different cancers, such as malignant melanoma and Hodgkin's lymphoma, most often administered intravenously. As a non-specific anti-melanoma drug that interferes with the cell cycle phase, the patient response is variable and frequently associated with significant toxicity and severe secondary effects. Nanoemulsions as drug delivery systems allow for (1) converting lipid-soluble components into water-soluble ones thus allowing their delivery into both polar and apolar matrices, potentially reducing toxicity; (2) reducing the particle size of existing drugs from the order of thousands to less than a hundred nanometer, presumably resulting in longer and deeper penetration [34, 105]. Making use of the cited features, a team of researchers converted an existing and highly lipid-soluble DAC suspension, associated with severe secondary effects and administration difficulties, into a more effective water-soluble one. The initial suspension was prepared by dissolving DAC in ethanol and adding sequentially soybean oil, Polysorbate 80, and HPLC-grade water, finalizing the mixture homogenization. The resulting suspension was then subjected to a microfluidization process to obtain a dacarbazine nanoemulsion (DAC-NE) (Table 2). The particle size of this DAC suspension, which was initially 5470 nm, was downsized, after microfluidization to 131 nm (Fig. 4(B), (C)), positioning the DAC-NE in the nanometer magnitude, to favor bioavailability. The PDI value was 0.421, indicative of homogeneous droplet size (Fig. 4. (A)). The newly formulated DAC-NE was incorporated in a hypoallergenic cream, intended for topical delivery, but also in an intramuscular injection. Mice xenograft models (Crl: NU/NU-nuBR) were subcutaneously injected with a human melanoma cell line (Malme 3 M cells) and then divided into five groups: control (untreated mice); four treatment groups treated with DAC suspension for topical administration, DAC suspension for intramuscular injection, DAC-NE for topical administration, and DAC-NE for intramuscular injection (IM) (Fig. 4. (D)). The topical application of the DAC-NE produced, in comparison with the DAC suspension, a 10-fold greater percent reduction of tumor size. In terms of efficacy, the effect of the DAC-NE administration both topical and intramuscular routes were comparable and superior to the existing DAC suspension. In addition, 12 weeks after treatment cessation, the animals treated with DAC-NE IM, remained tumor-free, in comparison with control or untreated animals (Fig. 4. (E)). In conclusion, the attained NE was demonstrated to be more efficacious in tumor treatment and growth prevention (via intramuscular and topical routes) compared to the existing DAC suspension. DAC-NE use might even result in fewer adverse effects since the available results demonstrate the possibility of replacing the conventional route of administration of intravenous (IV) DAC with more convenient routes such as topical or IM [34].

4.2.5. Nanoemulsion containing betulinic acid

Betulinic acid (BA) is a pentacyclic triterpene saponin compound that has been reported to exert anti-tumor and anti-inflammatory effects against lung adenocarcinoma, breast and prostate cancers, ovarian carcinoma, glioblastoma, and leukemia. Some other findings also suggest this active compound's potential as a prophylactic and curative agent in skin carcinomas management [106]. Despite its easy extraction from natural sources, the poor water solubility affects its biodisponibility. To overcome the drawbacks related to solubility and bioavailability, as well as to promote betulinic acid solubility, an O/W nanoemulsion was developed using a high-energy process (microfluidization) (Table 2). Flax-seed oil was used as the internal phase and a mixture of egg phosphatidylcholine and deionized water established the external phase [107]. The average BA-NE system droplet size ($n = 3$) was 145 ± 1.5 nm. The ZP of the uniformly distributed droplets (PDI 0.4) was measured, and the resultant value was -39.1 ± 1.2 mV. The disrupted angiogenic process equilibrium is involved in tumor progression and metastasis events, therefore, its inhibition could benefit cancer cure prognostics. For that reason, the ultimate purpose of this work was to access a novel BA-NE capacity to inhibit the tumor angiogenic process. In this sequence, the group examined this effect using *in vivo* chick embryo chorioallantoic membrane (CAM) assay, convenient given this model's extensive capillary network suitable for the angiogenic process

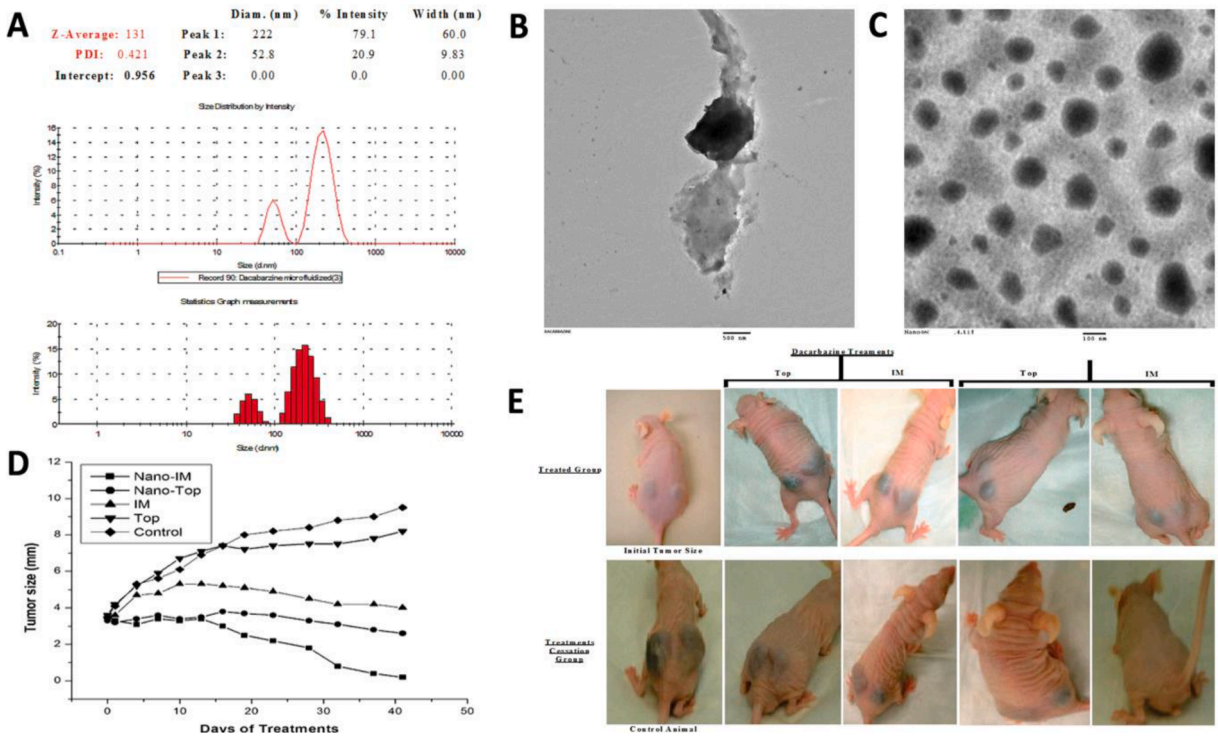

Fig. 4. (A) Z-average size distribution of nano-DAC particles, as determined by dynamic laser light scattering and statistical graph measurement revealing the heterogeneity of particle size within seemingly homogeneous distributions, emphasizing the impact of microfluidization. (B) Schematic structure of DAC characterized by transmission electron microscopy (TEM) using Philips EM400T. The evaluation of the NE preparation highlights large spherical particles exceeding 500 nm in size. (C) Schematic structure of the formulated nano-DAC, characterized by TEM using Philips EM400T. The morphological evaluation demonstrates spherical particles measuring less than 131 nm, showcasing formulation efficacy. (D) Pattern of tumor size growth in Malme 3 M xenograft mice after 40 days of DAC formulation treatment. Significant reductions in tumor growth are observed in nanoemulsion formulations of DAC compared to suspension-treated or untreated animals. (E) Nude mice with melanoma Malme 3 M cell line-derived xenografts for evaluating the effectiveness of DAC-NE. The upper portion depicts mice treated with both formulations (Top and IM), measuring tumor sizes during treatment. The lower portion illustrates tumor sizes measured 2 months after cessation of both treatments, providing insights into the enduring impact of the formulations [34]. DAC – Dacarbazine; IM – Intramuscular; TEM – Transmission electron microscopy; Top – Topical.

observation. Applying a blank-NE (no BA), in which components per se are irrelevant to the angiogenic process activity, seemed not to disturb the rapid capillary growth, with a normal vessel density observed. In contrast, a progressive new vessel density loss, indicative of anti-angiogenic properties, was visible for the BA-NE, demonstrative of superior efficacy. Histopathological tests performed on Balb/C mice skin confirmed the differences between untreated and treated groups, with reduced skin damage, inflammation, and tumor development observed in the latter. Through these observations, Dehelean and colleagues report that once incorporated into a NE, BA topical administration can perform anti-inflammatory and anti-carcinogenic activities both as a prophylactic and therapeutic agent against skin carcinoma [108].

4.2.6. Nanoemulsion containing triptolide

Triptolide (TPL) is employed in clinical practice, mainly via oral and intravenous routes, for its promising anticarcinogenic and immunosuppressive properties. However, its potential use is constrained by its significant secondary effects. These effects are most prevalent in the gastrointestinal tract (nausea, vomiting, diarrhea, duodenal ulcer), but are also noticeable in the cardiovascular, urogenital, bone marrow, and skin systems. Since it is a small molecule with a moderately lipophilic profile, and therapeutically effective at low doses, it is considered suitable for transdermal administration. To this end, a group of researchers incorporated this molecule, through magnetic stirring, in a NE carrier consisting of oleic acid (oil), propylene glycol (co-surfactant), Tween® 80 (surfactant), and water aiming for transdermal delivery (Table 2). To the final drug-loaded TPL-NE a 1 % (v/v) menthol, was added, to function as a permeation enhancer. A total of nine NE systems were formulated through the combination of various component ratios. All of these preserved droplet sizes between 10 and 150 nm, narrow PDI, and pH values within the physiological range (5.35 ± 0.04 to 6.58 ± 0.03). The viscosity values obtained were dispersed, being however greater in the formulations in which the percentage of the oil phase and surfactant was higher. The new formulations were subjected to physical and chemical stability tests (phase separation and clarity observation, triptolide concentration, and droplet size determination) over a period of 6 months, at the end of which no significative changes were observed. As confirmed by the *in vitro* permeation tests performed in diffusion equipment, on hairless male mice's abdominal skin, the skin permeation flux from the TPL-NE was superior and indicative of a prolonged release profile, when compared to an aqueous solution containing 0.025 % triptolide. The skin irritation tests also confirmed, given the edema or erythema absence, that the problems related to toxicity were overcome through the encapsulation of triptolide by this lipidic nanosystem. In conclusion, and as anticipated the produced O/W NE made it possible to achieve a carrier capable of offering a controlled, sustained, and prolonged delivery of TPL via transdermal route [109].

4.2.7. Nanoemulsion containing andrographolide

Andrographolide (AG) is an active natural compound, isolated from the leaves and stems of the medicinal plant *Andrographis paniculate*, that exhibits activities related to cancer treatment (NMSC, breast cancer, leukemia), inflammation, central nervous system disorders, and bacterial/viral infections treatment. More recently it has been recognized for its beneficial activities related to skin complications (skin cancer, skin damage caused by UV radiation, and skin pigmentation). However, its

low water solubility (around 0.10 mg/mL), impairs bioavailability, leads to poor efficacy, and complicates the development of products targeting these disorders. A team of researchers proposed the formulation of an AG-NE, prepared using a microfluidization technique, as a suitable carrier for transdermal delivery (Table 2). For the preparation of the cited NE, AG was solubilized in a mixture of oils (coconut, sesame, and jojoba oils). Tween® 80, lecithin, propylene glycol, ethanol, and a paraben concentrate were added and mixed until a clear solution was obtained. After adding water, the obtained mixture was mixed using a high-speed homogenizer and then homogenized via a microfluidizer. This team also investigated the existing correlation between the number of homogenization cycles and pressure *versus* resulting droplet size, PDI, and ZP values. After this analysis, some conclusions were drawn, in particular: an increase in the value of pressure and the number of homogenization cycles leads to a decrease in the values of droplet size, PDI, and more negative values of ZP. However, if extreme, the increase in pressure and the number of cycles has undesirable opposite effects, leading to an increment in all the previous parameters. The droplet size, ZP, and PDI analysis clarified that the optimized AG-NE (Op-AG-NE) formulation exhibited a droplet size in the nanometer range (176.6 ± 1.8 nm) with a moderately wide PDI (0.332 ± 0.004), a high negative ZP value (−11.78 ± 0.11 mV), and a pH value suitable for topical application (5.7 ± 0.1). To evaluate the *in vitro* AG release, the studies performed on modified Franz diffusion cells, on cellulose acetate membrane, using the Op-AG-NE, agreed with zero-order kinetic. Suggestive of a constant release, controlled exclusively by the diffusion rate of AG from the internal phase droplets, and independent of the AG concentration. These findings also suggest AG's prolonged action, which enables a convenient administration frequency reduction. The skin permeation studies, performed on newborn skin pig skin, also on modified Franz diffusion cells, proved that the formulation permits the permeation of the Op-AG-NE through the skin. Regarding the cytotoxicity tests performed in HFF-1 normal human skin fibroblasts cells, A375 human malignant melanoma cells, and A-431 epidermoid carcinoma cells it sought to compare the Op-AG-NE, blank NE (same preparation, no active ingredient), and AG solution effects. The results demonstrate that blank-NE shows no toxicity towards HFF-1 cells, AG solution exhibited toxicity to this same cell line while the Op-AG-NE cytotoxicity was, in comparison, only found at much higher concentrations and is therefore considered safe to normal human fibroblast cells. Additionally, this new formulation's inhibitory activities showed to be more selective against the A375 malignant melanoma and A-431 non-melanoma cell lines, compared to blank NE and AG-solution, in particular, because of the oil-droplets of these O/W NE that permit the endocytosis of the AG by the cancerous cells. It is also worth noting that the cell viability of HFF-1 cells exposed to Op-AG-NE was much higher than the one observed in the ones treated with AG solution. Further highlighting the extended-release profile of the NE, delaying the undesirable cytotoxicity effects on the normal cells. The conjugation of these findings legitimates the interest in this particular carrier as a promising cytotoxicity agent approach [110].

4.2.8. Nanoemulsion containing a carotenoid extract

Carotenoids belong to a class of liposoluble pigments, broadly distributed in nature, interesting for their well-documented bioactivities, such as immune system enhancement, free radical scavenging, and anticancer activities (colon, prostate, and breast cancers). Unfortunately, carotenoids are prone to degradation and instability namely through illumination, heating, oxygen, or acid exposure [111]. Therefore, the development of an efficient encapsulation method that improves the efficacy and biological activity of these unstable compounds is imperative. Among these techniques, NEs stand out because of their ease of production and extremely small droplet size. An undertaken study attempted to probe the melanoma inhibitory effects of carotenoids, extracted from pomelo leaves, by preparing a NE and comparing its effects with carotenoid extracts (CEs) on A375 melanoma cells. A transparent deep-yellow carotenoid nanoemulsion (CNE) was prepared through the sequential mixing and intermittent homogenization of soybean oil, vitamin E, Tween® 80, lecithin, and deionized water (Table 2). The resulting pre-mixture was subsequently ultrasonicated. Transmission electron microscopy imaging revealed round-shaped particles with an average droplet size of 16 nm. Storage stability was accessed every 15 days for over 3 months. During this period the samples stored a 4ºC presented a slight ZP increase and minor changes in the PDI value, indicative of high stability and uniform nanoparticle distribution maintenance, respectively. Contrarily at 25ºC and 40ºC, the pH value suffered a significative decline, explainable possibly by the hydrolysis of soybean oil into free fatty acids. Given these results, the maintenance of a storage temperature of 4 °C is indispensable, thus preventing disturbances (hydrolysis and lipid oxidation) to the stability of NE. MTT assay investigated the cell viability of A375 cells and fibroblasts CCD-986SK cells treated with CEs and CNE. Upon treatment, these formulations showed a dose-dependent decline in cell viability for both cell lines. Both CEs and CNE demonstrated slight toxicity towards CCD-986SK cells. As for A375 cells, CNE exhibited higher toxicity than CEs, evidenced by a lower IC_{50} value, at the same dose. It might postulate the prominence of this novel nanosystem as another possible melanoma treatment attempt [112].

4.2.9. Nanoemulsion containing Thymus vulgaris L. essential oil

Thymus vulgaris L. (thyme) essential oil (TEO) use has, during the past decades, received significant interest from the pharmaceutical industry due to its organoleptic properties and well-established pharmacological usage, among several other essential oils. By embedding this bioactive ingredient into an NE system, Nasr et al. sought to enhance not only the TEO physicochemical stability and bioavailability but also its transdermal permeability (Table 2). Although the use of surfactants stabilizes the NE system, after a certain period, the stability of these carriers may decrease leading to sedimentation, coagulation, creaming, and flocculation phenomena that ultimately limit their functional potential. By employing as a preventive strategy the surface modification of the nanoemulsions with a contrarily charged biopolymer this team expected to improve both the stability and applicability of these structures. Consequently, a bi-layered emulsion was produced by incorporating TEO, lecithin, Tween®80, and amphiphilic oligochitosan (AOC) (Fig. 5 (A)). The obtained physicochemical properties, in particular particle size and ZP values, are a confirmation of compliance with improved stability due to the presence of an AOC protective layer. As evidenced by the constancy of the AOC-coated TEO-NEs physicochemical properties during storage for a week (approximately 184.74 ± 1.27 nm particle size, +23.82 ± 0.55 mV ZP and a 0.19 PDI, within the desirable range for uniform monodispersed systems) (Fig. 5(C)). Relatively to its composition, the NEs targeted in this study comprised a primary or internal layer consisting of TEO and Tween® 80-lecithin and a secondary protective layer of AOC. A two-step process was used to prepare these NEs. In the first moment, primary NEs were prepared by adding TEO-Tween®80 (oily component) to a lecithin solution (aqueous component) under vigorous stirring using a low-energy emulsification process. In the second step, secondary NEs were obtained by coating the primary NEs with AOC, using electrostatic deposition. This involved depositing positively charged biopolymers onto the negatively charged surface of lecithin molecules, resulting in stable and uniform multilayered NEs, with scanning electron microscope images (SEM) confirming its spherical shape and uniform distribution (Fig. 5(B)). Overall, the AOC-NEs *in vitro* kinetic release experiments demonstrated that the AOC—NE had enhanced transdermal penetration (1.5 times greater than the primary nanoemulsion) (Fig. 5(D)) and anticancer efficacy, primarily attributed to their highly positive charge density that facilitated strong attachment to the negatively charged skin cell membrane, as well as the negatively charged phosphatidylserine (PS) expressed on the surface of melanoma cells. Therefore the surface modification with AOC provided sufficient time for the nanodroplets to penetrate these cells and induce apoptosis

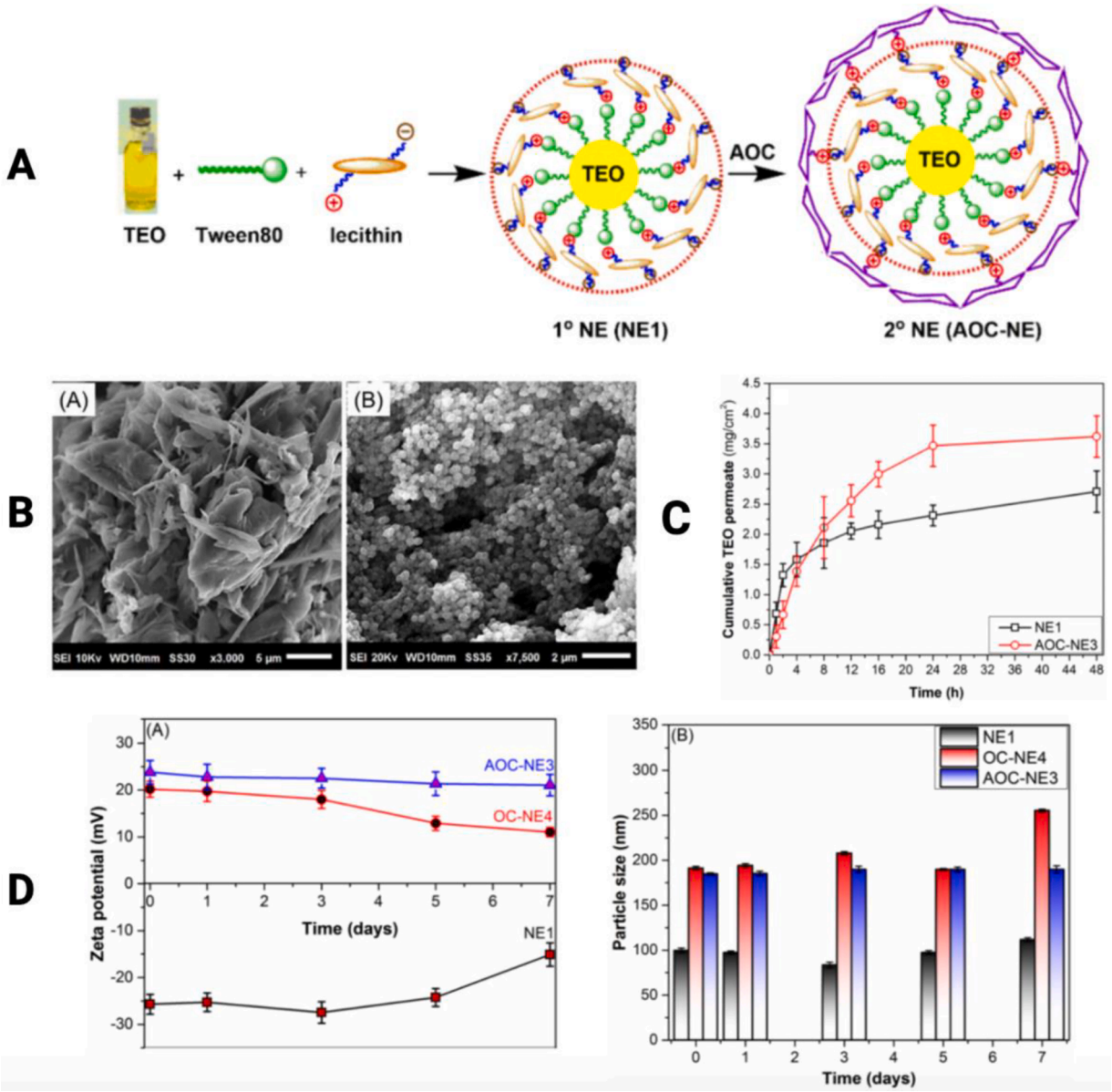

Fig. 5. (A) Schematic representation of TEO-based AOC-coated NE. (B) Representative SEM observation of AOC (left) and its secondary AOC-NE (right). (C) TEO-based nanoformulations skin permeability profile through a rat skin for 24 h. (D) Graph showing the AOC protective layer effect in the zeta potential (mV) and particle size (nm) values, during one week of storage at room temperature, compared to a bare NE. AOC – Amphiphilic oligochitosan; AOC-NE – Amphiphilic chitosan nanoemulsion; AOC-coated NE - Amphiphilic oligochitosan coated nanoemulsion; NE – Nanoemulsion; SEM - Scanning electron microscope; TEO - Thyme essential oil [113].

by delivering the TEO. While exhibiting excellent selectivity for melanoma cells over healthy cells. According to these findings, TEO delivery using this AOC-coated nanoemulsion technology offers a promising method to overcome the difficulties related to low skin permeability experienced by topical formulations designed for melanoma treatment [113].

4.2.10. Nanoemulsion-based gel containing chrysin

Chrysin is a flavone derived from passion flowers that exhibits potential anti-cancer activities, however, its applicability is limited as a result of inadequate solubility and poor biodisponibility. Nagaraja et al. adopted a novel approach to overcome these constraints and formulated an aqueous, gel-based nanoemulsion system with a hydrophobic chrysin core (Table 2). The construction of pseudo-ternary phase diagrams, with varying compositions of Caproyl® 90 (oil), Tween® 80 (surfactant), and Transcutol® HP (co-surfactant), permitted the authors to identify the ideal self-nanoemulsifying region that conducts to the production of the desired drug delivery systems (Fig. 6(A)). Topical delivery systems with oils and surfactants can easily modify the lipid arrangement of the *SC* and enhance the solubilization and partitioning of lipophilic actives. Self-emulsifying formulations however have limited applicability for topical delivery, likely due to an insufficient understanding of skin metabolomics and physical stability. For that reason, Pluronic® F127 was used as an emulsifier and stabilizer in the preparation of nanoemulgels to improve the final formulation's physicochemical and biopharmaceutic characteristics. The characterization studies conducted on the nanoemulsion indicated that the droplets present a negatively charged surface (ZP of −15 mV), a mean droplet size of 156.9 ± 3.4 nm, and a 0.26 PDI, in accordance with a monodisperse system. Both SEM and HR-TEM images confirm the droplets' uniform size distribution and spherical morphology. The data collected from *ex vivo* permeation studies performed through rat abdominal skin, using Franz-type

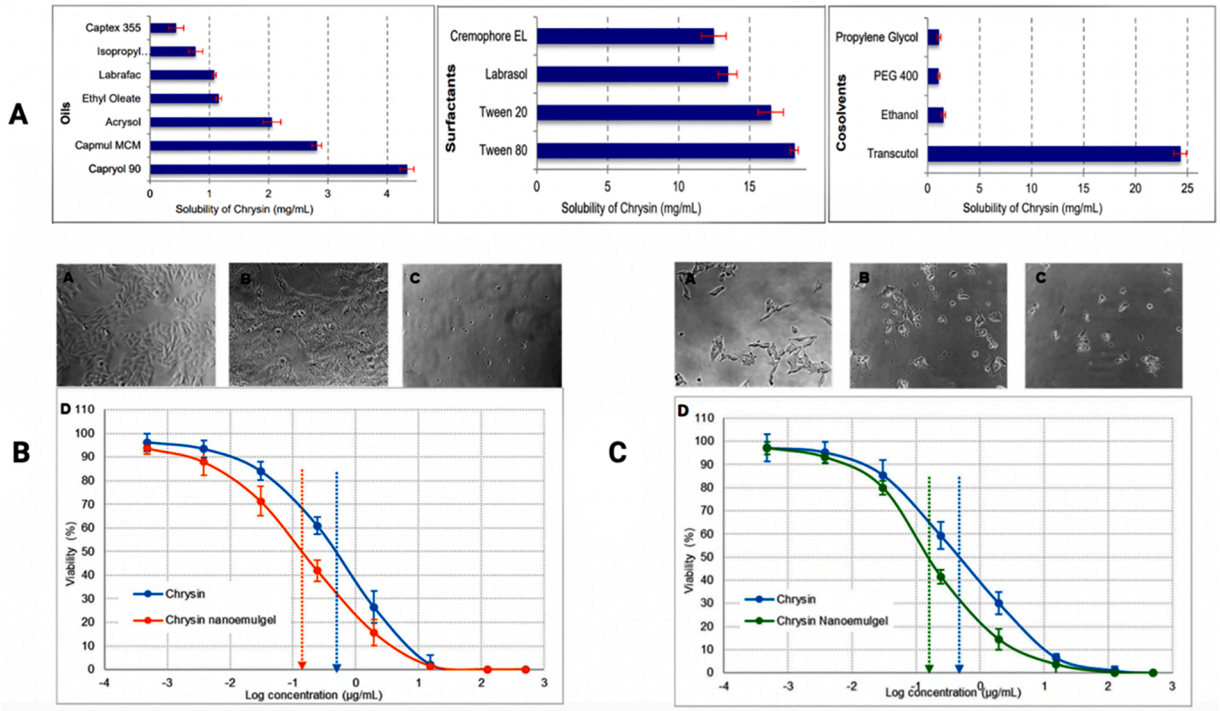

Fig. 6. (A) Evaluation of chrysin solubility in different oils, surfactants, and cosurfactants used to select the ideal final composition. (B) Morphological observation and growth inhibition of A375 cells under different treatments. Panel (A) shows the control cells that were left untreated. Panel (B) shows the cells treated with pure chrysin, while panel (C) shows the cells treated with chrysin nanoemulgel. Panel (D) displays the profile of *in vitro* cytotoxicity, where the viability of cells is plotted as a percentage against the logarithm of chrysin concentration. The results shown are based on the mean values obtained from three independent experiments, and the error bars indicate the standard error of triplicate analysis. (C) Displays the morphological observation and growth inhibition of SK-MEL-2 cells. Panel (A) represents the control cells, panel (B) cells that received pure chrysin treatment, and panel (C) the cells treated with chrysin nanoemulgel. Panel (D) displays the profile of *in vitro* cytotoxicity for chrysin, where the percentage viability of cells is plotted against the logarithm of chrysin concentration (µg/mL). The data presented are based on the mean values obtained from three independent experiments, with error bars indicating the standard error of triplicate analysis [114].

diffusion cell apparatus, clearly indicates an improved chrysin percutaneous delivery when delivered in the nanoemulgel form, when compared with that of a simple chrysin powder and Pluronic® gel base. *In vitro* cytotoxicity cell viability tests using A375 and SK-MEL-2 melanoma cells revealed that the cells treated with pristine chrysin retained normal cell morphology, whereas cells treated with the nanoemulgel displayed alterations and apoptotic changes such as rounding and shrinkage (Fig. 6(B) and (C)). In fact, the conversion of chrysin into nanoemulgel form was found to enhance its therapeutic response, as demonstrated by cytotoxicity studies, highlighting the potential benefits of this approach for the delivery of chrysin-based therapies. More so, the *in vitro* biocompatibility analysis in a mouse fibroblast cell line (L929) confirmed the cellular viability, and therefore the formulation was considered biocompatible and safe for topical application. Taking into account the results of this study, the nanoemulsion-inspired chrysin delivery system offers unique advantages as a possible valuable topical approach for skin cancer treatment [114].

4.2.11. Nanoemulsion containing caffeine

Evidence suggests that dermally applied caffeine can protect the skin against sun-induced skin cancer. Shakeel et al. developed and evaluated a nanoemulsion formulation of caffeine for transdermal drug delivery to further explore this potential (Table 2). As caffeine is a hydrophilic drug, various W/O nanoemulsion formulations of caffeine were prepared using the oil phase titration method. More so, this team of researchers aimed to achieve this using only non-irritating, pharmaceutically acceptable ingredients without resorting to the use of chemical enhancers. In this system, the nanoemulsion components themselves acted as permeation enhancers, making the addition of extra chemicals unnecessary. The solubility of caffeine was, in comparison to other oils, found to be highest in Lauroglycol-90, therefore it was selected as the oil phase. After a screening considering the highest solubilization capacity for the oil phase, Transcutol-HP was selected as the most convenient surfactant. While isopropyl acid (IPA) was the chosen co-surfactant. Morphological characterization showed that the mean droplet size of the prepared caffeine NEs was found in the range of 20.14–105.25 nm and all the formulations' PDI values were very low (0.105–0.177) indicative of the droplets' uniformity within the formulation. After conducting *in vitro* permeation studies, using rat abdominal skin, it was perceived that caffeine NE (CAF-NE) had a statistically significant effect ($p < 0.05$) compared to an aqueous drug solution (control). The highest steady-state flux and enhancement ratio values of the CAF- NEs formulation also demonstrated its potential efficacy against skin cancer. Additionally, histopathological studies provided further evidence (no apparent signs of skin erythema and edema) of the improved effectiveness and safety of transdermal delivery of the anticancer drug caffeine [115].

4.2.12. Lecithin-based nanoemulsion as a tetrahydrocurcumin topical delivery enhancer

Tetrahydrocurcumin (THC), a curcumin metabolite, presents recognized superior antioxidant properties. Therefore investigators speculate that its use may alleviate diseases resulting from oxidative stress. This includes the harmful UV radiation effects on the skin, such as hyperpigmentation but also melanoma initiation and progression. Although presenting many benefits, THC skin delivery is limited by its poor aqueous solubility. In their work, Tang et al. investigated both how THC impacts the melanogenic process under stressful conditions, and its H_2O_2-induced cytotoxicity protective capacities. As lecithin offers several distinctive benefits such as high biodegradability, a structure comparable to the SC lipid bilayer, a low skin irritation level, and moisture properties, a lecithin-based NE, where lecithin performs as an

emulsifier, was utilized as a topical delivery system (TDS) for this active ingredient (Table 2). To optimize the permeation properties and solubility capacity of the vehicle within the skin, tetrahydrocurcumin nanoemulsions (THC-NEs) were produced using Transcutol® and medium-chain triglycerides (MCT), employing high-speed and high-pressure homogenization techniques. The HaCaT cell assays and cell viability studies confirmed the THC protective effects from H_2O_2-induced cell death, reducing the ROS directly and in a concentration-dependent way. To assess the THC impact on the melanogenesis process, the melanin content was determined and compared between groups treated with alpha-melanocyte-stimulating hormone (α-MSH) (melanin production stimulator), THC co-cultured with α-MSH, and the control group (free of α-MSH), using a B16F10 melanoma cell model. It was shown that THC is capable of effectively inhibiting melanin production induced by α-MSH in B16F10 melanoma cells, as well as the gene expression of three other crucial enzymes involved in the melanin biosynthesis process namely, tyrosinase (TYR), tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2). Additionally, the topical delivery efficacy studies performed by means of an *in vitro* Franz diffusion cell model, using Strat-M® membrane, demonstrated that the THC-NE exhibited a significant permeation enhancement in comparison with the THC suspension. Such results reflect this THC-NE formulation's advantages given its anti-melanogenic benefits and its future potential topical application not solely in the cosmetic industry, as it mitigates hyperpigmentation, but perhaps as a novel skin cancer management tool [116].

Some other authors describe the interesting pro-apoptotic and anti-proliferative activities of a tocotrienol-based NE to be suitable as adjunctive therapy for non-melanoma skin cancers, due to its anticancer activity and efficacious cutaneous delivery [117]. Others propose the development of a nanoemulsion containing curcumin, an insoluble and unstable active, but advantageous for its anti-tumoral properties in preventing the occurrence of relapses and metastasis in melanoma cases, following surgery. In their research, Guerrero et al. present a novel fabrication approach for curcumin-loaded nanoemulsion known as CUR-NE (Table 2). These NEs presented dimensions of approximately 200 nm, exhibited low polydispersity (PDI ≤ 0.2), and carried a negative zeta potential (−30 mV) (Fig. 7(A)). The fabrication process employed safe excipients and resulted in a high curcumin content (95 %). *In vitro* experiments confirmed the biocompatibility of CUR-NE in non-cancerous human cells (HEK-293T) and selective cytotoxicity towards cancer cell lines, including melanoma (B16F10) cells (Fig. 7(C)). Notably, the nanoformulation exhibits remarkable effects on melanoma cells, enhancing intracellular curcumin accumulation and promoting the formation of ROS while inhibiting cell migration and invasion. *In vivo* studies conducted in mice showed that a single topical application of CUR-NEM after the surgical removal of primary tumors formed upon subcutaneous injection of syngeneic B16F10 cells effectively prevented tumor recurrence and spontaneous lung metastasis (Fig. 7(B)), in stark contrast to untreated animals, where a 70 % recurrence rate is observed. Importantly, the fluorescence signal of curcumin *in vivo* persisted for at least 15 days following CUR-NEM application, far exceeding the signal longevity when curcumin was administered in DMSO. Crucially, even when administered at a dose 22 times larger than that used topically after surgery, CUR-NEM does not adversely affect biochemical parameters. These findings underscore the safety and efficacy of CUR-NEM, suggesting its potential as an attractive option for topical post-surgery application to prevent tumor recurrence and metastasis in cancer patients (Fig. 7(D)) [118].

4.2.13. Nanoemulsion containing Zinc Phthalocyanine

Zinc Phthalocyanine (ZnPc) is a stable and nontoxic photosensitizing agent. However, its high lipophilicity, which contributes to its efficient photodynamic effects, hinders its topical and systemic administration. As the use of iontophoresis, a physical strategy for skin barrier transposition and satisfactory skin drug delivery depends on the use of a pharmaceutical dosage form that allows for the passage of an electric current, Dalmolin and Lopez produced O/W NEs with ZnPc incorporated

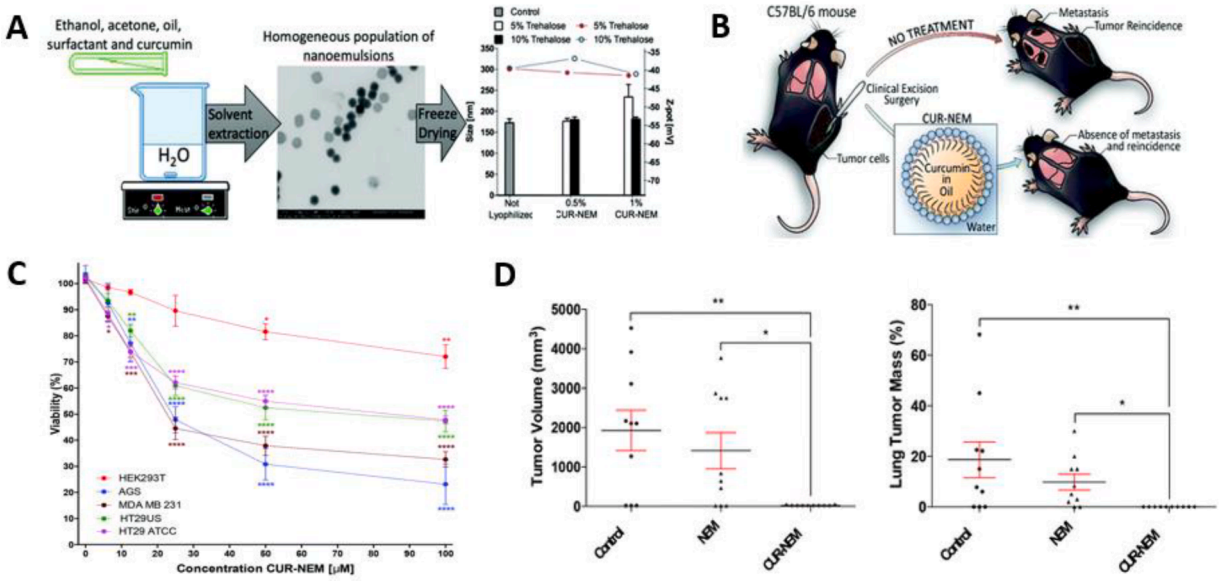

Fig. 7. (A) Schematic representation of the elaboration process for CUR-NE is depicted on the left. The center shows electron microscopy images illustrating the resulting nanoemulsions. On the right, the stability of formulations is presented before and after conversion to a dry powder. The bars represent the size of the formulations, while the lines represent the zeta potential. (B) Schematic representation illustrating the disparities in metastasis and tumor recurrence occurrences between mice that received no treatment and those treated with CUR-NE. (C) The viability of various human cancer cell lines, including AGS, MDA-MB-231, HT29 ATCC, and HT29 US, was assessed using the MTS assay after treatment with CUR-NEM for 24 h. The non-cancerous cell line HEK 293T was included for comparison. The inset graph displays results following treatment with different concentrations of nanoemulsions (NEM). ($n = 3$, $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$). (D) B16F10 cells were subcutaneously injected into C57BL/6 mice, allowing tumors to develop for 14 days before surgical removal. The resulting wounded zone post-surgery was either left untreated (control), treated with NEM, or treated with CUR-NEM. Mice were sacrificed 21 days post-surgery, and tumor growth at the initial site (a) and lung tumor mass (b) were evaluated. ($n = 10$, $^{*}P < 0.05$; $^{**}P < 0.01$).

in the internal oily phase (Fig. 8(C)). In addition to ZnPc, the oily phase was composed of MCTs, N-1,2-dioleoyloxy-3-trimethylammonium propane chloride (DOTAP), and egg phospholipids with 80 % phosphatidylcholine (Lipoid E80). Whereas the aqueous phase consisted of water, polysorbate 80, and Poloxamer 188. To prepare the final NE system the coarse emulsion was immediately ultrasonicated (Table 2). The physicochemical NE characterization unveils a system with narrow droplet size distribution (PDI=0.20 ± 0.02) and cationic properties (ZP=43 ± 7 mV) (Fig. 8 (A), (B)). *In vitro* skin permeation experiments using Franz diffusion cells confirmed that the synergistic use of this nanocarrier and iontophoresis increased the ZnPc skin permeation, compared to passive delivery. The *in vivo* studies in a melanoma murine model demonstrated that the combination of these strategies can also enhance the ZnPc penetration and homogenous drug distribution into the tumor (Fig. 8 (D)). In sum, this work results suggest that the ZnPc-NE in combination with iontophoresis may be considered a suitable platform for the delivery of this lipophilic drug in a melanoma scenario [119].

4.2.14. Nanoemulsion containing norcantharidin

Despite its promising potential as an antitumor agent, norcantharidin (NCTD), a derivative of natural blister beetles, faces limitations in clinical use due to its poor aqueous solubility. In order to address this constraint and take advantage of the promising NCTD properties, Martínez-Razo et al. produced an O/W nanoemulsion using ordinarily available cosmetic ingredients (Table 2). Almond oil (emollient), urea (hydrant), glyceryl monostearate (lubricant), glycerin (humectant), cetyl alcohol, stearic acid (texturizing agents), polysorbate 80 (hydrophilic surfactant), and Eumulgin B1® (autoemulsifier and hydrophobic surfactant), the latter particularly important since the solubility approach depended on pH interaction, composed the list of ingredients utilized. The synchronous use of all these ingredients is intended to achieve optimal skin responsiveness and prolonged drug delivery efficiency by moisturizing and hydrating the skin. In this work, ultrasonication, considered an effective and reliable high-energy preparation method, was employed as the second step in the preparation of nanoemulsions, following the production of an initial macroemulsion. Concretely, and according to the drug solubility assays, the incorporation of NCTD in a NE augmented its solubility about 10-fold, when compared to its solubility in water. Likewise, in this study, the values of droplet size, PDI, ZP, pH, and viscosity were also evaluated for NE characterization. The droplet size value showed no statistically significant alterations over 4 weeks (test period). The same was observed for the PDI values that remained between approximately 0.26 and 0.28 during the first and fourth week, respectively. As for the ZP value, it persisted close to zero during the whole evaluation period. Additional formulation characterization tests showed that these nanoformulations presented a pH of 6.5, a skin-safe value, and an integrated viscosity value (5489 Pa/s,) sufficient to enhance retention at the application site while maintaining good spreadability. Moreover, by characterizing the rheological behavior of this formulation, the authors concluded that it is a thixotropic non-Newtonian fluid. STEP-Technology® experimental data analysis used for accelerated stability assessment showed that upon centrifugation, as expected, the NEs stability underwent some decay, by forming a cream, a frequent destabilization phenomenon often observed in emulsions. The authors also report through *in vitro* drug release tests, performed using the Franz diffusion cell system, a consistent and prolonged NCTD release, ideal for topical administration. Although the *in vitro* experiments have shown that the NCTD NE can affect the growth and metabolism of melanoma B16F1 cells, additional *in vivo* and clinical studies are required to thoroughly evaluate the safety and effectiveness of the developed product. The overall purpose and novelty of this work lay in the development of a nanoformulation with high drug strength and optimal properties for topical delivery, through simple preparation methods, using easily accessible excipients. It is then the ambition that this formulation can be made more widely available in the future through preparation and distribution in community pharmacies [120].

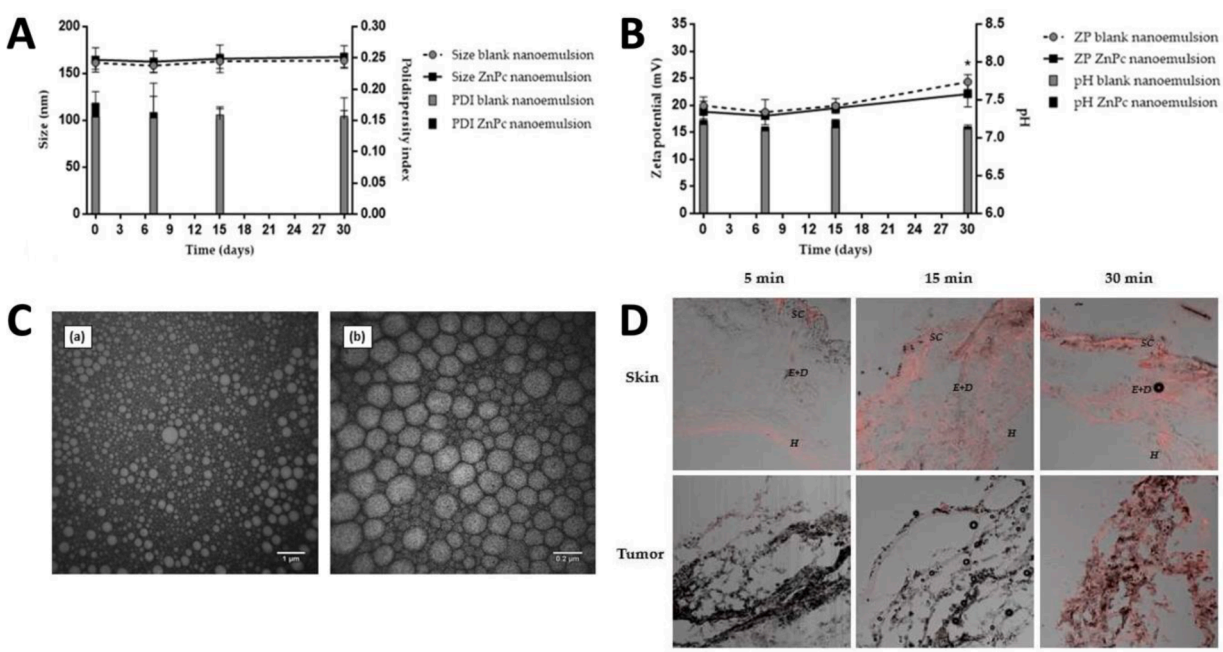

Fig. 8. (A) Temporal evolution of droplet size and PDI in blank and ZnPc nanoemulsions during a 60-day storage period at 4 °C. (B) Analysis of zeta potential and pH variations in blank and ZnPc nanoemulsions over a 60-day storage period at 4 °C, * indicates statistical difference in relation to time 0. (C) Transmission electron microscopy (TEM) images depicting the ZnPc nanoemulsion stained with negative contrast using 2 % uranyl acetate. (a) Captured at a magnification of 20,000×, whereas (b) reveals a higher magnification of 100,000×. (D) Representative confocal microscopy images depicting 20 μm-thick cryo-sections oriented perpendicular to the skin surface and tumor samples following various durations of ZnPc nanoemulsion iontophoresis. The images, generated by overlapping light and dark fields, highlight distinct skin layers and tumor structures, including the stratum corneum (SC), epidermis and dermis (*E* + D*)*, and hypoderm (H). ZnPc fluorescence provides detailed insights into the spatial distribution and penetration of the nanoemulsion within the skin and tumor samples [119]. TEM - Transmission electron microscopy; PDI - Polydispersity index; ZnPc - Zinc Phthalocyanine.

With this revision work a multitude of formulations has been investigated, revealing a spectrum of droplet sizes, polydispersity indices, and ZP values. An example to highlight is Liu et al.'s CNE, characterized by a small droplet size of 16 nm, which shows its potential for efficient skin penetration. In contrast, Guerrero et al.'s CUR-NE features a larger droplet size of 200 nm, offering unique perspectives on therapeutic characteristics. Nasr et al.'s TEO-NE distinguishes itself with a remarkable low PDI of 0.19, indicative of a uniform droplet distribution. Conversely, Tagne et al.'s DAC-NE exhibit a higher PDI of 0.421, suggestive of a broader size distribution. ZP values further contribute to the diversity, with Liu et al.'s CNE showcasing a remarkable −66.6 mV, potentially enhancing formulation stability. On the other hand, Tagne et al.'s DAC-NE presents ZP values of small absolute value, being close to neutrality, but although these formulations might not have electrostatic repulsion favoring their physical stability, many other factors could contribute to their stabilization, such as increased viscosity, droplets in the nanometric size range, and the presence of surfactants. Commonly utilized components such as ethanol, Tween® 80, and Transcutol® HP are recurrent, highlighting their versatility in nanoemulsion formulations, featured for example in formulations by Asasutjarit et al., Tagne et al., and Chen et al. It is also noteworthy that the majority of the nanoemulsions analyzed in these studies specifically target melanoma, underscoring the relevance of these findings to treat aggressive cutaneous cancer forms. This diverse array of findings encapsulates a nuanced understanding of nanoemulsion characteristics, providing valuable insights for the ongoing exploration of melanoma therapy.

The extensive body of scientific literature on nanoemulsions for the treatment of skin cancer underscores their growing importance and potential in oncotherapeutic strategies. This wealth of research not only highlights their increasing relevance but also suggests that they merit continued attention and investment. Upon reviewing the extensive scientific work on nanoemulsions for topical skin cancer treatment, it becomes evident that these formulations offer distinct advantages over currently available conventional therapies. Nanoemulsions, with their precisely tailored properties, including size, stability, and drug delivery capabilities, have the potential to revolutionize the approach to treating skin cancer. Their capacity to encapsulate and efficiently deliver therapeutic agents, such as chemotherapeutic drugs or natural active ingredients, directly to the affected skin site holds significant promise. Moreover, nanoemulsions facilitate enhanced penetration into the skin, ensuring that the active ingredients effectively reach their intended targets within the tumor microenvironment. This targeted and localized delivery minimizes systemic exposure and mitigates the potential for side effects—a critical limitation of many traditional cancer treatments. The conclusion drawn from this body of evidence strongly supports the superiority of nanoemulsions over conventional therapies for skin cancer [25,121]. Consequently, it calls for further sustained research and investment in this promising field of oncotherapeutics.

5. Overcoming challenges

It is noticeable that a great number of investigators and industries invested, in the past years, abundantly and with great depth in the research of nanotechnologies focused on cancer treatment. The generated data from the conducted work makes also evident the prominence of these nanosystems as future potential approaches to skin cancer treatment. Nevertheless, the translation into clinical practice although the numerous scientific publications concerning the use of these nanoformulations for cancer treatment is missing since many of them cannot successfully reach the *in vivo* clinical evaluation stage [3]. In terms of NEs particularly, despite all the emphasized characteristics that point to great prospects for their practical application the same problem is also observed. The first justification for this might be the fact that NEs undergo some decay despite the interesting long-term stability (months to years) emphasized by some authors (Table 1). Oswald ripening the major opponent to this desired stability forces the growth of the internal phase droplets, limiting the number of application possibilities [75]. Despite thousands of publications regarding nanoemulsions, their formulation is still generally based on a trial-and-error approach (Table 1) [78]. Future knowledge should also be orientated to the resolution of a few lacunes concerning NEs effectiveness like its tumor tissue permeation, bioadhesiveness, and controlled release of the drug, but also questions related to toxicity [3]. Since although less than that produced by the conventional chemotherapeutics used, the possible topical toxicity of these formulas should not be ruled out, and additional toxicity evaluation through *in vitro* and *in vivo* studies should be carried on. More multidisciplinary and rigorous studies should also be conducted regarding the scrupulous understanding of the underlying penetration mechanisms and interactions of these systems with the skin. The combination of these studies along with further formulation science and particle engineering advances will favor the obtention of much-needed clinically relevant information to the topical drug delivery field [12]. Additionally, the development of clear regulatory guidelines (Table 1) is also a requirement along with the clear identification of the breaches in current knowledge that difficult the translation of these therapies into clinical products with an economic interest (Fig. 9) [3].

6. Conclusion

NEs emerged as highly promising and distinctive platforms for drug delivery. They stand out due to their appealing visual properties, robust stability, unique rheological attributes, and the ability to be tailored to specific needs. These advantages set them apart from conventional methods and formulations currently in use. However, despite this position being proven inclusively by the results of several groups, in various scientific papers, some further steps have yet to be taken before NEs are undoubtedly seen as well-established tools for treating and managing skin cancers. The future development of NE technologies will require efforts regarding the deep comprehension of the relation between the formulation science inherent to NEs production and the diverse physicochemical and physiological challenges associated with the various pathologies and different routes of administration, in this case, particularly through the topical route. A complete understanding of the physiopathology underlying skin cancer development is also necessary. Furthermore, numerous other considerations must be met to facilitate the translation of promising NEs candidates, originating from research laboratories to the pharmaceutical marketplace. Including the careful selection of components, especially oil phase components, and emulsifiers, with respect to safety concerns and potential hypersensitivity reactions. Therefore, the formulation of NEs using biocompatible surfactants and excipients approved by the relevant health authorities is a prerequisite for any possible future pharmaceutical application. In addition to this, challenges concerning the manufacturing processes must also be considered, involving ease of large-scale synthesis, storage, and stability. This assessment must be parallelly addressed with the application and administration route in mind. Investment in the insurance of biocompatibility, efficient drug accumulation on tumor site, functionalization, maintenance of drug levels above the minimum cytotoxicity concentration, and of course regulatory considerations are some other aspects that need to be addressed. Undoubtedly the continued research, multidisciplinary approaches, and economic investment aiming to create increasingly sophisticated NE will lead to an extraordinary opportunity for clinical translation of these remarkable novel carrier systems to several fundamental indications. In particular, the encouraging results from the numerous studies reviewed herein demonstrate the potential of nanoemulsions as a versatile platform for skin cancer treatment. In conclusion, the comprehensive and robust body of literature presented in this review strongly suggests that the future of skin cancer treatment lies not only in the discovery of novel therapeutic agents but perhaps in the efficient and effective development of sophisticated delivery systems to the target site; and for this

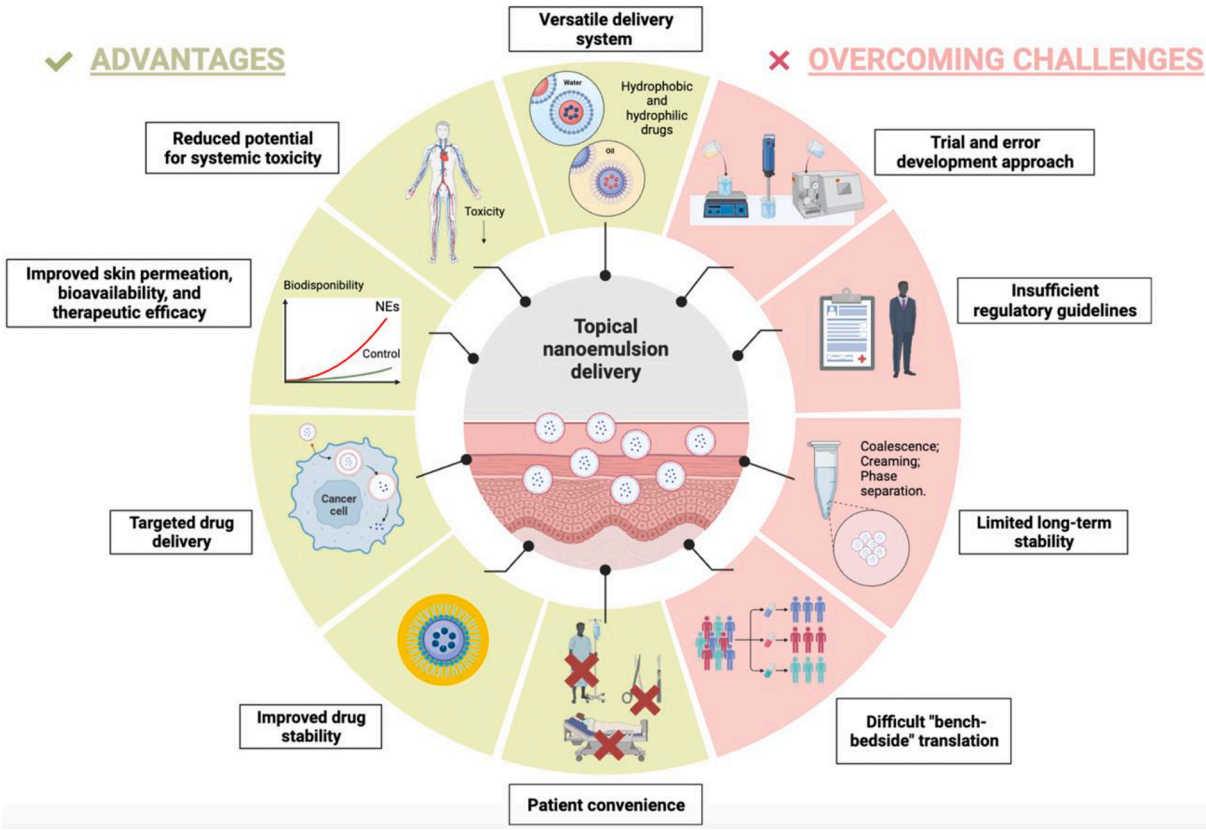

Fig. 9. Overview of the advantages and challenges associated with nanoemulsions utilization. Nanoemulsions offer unique advantages, that include enhanced solubility and stability, improved bioavailability, and targeted drug delivery. Despite the significant advantages of nanoemulsions, the development of this formulation is currently limited by a trial-and-error approach, insufficient regulatory guidelines, and often unsatisfactory long-term stability. Moreover, the bench-to-bedside translation of nanoemulsion formulations is complicated by issues such as toxicity concerns and limited knowledge of their biological behavior. Thus this figure highlights the various challenges associated with nanoemulsions, underscoring the need for a more systematic and informed approach to their development, in order to maximize their potential and overcome obstacles for safe and effective clinical translation.

emerges nanotechnology.

CRediT authorship contribution statement

Joana Duarte: Writing – original draft, Software. **Ankur Sharma:** Writing – review & editing, Conceptualization. **Esmaeel Sharifi:** Writing – review & editing, Conceptualization. **Fouad Damiri:** Writing – review & editing, Conceptualization. **Mohammed Berrada:** Writing – review & editing. **Moonis Ali Khan:** Writing – review & editing. **Sachin Kumar Singh:** Writing – review & editing, Supervision. **Kamal Dua:** Writing – review & editing, Supervision. **Francisco Veiga:** Supervision, Writing – review & editing, Conceptualization. **Filipa Mascarenhas-Melo:** Writing – review & editing. **Patrícia C. Pires:** Supervision, Writing – original draft, Writing – review & editing, Conceptualization. **Ana Cláudia Paiva-Santos:** Supervision, Writing – original draft, Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.
On behalf of all authors of the manuscript

Data availability

No data was used for the research described in the article.

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