Design of Experiments for the Development of Topical Drug Products

3

Nishtha Chaurawal and Kaisar Raza

Abstract

The systematic development of pharmaceutical products using quality tools is highly essential for attaining robust performance by minimizing variability. Design of experiment (DoE) is now considered as an indispensable tool in pharmaceutical development to obtain quality products. Among a variety of dosage forms, the topical dosage forms are useful for providing a localized therapeutic effect of the drugs. Such dosage forms include liposomes, ethosomes, niosomes, transfersomes, and many more, where the manufacturing of such dosage forms includes multifunctional excipients and a number of unit operations. Hence, the use of DoE provides greater flexibility and is highly effective for the efficient development of topical products with ultimate savings of the resources. The present chapter, therefore, provides a holistic account on the implementation of DoE approach for the manufacturing of topical drug products and also highlights the current challenges and opportunities associated with them.

Keywords

Quality by design · Design of experiment · Topical delivery · Liposomes · SLNs · D-optimal design · Box–Behnken design

N. Chaurawal · K. Raza (✉)
Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Ajmer, Rajasthan, India
e-mail: drkaisar@curaj.ac.in

S. Beg (ed.), *Design of Experiments for Pharmaceutical Product Development*,
https://doi.org/10.1007/978-981-33-4351-1_3

3.1 Introduction

Over the years, drug delivery systems are the epitome of the medicines arena. A drug delivery system is defined as a formulation that, after administration, enables the therapeutic agent to reach its site of action and shows therapeutic response without affecting the non-target tissues, cells, and organs. The area of drug delivery systems is broad, which also covers the targeted systems and controlled release systems of drug delivery [1]. Since ancient times, the drug is delivered by the most common traditional route of administration, i.e., oral and parenteral. For more than two decades, the research was preceding to overcome the problems (such as poor absorption, difficulty in dose intake, first pass metabolism) related to the traditional route of drug administration, which leads to the researchers to use the skin as a carrier for the drug delivery. The researchers found that the delivery of drugs via skin can successfully reduce the side effects related to other routes of administrations [2]. The delivery of therapeutic agents via skin was very challenging for the researchers. Since the late 1970s, intensive research has been carried out to understand the mechanism of drug penetration through the skin to enable the design of delivery systems for topical absorption [3]. Skin delivery can be of different types, such as transdermal delivery, dermal delivery, and topical delivery. In recent times, the demand for topical administration of drugs is increasing because of the high patient compliance and topical or systemic delivery of drugs whenever possible [4]. The topical delivery system is defined as the method of application of the formulation to the uppermost layer of the skin for the treatment of local skin diseases. In topical drug delivery, the skin penetration of drugs into the deeper tissues or systemic circulation is unnecessary for the therapeutic response of the drug [3]. There are several conventional topical formulations available in the market, as ascribed in Table 3.1. The topical administration of the active therapeutic agent mostly depends upon its physicochemical properties, its ability to permeate the skin, the structure of carrier, and skin conditions [5]. The formulation's role is also very

Table 3.1 Marketed conventional topical formulations

Drugs	Commercial products	Dosage forms	Indications	Manufacturer
Betamethasone propionate	Diprolene	Cream, ointment	Psoriasis	Merck Sharp & Dohme
Dithranol	Micanol	Cream	Psoriasis	Riemser
Diclofenac sodium	Voltinax	Gel	Anti-inflammatory, analgesic	Naxpar Ltd.
Tacrolimus	Aptac	Ointment	Fungal infections	Naxpar Ltd.
Capsaicin	Zostrix	Cream	Analgesic	Bradley Marketing Sol.
Menthol	Eucerin	Lotion	Dermatitis	Eucerin Ltd.
Neomycin sulfate	Neosporin	Ointment	antibacterial	Johnson & Johnson
Miconazole	Mycoban	Cream	Fungal infection	ICM Pharma

relevant in the topical delivery as the therapeutic efficacy of the drug can be altered by the interaction between the vehicle and the skin. The formulation assists the drug substance to reach the target site and also helps in the management of drug transport, active duration, and dosage integrity. To prepare and design an optimized formulation with the above characteristics, understanding the skin's structural features is also necessary along with the vehicle, which has a crucial role in the drug delivery mechanism. The vehicle super-saturation can improve the thermodynamic activity of the formulation for drug penetration through the skin. Therefore, topical delivery came out as a ray of hope in the treatment of various skin conditions like fungal infections, inflammation, bacterial infections, and skin cancers [6].

However, the conventional topical formulations were unable to provide the desired therapeutic efficacy and showed high degradation of the drug at the skin surface, making scientists research on nanocarriers based topical drug delivery. The nano-drug delivery systems have various applications over conventional drug delivery, such as allowing the drug to cross the skin membrane easily at a shallow dose and enhancing therapeutic efficacy. The nanocarriers encapsulate the drug, which can also retrain its degradation on the skin. Table 3.2 shows various types of nanocarriers designed by the researchers for the topical delivery of active molecules and their specific treatment [7]. Nanodelivery systems are suitable strategies for enhancing the rate and extent of drug penetration and improving drug absorption [8]. These all properties of nanocarriers such as potency, targeting ability, and permeability are depended upon its characterization studies, i.e., size, polydispersity index, percentage of components, and surface charge. Therefore, newer analytical and characterization techniques are necessary to determine these properties related to nanoformulations and control them during production and synthesis. The new drug delivery systems which can be utilized for the skin penetration of therapeutic agent were largely unexplored even after the numerous scientific research in this field. However, to overcome these hurdles, the scientific and systematic approaches were

Table 3.2 Topical drugs encapsulated in nanocarriers

Drugs	Nanocarriers	Indications
Benzyl peroxide	Liposomal gel	Acne
Prednisolone	Magnetic liposomes	Allergic dermatitis
Hydroxyzine	Liposomes	Skin allergy
Idoxuridine	Liposomal gel	Herpes simplex
Methotrexate	Ethosomes	Psoriasis
Dithranol	Liposomes and niosomes	Psoriasis
NB-002	Nanoemulsion	Fungal infection
Tacrolimus	Nanoparticles	Psoriasis
Cyclosporine A	Solid lipid nanoparticles	Atopic dermatitis
Capsaicin	Flexible membrane vesicles	Musculoskeletal pain
Coal tar	Lipid-coated microparticles	Psoriasis
Corticosteroid	Skin-lipid liposomes	UV induced erythema
Finasteride	Liposomes	Acne, androgenetic alopecia

employed by the researchers to optimize the process design and nanoformulations [9, 10]. In topical delivery, the incomplete understanding of process production is the main reason for the researchers to implement Quality by Design (QbD) approach to optimize nano-drug delivery systems, which can lead to the development of optimized nano-based formulations. The main advantage of the QbD concept is the development of high-quality drug products without extensive regulatory oversight [11]. QbD has implemented one of its components called the design of experiment (DoE) to develop an optimized pharmaceutical product. Besides product development, QbD has applications in the process design, robust manufacturing process, quality attributes, regulatory flexibility, and also improves the regulatory communication between industry and regulators. In the topical drug delivery, DoE provides real-time quality control with real-time release and decreases post-approval changes in the formulation. The various topical delivery formulations developed with the implementation of DoE have been discussed further in this chapter [12].

3.2 Advantages of Topical Drug Delivery

The approach related to drug delivery's topical route provides various advantages over other drug administration routes. Figure 3.1 represents the advantages of topical delivery. These include no systemic absorption of drugs, high concentration of drug at the target site, avoidance of the first pass metabolism sustained delivery of the drug, ease of administration, enhancement in bioavailability, a continuation of dosage regimen with short biological half-lives, non-invasive application of formulation, and patient compliance. Topical drug delivery also decreased the

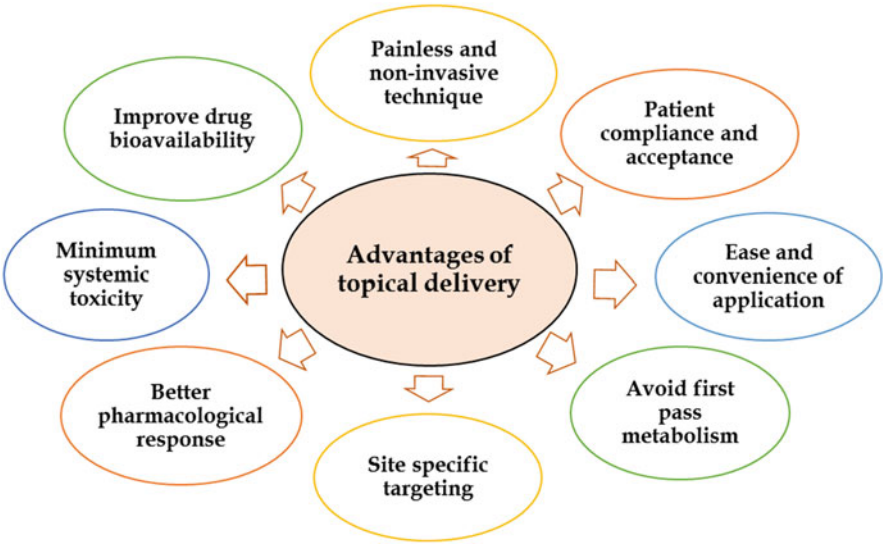

Fig. 3.1 Advantages of topical delivery

peak-associated side effects and ensured a steady-state profile, which helps in the increment of drug level above minimum therapeutic concentration [13]. The topical administration of drugs provides relief in a broad range of therapeutic indications. According to the researchers, various studies showed that the topical delivery of drugs exhibited sustained pharmacological action due to the high rate and extent of drug delivery in a reasonable time to achieve local therapeutic concentration [14]. This can enhance the absorption of drugs compared to other routes of administration [15]. The delivery of nanocarriers via topical administration also possesses several advantages: sustained release of the drug, skin targeting, increased uptake, a base to the bioactive compounds, avoiding direct contact of harmful drugs with the skin, and reduces adverse effects related to the drug. The most crucial application of topical delivery is to treat contagious skin diseases such as atopic dermatitis, skin cancer, and psoriasis. The encapsulation of bioactive agents into nanocarriers also reduces its degradation when coming in contact with the skin at the time of topical delivery [16].

3.3 Challenges in Topical Delivery

The skin is the largest organ of the body and consists of three main layers called the outermost epidermis layer (50–150 μm thick), inner dermis layer (about 250 μm thick), subcutaneous fat tissues. The nutrients maintain their vitality by crossing through dermal–epidermal junction because of the lack of blood vessels in the epidermis. The epidermis is further divided into five different layers named as stratum corneum (SC), stratum lucidum, stratum granulosum (granular layer), stratum spinosum (spinous layer), and stratum germinativum (basal layer). The epidermis with the absence of SC is called the viable epidermis, which is responsible for the barrier nature of skin for topical drug delivery, and most of the cutaneous disorders are also found in it [17]. The transportation of substances from the skin occurs mainly via three important routes by the passive diffusion process. The transportation routes are transcellular, intercellular, and trans-appendageal route [18]. As suggested by various researchers, the most effective penetration route for topical drug delivery is the trans-appendageal route, and some drugs also used the intercellular pathway between the corneocytes to cross the skin membrane [19].

The penetration of substances from hair follicles is also possible. It was demonstrated that skin penetration through the hair follicle pathway could enhance the penetration and absorption of bioactive agents for drug delivery. Hair follicles also work as a center for the depot formation, which can multiply the SC storage capabilities [20]. Figure 3.2 represents all the major routes of topical delivery of drugs. Instead of these routes of drug transportation via the skin, the protein transporters present in the skin also play a vital role in the transportation of substances. Therefore, the localization and expression of various tight junction proteins present at the epithelial layers and hair follicles have been altered during the disease stage of the skin, such as psoriasis. The main barrier in topical delivery is

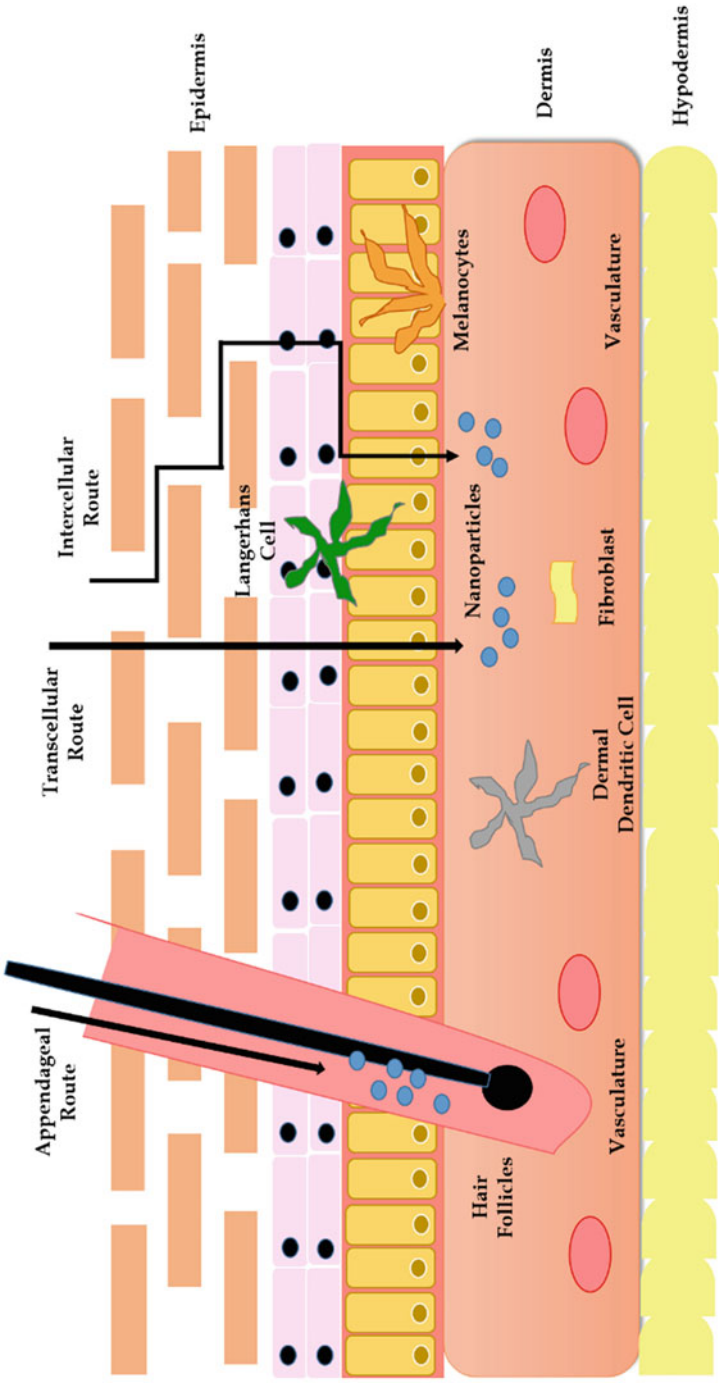

Fig. 3.2 Pictorial representation of topical drug delivery

the SC, which causes the challenge that the drug has to cross the SC and reach viable epidermis or systemic circulation to show the therapeutic efficacy.

Then another challenge is the balance between the drug penetration through SC and its buildup in the skin to ensure the therapeutic concentration of the drug [2]. Though molecules are unable to fully and readily pass through the SC membrane, they can only cross the membrane. The rate of percutaneous absorption of molecules is determined by the state of SC hydration [7]. Andrea and co-workers suggested that the skin permeability was increased by the removal of SC, whereas after removing the full epidermis, the magnitude of skin permeation enhanced by 1–2 orders of magnitude. The results manifested that skin permeability can be altered with different magnitudes by the influence of different skin layers [21]. The researchers also investigated that the permeation of active molecules through the SC is more in the diseased skin (e.g., psoriasis, dermatitis, and fungal infection) compared to the intact skin. The challenges in the penetration of the drugs across the skin are not only derived of the skin structure, but also depend on the nature of the topical delivery vehicle. One such factor in the resistance of permeability is the exogenous substances [22]. The characteristics of drugs/substances play an essential role in topical delivery. Although most of the substances have different physicochemical properties such as poor aqueous solubility, high permeability, and different molecular structures, which make them rigid to cross skin membrane, to penetrate the skin layer effectively, the drug should possess log partition coefficient value (log P) between 1 and 4 and a molecular weight less than 500 Da [23]. Despite all the above challenges, the permeation of drugs across the skin can be enhanced by various chemical and physical techniques such as radiofrequency, iontophoresis, electroporation, microneedles, magnetophoresis, and ultrasound. These techniques are very advantageous in topical delivery, but their utilization is limited due to their toxicity and lack of feasibility. However, the delivery of peptides, proteins, nucleotide fragments, vaccines, or cancer therapeutics does not still fit in the criteria of the attractive prospects of topical delivery via different techniques and strategies [24]. These challenges or drawbacks are the significant factors for the implementation of nanodelivery in the topical application, which could also expand the range of drugs available for the topical administration [25].

3.4 Role of Nanocarriers in Topical Delivery

As discussed above, conventional topical formulations have numerous side effects and limitations related to efficacy, safety, and patient compliance. The skin is a comprehensive and easy route for drug application, but it is not evident that drug is reaching its site of action. To overcome these side effects, the researchers' designed novel drug delivery systems without reducing the efficacy of therapeutic agents. The therapeutic strategies for the management of skin disease have also been available due to the new delivery of a drug [26, 27]. The nanocarriers provide satisfactory pharmacokinetic data such as low absorption of the drug as compared to conventional formulations. In general, the use of nanocarriers offers several advantages over

other drug delivery systems. These are used to enhance the stability of drugs by chemical or physical means, increase the solubility of hydrophobic drugs, deliver higher concentrations of drugs to the target site due to and enhanced permeation and retention (EPR) effect, and offers sustained and controlled release of the drug. The nanocarriers are modified with cell-specific ligands and anticipate the targeted treatment of drugs. Nanocarriers enhance the permeation of therapeutic agents through the SC by accumulating in the hair follicles and provide drug release into the deeper skin layers. There are various nanoparticles for topical delivery, such as lipid-based nanocarriers, polymeric nanoparticles, emulsified nanocarriers, dendrimers, and organic nanoparticles [28]. When the nanocarriers are formulated with the implementation of DoE, the above applications become more dominant, which have been discussed further in this chapter.

3.5 Applications of DoE in the Development of Topical Drug Delivery Systems

3.5.1 Lipid-Based Systems

3.5.1.1 Liposomes

Liposomes are the lipid-based vesicular drug delivery systems made up of one or more lipid bilayers. They are biocompatible and biodegradable and prepared by natural phospholipids with mixed lipid chains. They can entrap hydrophilic, lipophilic, and amphiphilic drugs. The drug can be either intercalated into a lipid bilayer or encapsulated in the aqueous phase. The physicochemical properties of the drug and lipid composition decide the location of drugs in liposomes [29]. The topical administration of drugs using liposomal formulation is a promising area for the concern of skin diseases. At the International Pharmaceutical Federation Congress (1979), the first report enlightened the evidence of altered drug deposition during the topical delivery of drugs via liposomal formulation was presented [30]. This report provided the base for further research articles indicating that entrapment of drugs into liposomes enhanced the skin deposition of drugs and reduced its absorption into the blood. The liposomes might reduce the percutaneous absorption of the drug by increased local action. These studies claim that liposomes can be useful for drug delivery by topical route [31]. Therefore, it is necessary to prepare the liposomes with optimized liposomal composition, high-throughput production, and better in vivo kinetics. QbD implementation in liposomal design and production can be suitable for optimizing elements, identifying suitable composition, and high screening methodologies. The various type of designs is utilized for the same [32]. All lipid-based nanocarriers with their DoE are enlisted in Table 3.3. The first optimization studies for liposomes were reported in 1988 by the well-known researcher Gregoriadis [58].

Singh et al. in 2005 reported that the optimization of formulation with traditional approaches is unpredictable, uneconomical, invariable, and time-consuming. Therefore, the use of systemic optimization techniques with response surface methodology

Table 3.3 List of experimental designs used in lipid-based nanocarriers

Drug	Nanocarriers	Lipids/surfactant	Experimental design	References
Nimesulide	Liposomes	Phospholipon 90G	Factorial design	Singh et al. [33]
Benzocaine	Liposomes	Phosphatidylcholine	D-optimal design	Mura et al. [34]
5-fluorouracil	Liposomes	phosphatidylcholine	Full factorial design	Hussain et al. [35]
Tretinoin	Liposomes	Phospholipon 85G	Full factorial design	Bavarsad et al. [36]
CoQ10 and PTA	Liposomes	Soy phosphatidylcholine	Central composite design	Çelik et al. [37]
Papain	Liposomes	Soy phosphatidylcholine	Box–Behnken design	Chen et al. [38]
Lornoxicam	Liposomes	Soy lecithin	Central composite design	Joseph et al. [39]
Acetazolamide	Liposomes	Soy phosphatidylcholine	D-optimal design	Naguib et al. [40]
Diacerein	Elastosomes	Span 60	Full factorial design	Aziz et al. [41]
Sodium stibogluconate	Liposomes	Phospholipon 90G	Box–Behnken design	Dar et al.
Insulin	Liposomes	Phosphatidylcholine	Fractional factorial design	Dawoud et al. (2018)
Clotrimazole	Ethosomes	Soy lecithin	Factorial design	Akhtar and Pathak [42]
Psoralen	Ethosomes	Soy lecithin	Factorial design	Pathak and Kumari [43]
Tramadol	Ethosomes	Phospholipon 90G	Factorial design	Ahmed et al. [44]
Triamcinolone	Ethosomes	Soy lecithin	Box–Behnken design	Akhtar et al. [45]
Piroxicam	Proniosomes	Span 60	Box–Behnken design	Solanki et al. [46]
Methotrexate	Niosomes	Span 60	Box–Behnken design	Abdelbary and Aboughaly [47]
Diacerein	Niosomes	Tween 40 and Tween 60	Box–Behnken design	El-Say et al. [48]
Coenzyme Q10	Proniosomes	Span 85	*I*-optimal mixture design	Yadav et al. [49]
Acyclovir	Niosomes	Tween 60 and Span 60	Full factorial design	Jacob et al. [50]
Diacerein	Niosomes	Span 60	Box–Behnken design	Moghddam et al. [51]
Methotrexate	Niosomes	Span 20 and Tween 40	Box–Behnken design	Zidan et al. [52]

(continued)

Table 3.3 (continued)

Drug	Nanocarriers	Lipids/surfactant	Experimental design	References
Halobetasol propionate	SLNs	Tween 80	Full factorial design	Bikkad et al. [53]
Terbinafine Hcl	SLNs	Compritol 888 ATO	Full factorial design	Vaghasiya et al. [54]
Isotretinoin	SLNs	Phospholipon 90G	Face-centered cubic design	Raza et al. [55]
Auraptene	SLNs	Glyceryl palmitostearate	Central composite design	Daneshmand et al. [56]
Fluocinolone acetonide	NLCs	Compritol 888 ATO	Box–Behnken design	Pradhan et al. [57]

(RSM) can overcome these side effects and help choose the best formulation. The researchers employed full factorial design with two independent variables at three levels, and the response variables selected were percent drug diffused, percent drug entrapment (EE%), and percent drug leakage for the optimization of the liposomal formulation. The other process variables were kept constant throughout the study. The Design-Expert software was selected, and a response surface plot was formed to fit the second-order polynomial equation. The results concluded that the full factorial design was successful in optimizing liposomal formulation, and liposomes showed high potential carriers for the topical delivery of nimesulide. The linearity with negligible deviation was shown between the observed and predicted values. The prophecy of the best liposomal formulation can be achieved using optimization methods [33]. Mura et al. [34] implemented a D-optimal design for the development of benzocaine liposomes. The primary purpose of the design implementation was to identify the essential factors influencing the response. Afterward, Doehlert design was applied for the response surface plot of the preliminary phase factors. Then, ANOVA was used for the validation of the regression model. Then, the formulation factors and their best level for response optimization were identified by the graphical analysis of the effects. The results suggested that benzocaine's permeability to the skin layer was increased because of the formulation optimization. The in vivo studies of optimized formulation indicated the enhancement of the benzocaine therapeutic efficacy compared to the previous best benzocaine liposomal formulation. This confirmed the relationship between the intensity of anesthetic effect and the in vitro drug permeation rate of benzocaine and demonstrating the actual effectiveness of the proposed approach for the liposomal formulation optimization [34].

Li et al. [59] prepared daptomycin loaded flexible nanoliposomes for topical skin therapy. They applied the Design-Expert version 8.1 to design the experimental groups and analyze the results. The results depicted that the optimized nanoliposomes exhibited narrow particle size distribution, high stability, and increase drug loading capacity. The in vitro skin permeability data showed a high diffusion capacity of the daptomycin into the skin compared with the naive drug.

Further, the optimized nanoliposomes indicated that the drug could rapidly achieve high-level concentration in the skin and underlying tissues. Hence, they may serve the potential clinical applications of daptomycin using a topical delivery approach to current intravenous delivery [59]. Hussain et al., in 2015, designed 5-fluorouracil loaded elastic liposomes for the management of skin cancer. The elastic liposomes were optimized by employing full factorial experimental design using Design-Expert software. A total of nine liposomal formulations were prepared using dependable variables against independent variables. The study results indicated that the optimized 5-fluorouracil loaded elastic liposomes had desired quality and pharmacokinetics compared to the plain drug. The liposomes manifested optimum permeation ability and overcame therapeutic limitations for the drug's topical delivery, which was absent in the conventional dosage form [35].

Bavarsad et al. in 2016 reported penetration-enhancer vesicles (PEV) for topical delivery of tretinoin using Transcutol, and a full factorial design was employed for the formulation optimization. The three-dimensional response surface plot stated that by increasing the phospholipid concentration up to 20%, the drug release was decreased in 15 min. The optimized PEV exhibited high incorporation efficiency and enhanced skin drug penetration compared to the conventional cream formulation due to the solubilizing properties of the Transcutol. The optimum PEV showed less hyperkeratosis without hyperplasia, high patient compliance, and caused mild adverse effects such as peeling, erythema, and burning in comparison to the conventional cream formulation [36]. Çelik et al. [37] developed coenzyme Q10 (CoQ10) and D-panthenyl triacetate (PTA)-loaded liposomes for topical delivery. For this purpose, a central composite design was used with three factors studied at five different levels. The experimental design aided in the preparation of CoQ10 and PTA liposomes successfully for the first time, and the results showed enhancement in EE% and drug loading capacity. Hence, the report concluded that the encapsulation of these two compounds could be a promising way for the effective delivery of both compounds simultaneously [37]. Chen et al. [38] prepared novel elastic liposomes for skin delivery of papain by implementing three-level three-variable Box–Behnken Design. The Design-Expert software was used for data prediction. The results showed an increment in EE%, high drug deposition in skin, and higher cumulative amounts and fluxes of optimized papain loaded liposomes compared to the papain solution. The topical delivery of liposomes could inhibit the hypertrophic scar in rabbit ears, including decreased collagen fibers, decreased microvessels, and signaling pathway regulation. The optimized elastic liposomes were found to be effective in topical scar treatment [38].

Naguib et al. [40] prepared acetazolamide loaded penetration-enhancing hybridized vesicles for the topical delivery of glaucoma. The formulation was optimized by applying a highly accurate and robust D-optimal mixture statistical design. The prepared and optimized liposomes are evident in high physicochemical and biological properties, large particle size, high drug release, increased EE%, excellent stability, and high drug bioavailability compared to conventional liposomes. The optimized acetazolamide loaded liposomes also showed lesser side effects as compared to the conventional acetazolamide loaded liposomes [40].

Joseph et al. [39] developed lornoxicam loaded liposomes for topical delivery. The central composite design was implemented for formulation optimization. The two variables (phospholipid and cholesterol content) and two responses (EE% and in vitro drug release) and the Design-Expert software were selected for the optimization. The response surface plot was formed to evaluate the independent variables' response to the dependent variables (Fig. 3.3). The plot showed that the EE% was the same at the medium content of phospholipid and cholesterol, but as their concentration decreases, the EE% was also decreased and vice versa. The highest and lowest levels increased the drug release, while at other levels, the drug release was decreased. Thus, the study concluded that there was an enhancement in the half-life of the optimized formulation. The optimized liposomes had stability limitation for 3–4 weeks and large particle size compared to the naïve drug. The ex vivo permeation studies and in vitro skin toxicity studies showed that the low doses could be efficient for the topical delivery of the optimized formulation, which can enhance the efficacy of the drug with fewer side effects [39].

Aziz et al., in 2018, developed diacerein loaded elastosomes for topical delivery to treat osteoarthritis. The full factorial design was implemented for the optimization of elastosomes, and the results showed high skin permeation and retention capacity of diacerein compared to the plain drug. Thus, it is concluded that the topical delivery of the drug can reduce its adverse effects on oral administration [41].

Dar et al., in 2018, developed sodium stibogluconate loaded nano-deformable liposomes for topical treatment of leishmaniasis. A Box–Behnken design was selected with three levels and three factors to optimize the formulation using RSM. The study's result stated that the surface area of the optimized formulation decreased with an increase in the vesicle size of the liposomes. The optimized topical liposomes reduced the side effects, which were seen in the intralesional or intravenous delivery of the sodium stibogluconate [60]. Dawoud et al. [61] prepared insulin chitosan mucoadhesive liposomal gel for wound healing. The Box–Behnken design was implemented with three critical process parameters for liposomes optimization. The optimization of liposomes availed high stability and sustained release profile to the insulin for the wound healing. Chen et al. in 2020 prepared zedoary turmeric oil and tretinoin encapsulated liposomal gel for topical delivery of psoriasis using orthogonal design. The results suggested that the optimized formulation allowed high EE%, solubility, and stability. The optimized liposomal gel increased the tretinoin content in the skin and reduced administration frequency compared to the naïve drug [61].

3.5.1.2 Ethosomes

Touitou and his co-workers have discovered a new type of vesicular system named ethosomes. Ethosomes differ from the liposomes in their structure, mechanism of action, and mode of application [62]. Ethosomes are defined as the non-invasive vesicular drug delivery systems and carry drugs deep into the skin layers or systemic circulation. These are malleable, soft lipid vesicles containing phospholipids, alcohol (in high concentration), and water. Ethosomes may vary in the size range from 10 nm to few microns. Ethosomes are well-established nanocarriers for local

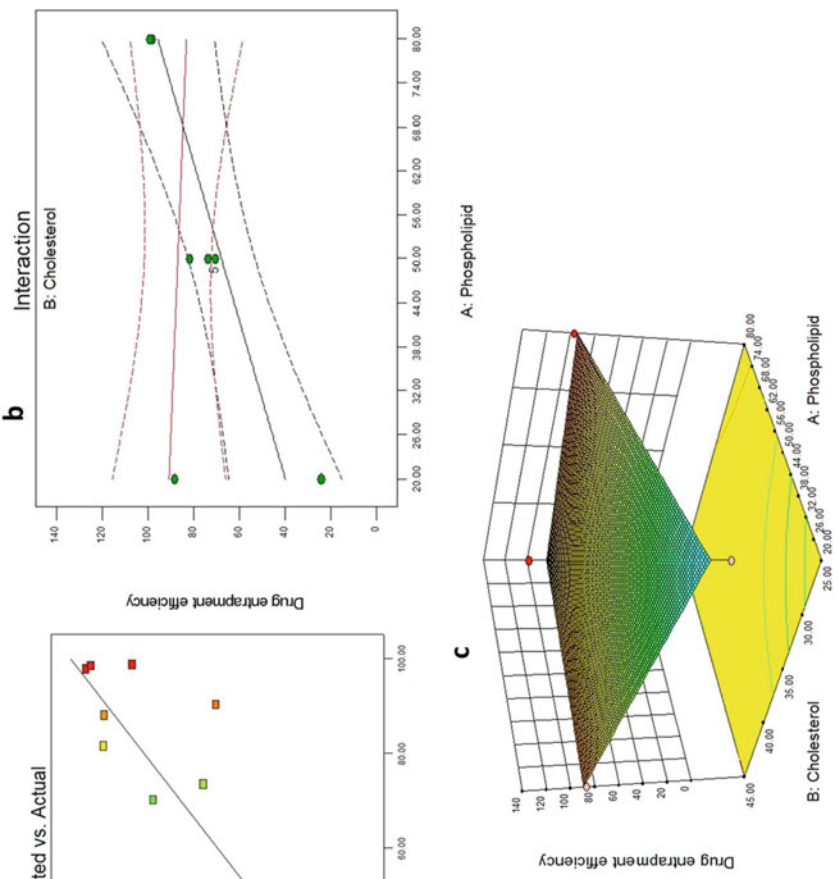

Fig. 3.3 (a) Relationship between the predicted and actual values, (b) interaction between the variables and responses, (c) 3D surface plots for variables of (**a**) entrapment values (**b**) drug release. Figure source: Joseph et al. [39]

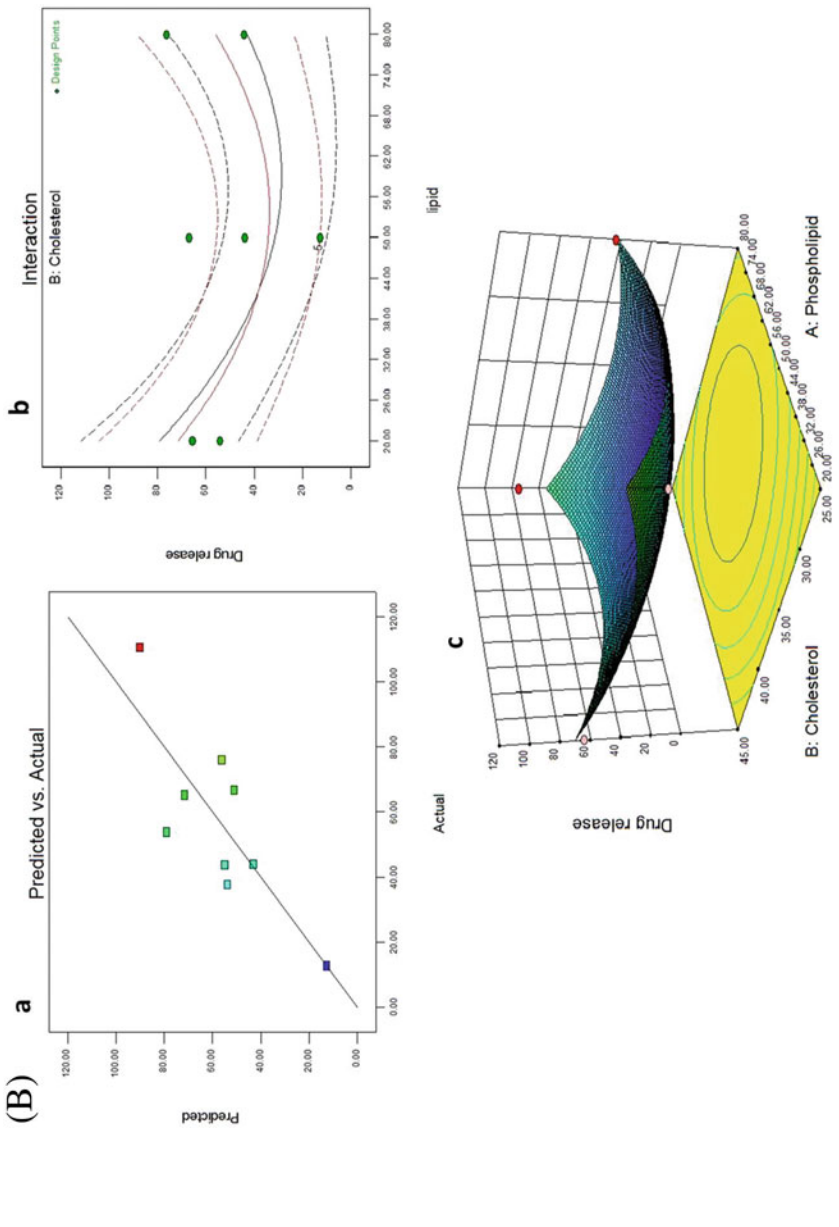

Fig. 3.3 (continued)

delivery of drugs and can penetrate the skin layers with high transdermal flux. The high concentration of ethanol (20–50%) in the ethosomes is the reason for its high permeation ability because the ethanol can disturb the organization of skin lipid bilayer. The ethosomes also possess the same stability as conventional vesicles [63]. Akhtar et al., in 2012, designed Cavamax W7 composite ethosomal gel for topical delivery of clotrimazole using factorial design. The design was validated by the Design-Expert software version 8.0.5. The response surface plot showed that EE % was increased with an increase in the concentration of soya lecithin and Cavamax W7. The optimized ethosomes were found to be stable, offered better efficiency, and higher steady-state flux than the reference ethosomes. The in vivo study of optimized ethosomes indicated the high permeability of the vesicles into the skin layers. Thus, the Cavamax W7 ethosomes were discovered as the superior nanocarrier systems for the clotrimazole topical delivery [42].

Pathak and Kumari [43] prepared Cavamax W7 composite ethosomal gel of psoralen by implementing the factorial design, and the design was validated by the Design-Expert software version 8.0.5. The response surface plot found that EE% was increased at the highest level of soy lecithin and Cavamax W7. The results concluded that the permeation of optimized formulation was more than that of the reference ethosomes, even without using a permeation enhancer. The optimized psoralen ethosomes had the potential to minimize the adverse effects associated with photosensitivity of psoralen and were the efficient carrier for the topical delivery of the drug [43]. Ahmed et al. developed tramadol loaded nanoethosomal transgel for the topical delivery. The three-factor three-level Box–Behnken design was used for formulation optimization. It was observed that the vesicle size had a direct positive relationship with the phospholipid concentration, and the permeability of the system was increased with a decrease in particle size. Therefore, the optimized ethosomal gel possessed high analgesic efficacy, enhanced skin permeability and bioavailability, improved patient compliance, and avoided frequent dosing compared to the oral marketed formulation [44].

Garg et al. [64] prepared methoxsalen loaded ethosomes-based hydrogel for the treatment of vitiligo. The two-factor, three-level face-centered design was implemented for the optimization of ethosomes. The response surface plot showed that at a higher level of ethanol and lower phospholipid levels, the vesicles' size decreased. Hence, the QbD approach in the optimization of formulation provided detailed knowledge about the ethosomal gel quality and the formulation attributes. Overall, the optimized formulation imparted better therapeutic efficiency and higher flux in comparison to the commercial product and hydroethanolic formulation [64]. Akhtar et al. [45] designed nanoethosomal glycolic vesicles of triamcinolone acetonide to manage atopic dermatitis using a three-level Box–Behnken experimental design. The design was applied to investigate the sensitivity of the responses against the variations in the experimental variables. The research group concluded that the optimized formulation not only provided an enhancement in the drug entrapment, but also offered better stability, higher drug release flux, and uniform, and more profound permeability compared to the reference ethosomes. The carbomer gel of the optimized ethosomal glycolic vesicles was found to be clear

and transparent at a pH range of skin. Overall, the nanoethosomal vesicles were suitable for the topical delivery of triamcinolone acetonide [45].

3.5.1.3 Niosomes

Niosomes are the vesicular systems composed of non-ionic surfactants and are multilamellar. They do not contain phospholipids, making them different from the liposomes [65]. Niosomes can be used as the drug delivery vehicles to deliver the drugs to the lung, ocular system, skin, brain, liver, tumor organ, etc. They are very successful in the topical delivery of drugs as they increase the SC properties by enhancing their smoothness or decreasing transepidermal water loss [66]. They have various topical delivery applications such as drug stability, sustained drug release, high skin penetration, and the ability to encapsulate both hydrophilic and lipophilic drugs [67]. DoE implementation in the niosomes can avoid excessive effort, time, and materials for the formulation development and provide significance of different variables.

Solanki et al. formulated piroxicam loaded proniosomes for the topical delivery employing the three-factor three-level Box–Behnken statistical design. The response surface plots depicted that on decreasing the concentration of cholesterol, EE% of the niosomes was also decreased. The optimized niosomes were found to be less leaky and small particle size. The study concluded that the optimized proniosomes exhibited all the desired properties for topical delivery [46]. Abdelbery et al., in 2015, prepared methotrexate loaded niosomes for the treatment of psoriasis. A three-level three-factor Box–Behnken design was applied for the formulation optimization, and the independent variables selected were the methotrexate concentration in hydration medium, the total weight of niosomal components, and surfactant. The dependent variables selected were EE% and particle size. The EE% was reported to decrease along with an increase in hydration volume. The reason for increment in particle size was the excessive amount of drugs inside the niosomes. The in vivo study displayed that the niosomes were non-irritant after applied topically. Thus, it is concluded that the methotrexate loaded niosomes had the therapeutic efficacy to treat psoriasis, and preclinical applications on animal models were necessary to establish the safety of the niosomes [47].

El-say et al. developed niosomal gel for the topical delivery of diacerein using the Box–Behnken design model. The three independent variables selected were the percentage of stearylamine, hydrophilic–lipophilic balance, and concentration of surfactant and sonication time. The dependent variables selected were the EE%, particle size, and percentage of diacerein release. The stat graphics model with two ANOVA was used for the statistical analysis. It was found that the increase in sonication time can reduce the vesicle size, and a decrease in both percentages of stearylamine and surfactant might decrease the EE%. Also, the optimized formulation resulted in a suitable topical application and exhibited high pharmacodynamic and anti-inflammatory activity compared to the conventional gel of diacerein [48]. Jacob et al. developed acyclovir loaded niosomal gel for the topical application, and 3^2 full factorial design was implemented for the niosomes optimization. From the contour response plot, it was observed that there is no significant difference in the

particle size between various formulations. The vesicle size and EE% were changed while changing the concentration of surfactant and cholesterol. The study's result stated that the formulation manifested high bioavailability and sustained release of drugs compared to conventional dosage form [50].

Moghddam et al. prepared diacerein loaded niosomes for the treatment of psoriasis by applying 3^3 Box–Behnken design. The optimized niosomal formulation exhibited high EE%, reasonable particle size, and homogenous surface morphology. The developed formulation was found to be the better carrier for diacerein delivery [51]. Arafa et al. in 2017 employed Minitab software for the optimization of pregabalin loaded niosomes. The data exhibited that EE% of the formulation had a direct relationship with the cholesterol ratio. However, the in vitro release study showed that the drug delivery followed Higuchi and Fickian release model. The ex vivo permeation of niosomes was increased as compared to the hydrogels. Therefore, it is investigated that the hydrogels loaded niosomes are the only capable carrier for the controlled release of pregabalin [68].

Al-Mahallawi et al. [69] designed methotrexate loaded ultra-permeable niosomes by implementing a 3^3 Box–Behnken experimental design with Design-Expert software to evaluate the design. The independent variables selected were the Cremophor RH 40 percentage, stabilizer percentage, and sonication time, and the dependent variables evaluated were the EE%, vesicle size, and polydispersity index. The response surface plot depicted that the sonication time and stabilizer percentage had a negative impact on the EE%, and increasing stabilizer percentage might enhance the vesicle size of the niosomes. Thus, the results concluded that the optimized formulation showed better stability profile and in vivo data evaluated the high amount of drug deposition in the rat skin compared to the methotrexate solution [69].

3.5.1.4 Solid Lipid Nanocarriers (SLNs)

SLNs were first discovered in 1991; they are the lipid nanocarriers containing the properties of both lipid-based systems and the polymeric nanoparticle systems. SLNs encapsulate drugs at the lipid matrix composed of a solid lipid or mixture of solid lipids. They have various advantages such as drug protection against enzymes or degradation, controlled drug release, biocompatibility. SLNs are better nanocarriers for skin delivery as they enhanced the permeation of encapsulated drug [70].

Bikkad et al. developed halobetasol propionate loaded SLNs for the topical delivery using 3^2 full factorial design. Amount of Tween 80 and the content of glycerol monostearate were selected as the independent factors, and particle size and EE% were the dependent factors. The Design-Expert software was used for data analysis, and the response surface plot showed that the particle size was increased with decrease in the ratio of Tween 80: glyceryl monostearate and EE% increased with increase in the ratio of Tween 80 and glyceryl monostearate. The optimized formulation exhibited controlled drug release, less skin irritation, better stability, lower side effects, and high therapeutic efficacy compared with the conventional gel [53]. Raza et al. in 2013 developed isotretinoin loaded SLNs for the treatment of skin acne. They employed a face-centered cubic design for the optimization of response

variables. From the response surface plot, it resulted that the phospholipid had a positive relationship with the particle size and zeta potential. The optimized SLNs were highly stable, with a low aggregation tendency of the dispersed phase. The SLNs could be the better option for the isotretinoin topical delivery [55].

Daneshmand et al. [56] prepared SLNs for the auraptene delivery as an anti-inflammatory agent. A central composite design with a five-factor three responses was used for the analysis of variables. The optimized formulation exhibited high EE % and low skin irritancy. The in vitro data provided controlled release of the drug as compared to the conventional cream. The optimized SLNs revealed high anti-inflammatory efficacy and maximum local effect of the drug at the skin with no sensitization [56].

3.5.2 Emulsion Based Systems

3.5.2.1 Microemulsion

Hoar and Schulman, in 1943, prepared a single-phase, uniform, non-conducting, transparent solution called microemulsions. Microemulsions are thermodynamically stable, transparent mixtures of oil, water, and surfactant. These have various advantages such as high stability, better bioavailability, deliver both hydrophilic and lipophilic drugs. Microemulsions are different from other nanocarriers in such a way that they are amphiphilic and have multi-drug delivery [71]. Table 3.4 shows the list of designs used in these systems. Microemulsion can enhance drug penetration from the skin, making it a suitable option for the topical delivery of drugs. In

Table 3.4 List of experimental designs used in emulsified systems and polymeric systems

Drug	Nanocarriers	Experimental design	References
Aceclofenac	Microemulsion	D-optimal design	Todosijević et al. [72]
Lidocaine and prilocaine	Microemulsion	D-optimal design	Negi et al. [73]
Clobetasol propionate	Microemulsion	D-optimal design	Patel et al. [74]
Sertaconazole	Microemulsion	Full factorial design	Radwan et al. [75]
Rotigotine	Microemulsion	Central composite design	Wang et al. [76]
Synthetic chalcone	Nanoemulsion	Full factorial design	de Mattos et al. [77]
Kojic monooleate	Nanoemulsion	D-optimal design	Afifah et al. [78]
Metronidazole	Nanoemulsion	D-optimal design	Yu et al. [79]
Chlorpromazine	PNPs	Box–Behnken design	Alvarez-Trabado et al. [80]
Cetrimide	PNPs	Central composite design	Marto et al. [81]
Terconazole	Micelle	Full factorial design	Abd-Elsalam et al. [82]
Simvastatin	Micelle	Full factorial design	Varshosaz et al. [83]
Vinpocetine	Micelle	Orthogonal design	Zhou et al. [84]

recent times, researchers take an interest in the in silico techniques such as DoE for the prediction of microemulsion phase behavior.

Patel et al. designed clobetasol propionate loaded microemulsion gel for the treatment of vitiligo. The D-optimal design was implemented for the optimization of formulation, and the independent variables selected were the amount of mixture of surfactant and co-surfactant (S_{mix}) and oil concentration. The dependent variables selected were the globule size and solubility of the drug. The response surface plot evaluated that the decrease in globule size is directly proportional to the amount of oil proportion. The results concluded that the optimized microemulsion exhibited better skin permeation, high skin retention, and less skin irritation than the marketed formulation. Therefore, the optimized topical microemulsion gel could be a useful option for the treatment of vitiligo [74]. Negi et al. in 2014 developed lidocaine and prilocaine loaded microemulsion hydrogel for topical delivery using D-optimal mixture design. The formulation variables selected were the Smix, water, and oil, and the response variables chosen were skin retention, globule size, percent cumulative permeation, and skin permeation flux. The response analysis was carried out, and the response surface plot (Fig. 3.4) evaluated that with an increase in the water level, the globule size was decreased, and percent skin retention of both the drugs increased. The thermodynamic activity of the drug decreased with an increase in the surfactant concentration and the drug's affinity.

The response surface plot proved that the maximum water content and minimum S_{mix} could enhance the globule size, % skin retention, and permeation flux and percutaneous absorption. The results concluded that the optimized microemulsion hydrogel showed high skin absorption and skin permeation compared to the commercial cream [73]. Radwan et al. prepared sertaconazole loaded microemulsion gel as an antifungal agent. The formulation was optimized, employing factorial design and Design-Expert software. The results indicated that the optimized microemulsions impart a high skin retention activity and better antimycotic activity as compared to the marketed formulation. Moreover, optimized microemulsion did not show any histopathological changes on rat skin as proved to be non-irritant as compared to marketed cream. Thus, the dermal delivery could be achieved by the sertaconazole loaded optimized microemulsion [75].

3.5.2.2 Nanoemulsions

Nanoemulsions are the emulsified carriers composed of oil, surfactant, and water. Their droplet size is smaller than the microemulsion. They are usually opaque to blue-white [71]. Nanoemulsions have various advantages compared to a conventional emulsion, such as increased interfacial area, better stability, high solubility, and improved bioavailability.

Yu et al. applied a D-optimal design for the optimization of metronidazole loaded nanoemulsions with anti-rosacea properties. The droplet size, % skin retention, and metronidazole content were selected as the independent variables, and the oil content, water, and S_{mix} were used as the dependent variables. The response surface plot evaluated that at the moderate levels, optimized nanoemulsion showed less droplet size and better skin retention properties and high therapeutic efficacy

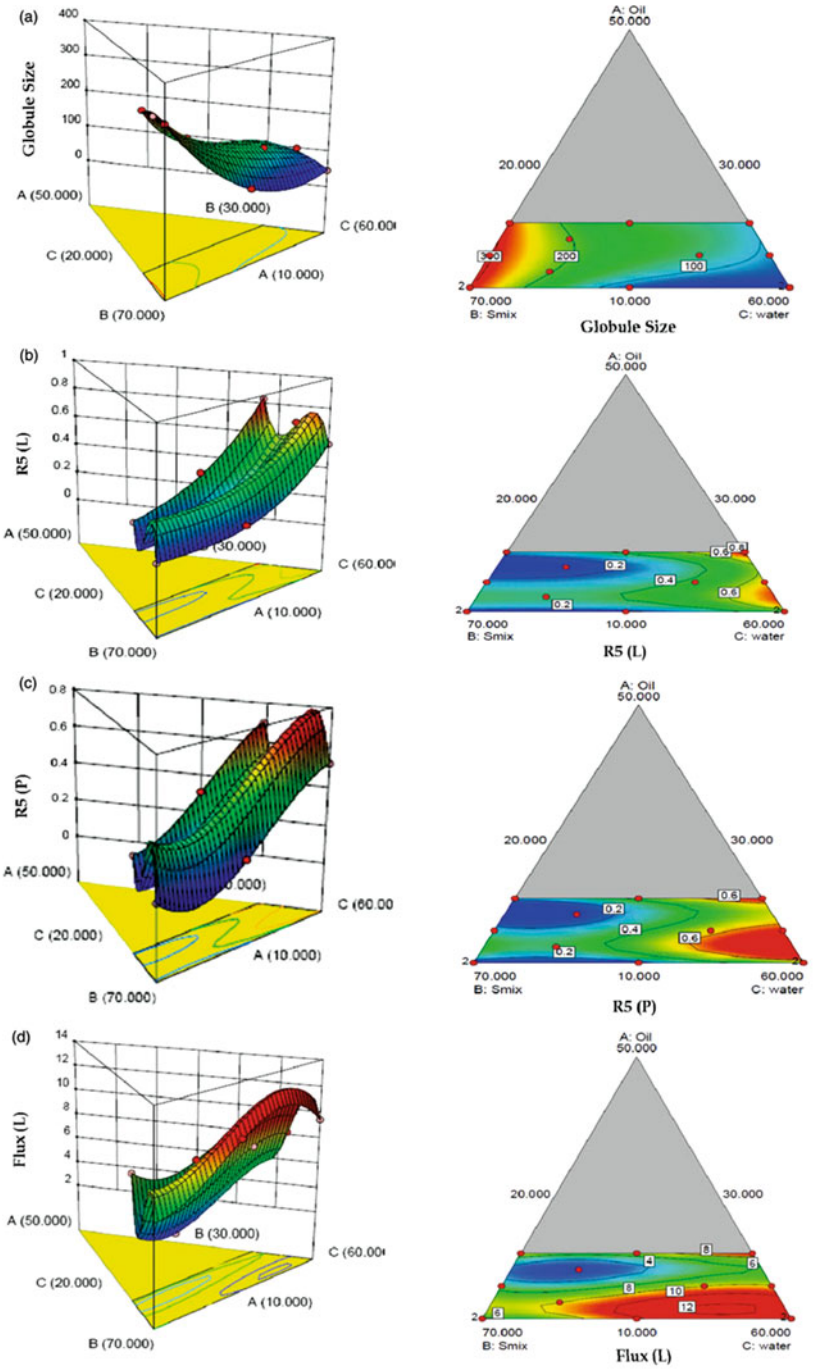

Fig. 3.4 3D response surface plots and 2D contour plots of (**a**) globule size of microemulsion, (**b**) skin retention of lidocaine, (**c**) skin retention of prilocaine, (**d**) permeation flux of lidocaine, (**e**) permeation flux of prilocaine, (**f**) skin permeation of lidocaine, (**g**) skin permeation of prilocaine (adapted from [73])

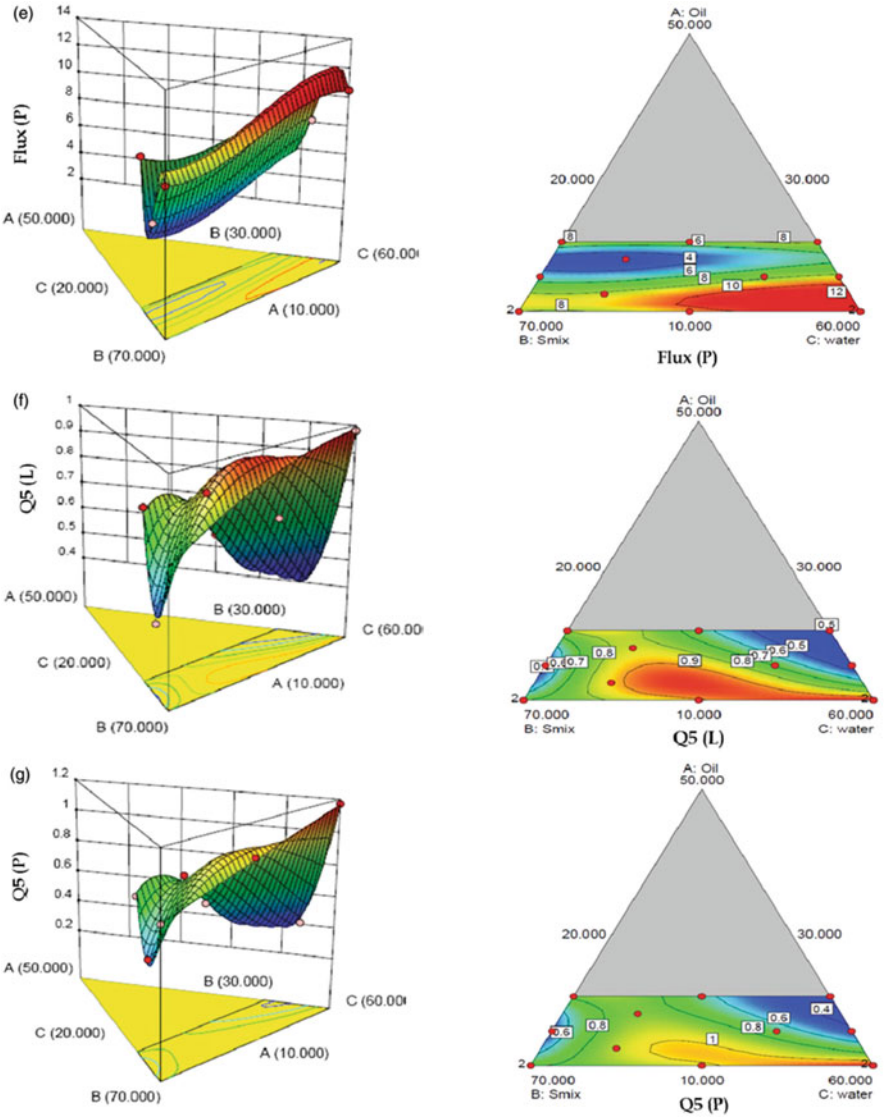

Fig. 3.4 (continued)

[79]. Afifah et al., in 2018, prepared kojic monooleate loaded nanoemulsions for the treatment of hyperpigmentation in the skin. A five-factor one-response D-optimal design was used for the evaluation of nanoemulsions. The three-dimensional surface plot stated that the droplet size was decreased with increasing xanthan gum and

surfactant content. The in vitro data of the optimized formulation suggested that the microemulsion was less toxic and safe for the cosmeceutical applications [78].

3.5.3 Polymer-Based Systems

3.5.3.1 Polymeric Nanoparticles (PNPs)

PNPs are biomaterials with immense potential, simple design, higher biocompatibility and biomimetic properties. In the drug delivery systems, the main characteristics of PNPs are controlled particle size and excellent solubility and elasticity. PNPs are of two types, i.e., nanocapsules and nanospheres. They can reach its target site at the desired time and level [85]. Topical delivery of PNPs can provide protection to drugs from degradation, reduce side effects of toxic drugs via controlled disease, and increase skin penetration [86].

Marto et al., in 2018, developed novel human neutrophil elastase loaded starch nanocapsule implementing the central composite design. The design was used to evaluate the response of independent variables on the dependent variables. The independent variables selected were the amount of drug, Tween 80 content, lipid content, and percentage of surfactant and drug loading, particle size, zeta potential, and EE% are the dependent variables. The MODDE software was used for the data evaluation, and it was found that the enhancement in the content of lipid and Tween 80 led to a decrease in the particle size and increase in the EE%. The result of the study concluded that the optimized nanocapsules exhibited high efficacy and skin permeation activity and showed a promising carrier for topical delivery [81]. Pizarro et al. prepared fluorometholone loaded nanoparticles for inflammatory disorders. The formulation was optimized using three factors (concentration of drug, surfactant, and polymer) and three levels (particle size, zeta potential, polydispersity index, and EE%) central composite design matrix. The response surface plot showed that the particle size increased with an increase in polymer concentration and decreased with an increase in the surfactant concentration. The zeta potential and polydispersity index exhibited that the formulation is monodispersed in nature with very high EE%. The skin permeation and anti-inflammatory studies showed that the optimized formulation had better skin permeability than commercial formulation. However, the optimized fluorometholone NPs were found to be successful for the topical delivery to reach the posterior segment of the eye [87].

3.5.3.2 Micelles

Micelles are polymeric nanocarriers composed of mainly surfactants and polymers. The micelles are formed only, when the surfactant concentration reaches to or above the critical micellar concentration and these nanostructures consist of a solvophobic core and solvophilic shell. The hydrophobic drugs can encapsulate in the core of polymeric micelles, and shell provides stability to the micelles. The micelles shell has the ability to prevent protein adsorption and opsonization. However, micelles have various advantages in topical drug delivery, such as high biocompatibility,

better drug deposition, and high skin permeation [88]. The polymeric micelles have been reported to increase the drug's solubility at the skin site, sustained drug release.

Varshosaz et al. developed chitosan gel containing nanomicelles for the effective topical delivery of simvastatin for wound healing using irregular full factorial design. The independent variables chosen for the optimization were the hydration temperature, polymer/drug ratio, organic solvent and hydration time, and the responses were the EE%, particle size, zeta potential, drug release, and drug loading efficiency. From the response surface plot (Figs. 3.5 and 3.6), the drug release was found to be rapid at first and then followed by sustained release. The hydration temperature affected the particle size. The EE% was increased with an increase in the drug content. Thus, the study concluded that the optimized formulation showed high wound healing ability and could be a promising carrier for topical delivery [83]. Zhou et al. prepared vinpocetine loaded micelles with the implementation of orthogonal design to optimize micelles. They found that the optimized micelles

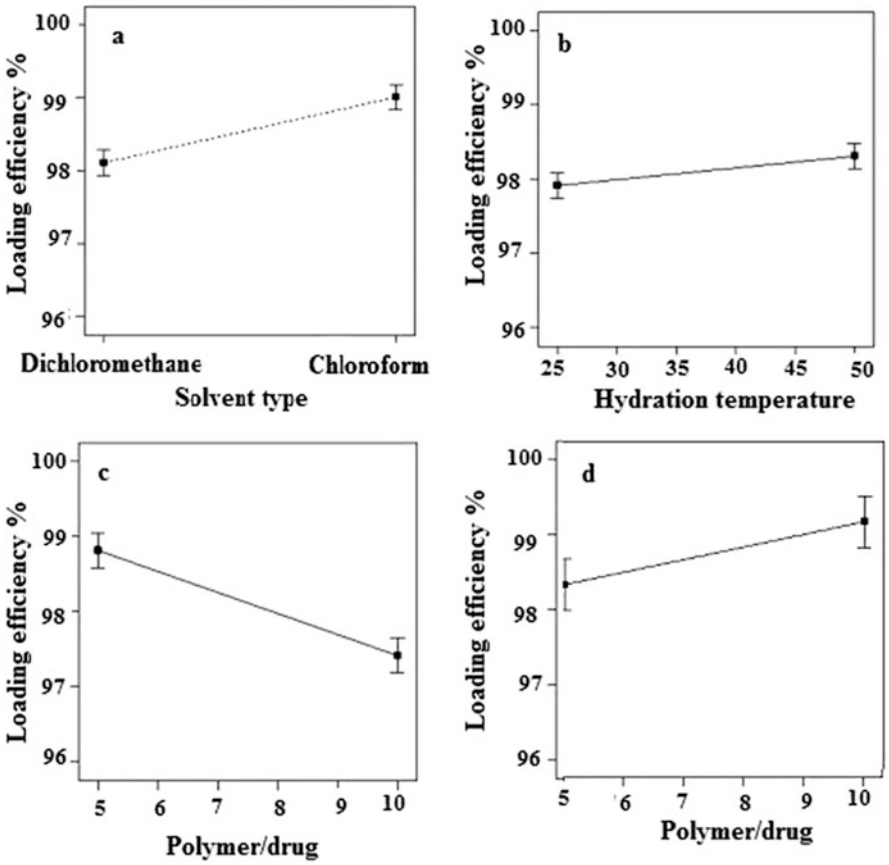

Fig. 3.5 The effects of different studied parameters on loading efficiency of nanomicelles

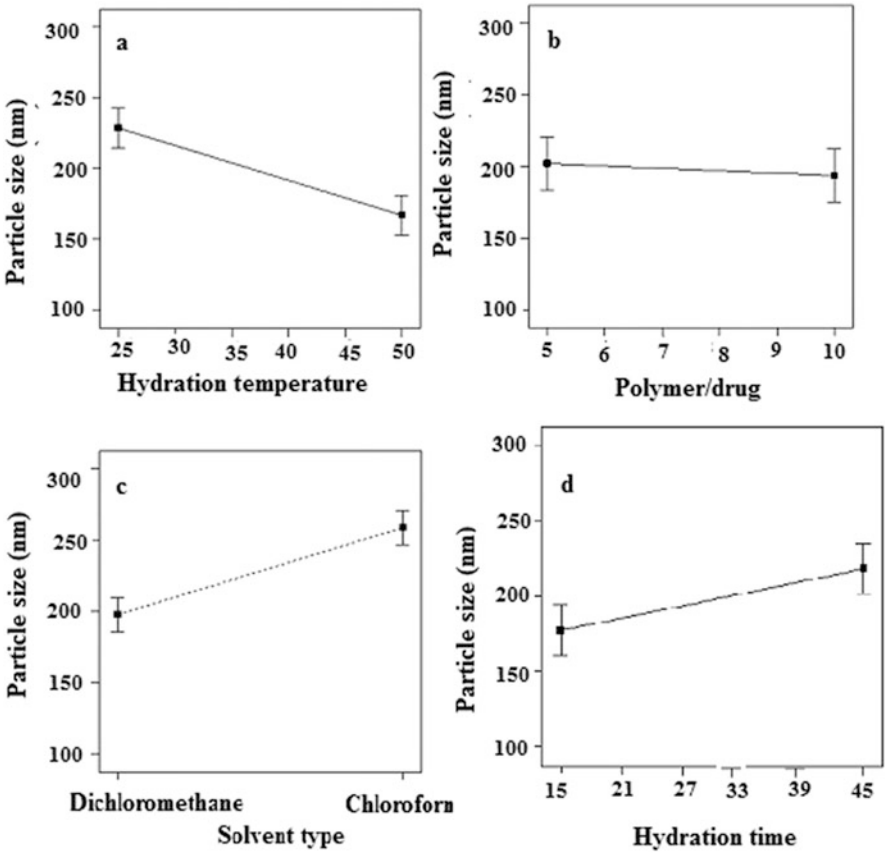

Fig. 3.6 The effects of different studied parameters on the particle size of nanomicelles. Figures source: Varshosaz et al. [83]

exhibited a sustained release profile and prolonged plasma concentration compared to the vinpocetine injection [84].

3.6 Conclusion

The skin is the easiest, non-invasive, and an appealing route for the drug administration, which initiates the research towards the topical drug delivery system. The main drawback of the topical delivery is the SC barrier which resists the entry of bioactive agents. Henceforth, nanosystems such as liposomes, niosomes, SLNs, microemulsions, PNPs become the widely formulated technologies to overcome these side effects and improve the efficacy of drugs at the skin. It requires many efforts to prepare a nanosystem, either raw materials, preparation methods, or characterization techniques. At the duration of the formulation of nanosystems,

various process and formulation parameters are required, and their optimization is crucial for the desired quality of the product for topical delivery. Thus, the DoE is a vital optimization technique for the optimization of nanosystems and ensures desirable product design with a highly potential final topical product.

References

1. Lavik EB, Kuppermann BD, Humayun MS (2012) Drug delivery, 5th edn. Elsevier, London. https://doi.org/10.1016/B978-1-4557-0737-9.00038-2
2. Petrilli R, Lopez RFV (2018) Physical methods for topical skin drug delivery: concepts and applications. Braz J Pharm Sci 54:1–19. https://doi.org/10.1590/s2175-97902018000001008
3. Touitou E (2002) Drug delivery across the skin. Expert Opin Biol Ther 2:723–733. https://doi.org/10.1517/14712598.2.7.723
4. Lee H, Song C, Baik S, Kim D, Hyeon T, Kim DH (2018) Device-assisted transdermal drug delivery. Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2017.08.009
5. Simões A, Veiga F, Figueiras A, Vitorino C (2018) A practical framework for implementing quality by design to the development of topical drug products: Nanosystem-based dosage forms. Int J Pharm 548:385–399. https://doi.org/10.1016/j.ijpharm.2018.06.052
6. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, Wurm EMT, Yoong C, Robertson TA, Soyer HP, Roberts MS (2011) Nanoparticles and microparticles for skin drug delivery Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev 63:470–491. https://doi.org/10.1016/j.addr.2011.01.012
7. Katare O, Raza K, Singh B, Dogra S (2010) Novel drug delivery systems in topical treatment of psoriasis: rigors and vigors. Indian J Dermatol Venereol Leprol 76:612–621. https://doi.org/10.4103/0378-6323.72451
8. Marto J, Gouveia LF, Gonçalves LM, Gaspar DP, Pinto P, Carvalho FA, Oliveira E, Ribeiro HM, Almeida AJ (2016) A quality by design (QbD) approach on starch-based nanocapsules: a promising platform for topical drug delivery. Colloids Surf B Biointerfaces 143:177–185. https://doi.org/10.1016/j.colsurfb.2016.03.039
9. Kraft JC, Freeling JP, Wang Z, Ho RJY (2014) Emerging research and clinical development trends of liposome and lipid nanoparticle drug delivery systems. J Pharm Sci. https://doi.org/10.1002/jps.23773
10. Li J, Qiao Y, Wu Z (2017) Nanosystem trends in drug delivery using quality-by-design concept. J Control Release. https://doi.org/10.1016/j.jconrel.2017.04.019
11. Rathore AS (2009) Roadmap for implementation of quality by design (QbD) for biotechnology products. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2009.06.006
12. Pramod K, Tahir M, Charoo N, Ansari S, Ali J (2016) Pharmaceutical product development: a quality by design approach. Int J Pharm Investig 6:129. https://doi.org/10.4103/2230-973x.187350
13. Sharma G, Thakur K, Raza K, Singh B, Katare OP (2017) Nanostructured lipid carriers: a new paradigm in topical delivery for dermal and transdermal applications. Crit Rev Ther Drug Carrier Syst 34:355–386. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2017019047
14. Robert MEM, Kalia YN (2006) New developments in topical antifungal therapy. Am J Drug Deliv. https://doi.org/10.2165/00137696-200604040-00006
15. Maibach HI (2000) Introduction. In: Dermatopharmacology of topical preparations. Springer, Berlin, pp 1–2. https://doi.org/10.1007/978-3-642-57145-9_1
16. Gupta M, Agrawal U, Vyas SP (2012) Nanocarrier-based topical drug delivery for the treatment of skin diseases. Expert Opin Drug Deliv 9:783–804. https://doi.org/10.1517/17425247.2012.686490

17. Boer M, Duchnik E, Maleszka R, Marchlewicz M (2016) Structural and biophysical characteristics of human skin in maintaining proper epidermal barrier function. Postep Dermatol Alergol 33(1):1–5. https://doi.org/10.5114/pdia.2015.48037
18. Ruela ALM, Perissinato AG, de Lino ME, Mudrik PS, Pereira GR (2016) Evaluation of skin absorption of drugs from topical and transdermal formulations. Braz J Pharm Sci 52:527–544. https://doi.org/10.1590/s1984-82502016000300018
19. Fluhr J, Lademann J (2009) Editorial: Emphasis on skin physiology. Skin Pharmacol Physiol. https://doi.org/10.1159/000235823
20. Lauterbach A, Müller-Goymann CC (2015) Applications and limitations of lipid nanoparticles in dermal and transdermal drug delivery via the follicular route. Eur J Pharm Biopharm 97:152–163. https://doi.org/10.1016/j.ejpb.2015.06.020
21. Luís A, Ruela M, Perissinato AG, Esselin M, Lino DS (2016) Evaluation of skin absorption of drugs from topical and transdermal formulations. Braz J Pharm Sci 52:527–544
22. Singh Malik D, Mital N, Kaur G (2016) Topical drug delivery systems: a patent review. Expert Opin Ther Pat 26:213–228. https://doi.org/10.1517/13543776.2016.1131267
23. Ng KW (2018) Penetration enhancement of topical formulations. Pharmaceutics 10:10–12. https://doi.org/10.3390/pharmaceutics10020051
24. Cevc G, Blume G (1992) Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. BBA-Biomembranes 1104:226–232. https://doi.org/10.1016/0005-2736(92)90154-E
25. Roberts MS, Mohammed Y, Pastore MN, Namjoshi S, Yousef S, Alinaghi A, Haridass IN, Abd E, Leite-Silva VR, Benson HAE, Grice JE (2017) Topical and cutaneous delivery using nanosystems. J Control Release 247:86–105. https://doi.org/10.1016/j.jconrel.2016.12.022
26. Castro GA, Coelho ALLR, Oliveira CA, Mahecha GAB, Oréfice RL, Ferreira LAM (2009) Formation of ion pairing as an alternative to improve encapsulation and stability and to reduce skin irritation of retinoic acid loaded in solid lipid nanoparticles. Int J Pharm 381:77–83. https://doi.org/10.1016/j.ijpharm.2009.07.025
27. Gupta M, Vyas SP (2012) Development, characterization and in vivo assessment of effective lipidic nanoparticles for dermal delivery of fluconazole against cutaneous candidiasis. Chem Phys Lipids 2012:454–461. https://doi.org/10.1016/j.chemphyslip.2012.01.006
28. Goyal R, Macri LK, Kaplan HM, Kohn J (2016) HHS Publ Access 2016:77–92. https://doi.org/10.1016/j.jconrel.2015.10.049.Nanoparticles
29. Bangham AD, Standish MM, Weissmann G (1965) The action of steroids and streptolysin S on the permeability of phospholipid structures to cations. J Mol Biol 13:253–259. https://doi.org/10.1016/S0022-2836(65)80094-8
30. Mezei M, Gulasekharam V (1980) Liposomes - a selective drug delivery system for the topical route of administration I. Lotion dosage form. Life Sci 26:1473–1477. https://doi.org/10.1016/0024-3205(80)90268-4
31. Egbaria K, Weiner N (1990) Liposomes as a topical drug delivery system. Polym Mater Sci Eng Proc ACS Div Polym Mater Sci Eng 63:59–63
32. Wong A (2010) Quantitative modeling of the high-throughput production and in vivo kinetics of (drug-encapsulating) liposomes. PLoS One 5:e0010280. https://doi.org/10.1371/journal.pone.0010280
33. Singh B, Mehta G, Kumar R, Bhatia A, Ahuja N, Katare O (2005) Design, development and optimization of nimesulide-loaded liposomal systems for topical application. Curr Drug Deliv 2:143–153. https://doi.org/10.2174/1567201053585985
34. Mura P, Capasso G, Maestrelli F, Furlanetto S (2008) Optimization of formulation variables of benzocaine liposomes using experimental design. J Liposome Res 18:113–125. https://doi.org/10.1080/08982100802118540
35. Hussain A, Haque MW, Singh SK, Ahmed FJ (2016) Optimized permeation enhancer for topical delivery of 5-fluorouracil-loaded elastic liposome using design expert: part II. Drug Deliv 23:1242–1253. https://doi.org/10.3109/10717544.2015.1124473

36. Bavarsad N, Akhgari A, Seifmanesh S, Salimi A, Rezaie A (2016) Statistical optimization of tretinoin-loaded penetration-enhancer vesicles (PEV) for topical delivery. DARU J Pharm Sci 24:1–12. https://doi.org/10.1186/s40199-016-0142-0
37. Çelik B, Sağıroğlu AA, Özdemir S (2017) Design, optimization and characterization of coenzyme Q10- and D-panthenyl triacetate-loaded liposomes. Int J Nanomedicine 12:4869–4878. https://doi.org/10.2147/IJN.S140835
38. Chen YY, Lu YH, Ma CH, Tao WW, Zhu JJ, Zhang X (2017) A novel elastic liposome for skin delivery of papain and its application on hypertrophic scar. Biomed Pharmacother 87:82–91. https://doi.org/10.1016/j.biopha.2016.12.076
39. Joseph J, Vedha Hari BN, Ramya Devi D (2018) Experimental optimization of Lornoxicam liposomes for sustained topical delivery. Eur J Pharm Sci 112:38–51. https://doi.org/10.1016/j.ejps.2017.10.032
40. Naguib SS, Hathout RM, Mansour S (2017) Optimizing novel penetration enhancing hybridized vesicles for augmenting the in-vivo effect of an anti-glaucoma drug. Drug Deliv 24:99–108. https://doi.org/10.1080/10717544.2016.1233588
41. Aziz DE, Abdelbary AA, Elassasy AI (2018) Fabrication of novel elastosomes for boosting the transdermal delivery of diacerein: statistical optimization, ex-vivo permeation, in-vivo skin deposition and pharmacokinetic assessment compared to oral formulation. Drug Deliv 25:815–826. https://doi.org/10.1080/10717544.2018.1451572
42. Akhtar N, Pathak K (2012) Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. AAPS PharmSciTech 13:344–355. https://doi.org/10.1208/s12249-012-9754-y
43. Pathak K, Kumari S (2013) Cavamax W7 composite psoralen ethosomal gel versus cavamax W7 psoralen solid complex gel for topical delivery: a comparative evaluation. Int J Pharm Investig 3:171. https://doi.org/10.4103/2230-973x.121284
44. Ahmed S, Sarim Imam S, Zafar A, Ali A, Aqil M, Gull A (2016) In vitro and preclinical assessment of factorial design based nanoethosomes transgel formulation of an opioid analgesic. Artif Cells Nanomed Biotechnol 44:1793–1802. https://doi.org/10.3109/21691401.2015.1102742
45. Akhtar N, Verma A, Pathak K (2017) Feasibility of binary composition in development of nanoethosomal glycolic vesicles of triamcinolone acetonide using Box-behnken design: in vitro and ex vivo characterization. Artif Cells Nanomed Biotechnol 45:1123–1131. https://doi.org/10.1080/21691401.2016.1202261
46. Solanki AB, Parikh JR, Parikh RH (2007) Formulation and optimization of piroxicam proniosomes by 3-factor, 3-level Box-Behnken design. AAPS PharmSciTech 8. https://doi.org/10.1208/pt0804086
47. Abdelbary AA, Aboughaly MHH (2015) Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: application of Box-Behnken design, in-vitro evaluation and in-vivo skin deposition study. Int J Pharm 485:235–243. https://doi.org/10.1016/j.ijpharm.2015.03.020
48. El-Say KM, Abd-Allah FI, Lila AE, Hassan AESA, Kassem AEA (2016) Diacerein niosomal gel for topical delivery: development, in vitro and in vivo assessment. J Liposome Res 26:57–68. https://doi.org/10.3109/08982104.2015.1029495
49. Yadav NK, Nanda S, Sharma G, Katare OP (2016) Systematically optimized coenzyme q10-loaded novel proniosomal formulation for treatment of photo-induced aging in mice: characterization, biocompatibility studies, biochemical estimations and anti-aging evaluation. J Drug Target 24:257–271. https://doi.org/10.3109/1061186X.2015.1077845
50. Jacob S, Nair AB, Al-Dhubiab BE (2017) Preparation and evaluation of niosome gel containing acyclovir for enhanced dermal deposition. J Liposome Res 27:283–292. https://doi.org/10.1080/08982104.2016.1224897
51. Moghddam SRM, Ahad A, Aqil M, Imam SS, Sultana Y (2016) Formulation and optimization of niosomes for topical diacerein delivery using 3-factor, 3-level Box-Behnken design for the

management of psoriasis. Mater Sci Eng C 69:789–797. https://doi.org/10.1016/j.msec.2016.07.043
52. Zidan AS, Mokhtar Ibrahim M, Megrab NAE (2017) Optimization of methotrexate loaded niosomes by Box–Behnken design: an understanding of solvent effect and formulation variability. Drug Dev Ind Pharm 43:1450–1459. https://doi.org/10.1080/03639045.2017.1318907
53. Bikkad ML, Nathani AH, Mandlik SK, Shrotriya SN, Ranpise NS (2014) Halobetasol propionate-loaded solid lipid nanoparticles (SLN) for skin targeting by topical delivery. J Liposome Res 24:113–123. https://doi.org/10.3109/08982104.2013.843192
54. Vaghasiya H, Kumar A, Sawant K (2013) Development of solid lipid nanoparticles based controlled release system for topical delivery of terbinafine hydrochloride. Eur J Pharm Sci 49:311–322. https://doi.org/10.1016/j.ejps.2013.03.013
55. Raza K, Singh B, Singal P, Wadhwa S, Katare OP (2013) Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne. Colloids Surf B Biointerfaces 105:67–74. https://doi.org/10.1016/j.colsurfb.2012.12.043
56. Daneshmand S, Jaafari MR, Movaffagh J, Malaekeh-Nikouei B, Iranshahi M, Seyedian Moghaddam A, Tayarani Najaran Z, Golmohammadzadeh S (2018) Preparation, characterization, and optimization of auraptene-loaded solid lipid nanoparticles as a natural anti-inflammatory agent: in vivo and in vitro evaluations. Colloids Surf B Biointerfaces 164:332–339. https://doi.org/10.1016/j.colsurfb.2018.01.054
57. Pradhan M, Singh D, Murthy SN, Singh MR (2015) Design, characterization and skin permeating potential of fluocinolone acetonide loaded nanostructured lipid carriers for topical treatment of psoriasis. Steroids 101:56–63. https://doi.org/10.1016/j.steroids.2015.05.012
58. Gregoriadis G (1988) Liposomes as a drug delivery system: optimization studies. Adv Exp Med Biol 1988:151–159. https://doi.org/10.1007/978-1-4684-7908-9_12
59. Li C, Zhang X, Huang X, Wang X, Liao G, Chen Z (2013) Preparation and characterization of flexible nanoliposomes loaded with daptomycin, a novel antibiotic, for topical skin therapy. Int J Nanomedicine 8:1285–1292. https://doi.org/10.2147/IJN.S41695
60. Dar MJ, Din FU, Khan GM (2018) Sodium stibogluconate loaded nano-deformable liposomes for topical treatment of leishmaniasis: macrophage as a target cell. Drug Deliv 25:1595–1606. https://doi.org/10.1080/10717544.2018.1494222
61. Dawoud MHS, Yassin GE, Ghorab DM, Morsi NM (2019) Insulin mucoadhesive liposomal gel for wound healing: a formulation with sustained release and extended stability using quality by design approach. AAPS PharmSciTech 20:1–15. https://doi.org/10.1208/s12249-019-1363-6
62. Touitou E, Godin B (2007) Ethosomes for skin delivery. J Drug Deliv Sci Technol 17:303–308. https://doi.org/10.1016/S1773-2247(07)50046-8
63. Aggarwal D, Nautiyal U (2016) Ethosomes: a review. Int J Pharm Med Res 4:354–363
64. Garg BJ, Garg NK, Beg S, Singh B, Katare OP (2016) Nanosized ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo: Formulation optimization, in vitro evaluation and preclinical assessment. J Drug Target 24:233–246. https://doi.org/10.3109/1061186X.2015.1070855
65. Rajera R, Nagpal K, Singh SK, Mishra DN (2011) Niosomes: a controlled and novel drug delivery system. Biol Pharm Bull 34:945–953. https://doi.org/10.1248/bpb.34.945
66. Hamishehkar H, Rahimpour Y, Kouhsoltani M (2013) Niosomes as a propitious carrier for topical drug delivery. Expert Opin Drug Deliv 10:261–272. https://doi.org/10.1517/17425247.2013.746310
67. Marianecci C, Di Marzio L, Rinaldi F, Celia C, Paolino D, Alhaique F, Esposito S, Carafa M (2014) Niosomes from 80s to present: the state of the art. Adv Colloid Interf Sci 205:187–206. https://doi.org/10.1016/j.cis.2013.11.018
68. Arafa MG, Ayoub BM (2017) DOE optimization of nano-based carrier of pregabalin as hydrogel: new therapeutic & chemometric approaches for controlled drug delivery systems. Sci Rep 7:1–15. https://doi.org/10.1038/srep41503

69. Al-Mahallawi AM, Fares AR, Abd-Elsalam WH (2019) Enhanced permeation of methotrexate via loading into ultra-permeable niosomal vesicles: fabrication, statistical optimization, ex vivo studies, and in vivo skin deposition and tolerability. AAPS PharmSciTech 20:1–10. https://doi.org/10.1208/s12249-019-1380-5
70. Sala M, Diab R, Elaissari A, Fessi H (2018) Lipid nanocarriers as skin drug delivery systems: properties, mechanisms of skin interactions and medical applications. Int J Pharm 535:1–17. https://doi.org/10.1016/j.ijpharm.2017.10.046
71. Callender SP, Mathews JA, Kobernyk K, Wettig SD (2017) Microemulsion utility in pharmaceuticals: implications for multi-drug delivery. Int J Pharm 526:425–442. https://doi.org/10.1016/j.ijpharm.2017.05.005
72. Todosijević MN, Cekić ND, Savić MM, Gašperlin M, Ranđelović DV, Savić SD (2014) Sucrose ester-based biocompatible microemulsions as vehicles for aceclofenac as a model drug: formulation approach using D-optimal mixture design. Colloid Polym Sci 292:3061–3076. https://doi.org/10.1007/s00396-014-3351-4
73. Negi P, Singh B, Sharma G, Beg S, Raza K, Katare OP (2016) Phospholipid microemulsion-based hydrogel for enhanced topical delivery of lidocaine and prilocaine: QbD-based development and evaluation. Drug Deliv 23:951–967. https://doi.org/10.3109/10717544.2014.923067
74. Patel HK, Barot BS, Parejiya PB, Shelat PK, Shukla A (2013) Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo: ex vivo permeation and skin irritation studies. Colloids Surf B Biointerfaces 102:86–94. https://doi.org/10.1016/j.colsurfb.2012.08.011
75. Radwan SAA, ElMeshad AN, Shoukri RA (2017) Microemulsion loaded hydrogel as a promising vehicle for dermal delivery of the antifungal sertaconazole: design, optimization and ex vivo evaluation. Drug Dev Ind Pharm 43:1351–1365. https://doi.org/10.1080/03639045.2017.1318899
76. Wang Z, Mu HJ, Zhang XM, Ma PK, Lian SN, Zhang FP, Chu SY, Zhang WW, Wang AP, Wang WY, Sun KX (2015) Lower irritation microemulsion-based rotigotine gel: Formulation optimization and in vitro and in vivo studies. Int J Nanomedicine 10:633–644. https://doi.org/10.2147/IJN.S74079
77. de Mattos CB, Argenta DF, de Melchiades GL, Cordeiro MNS, Tonini ML, Moraes MH, Weber TB, Roman SS, Nunes RJ, Teixeira HF, Steindel M, Koester LS (2015) Nanoemulsions containing a synthetic chalcone as an alternative for treating cutaneous leshmaniasis: optimization using a full factorial design. Int J Nanomedicine 10:5529–5542. https://doi.org/10.2147/IJN.S83929
78. Afifah SN, Azhar S, Ashari SE, Salim N (2018) Development of a kojic monooleate-enriched oil-in-water nanoemulsion as a potential carrier for hyperpigmentation treatment. Int J Nanomedicine 13:6465–6479. https://doi.org/10.2147/IJN.S171532
79. Yu M, Ma H, Lei M, Li N, Tan F (2014) In vitro/in vivo characterization of nanoemulsion formulation of metronidazole with improved skin targeting and anti-rosacea properties. Eur J Pharm Biopharm 88:92–103. https://doi.org/10.1016/j.ejpb.2014.03.019
80. Alvarez-Trabado J, López-García A, Martín-Pastor M, Diebold Y, Sanchez A (2018) Sorbitan ester nanoparticles (SENS) as a novel topical ocular drug delivery system: design, optimization, and in vitro/ex vivo evaluation. Int J Pharm 546:20–30. https://doi.org/10.1016/j.ijpharm.2018.05.015
81. Marto J, Ruivo E, Lucas SD, Gonçalves LM, Simões S, Gouveia LF, Felix R, Moreira R, Ribeiro HM, Almeida AJ (2018) Starch nanocapsules containing a novel neutrophil elastase inhibitor with improved pharmaceutical performance. Eur J Pharm Biopharm 127:1–11. https://doi.org/10.1016/j.ejpb.2018.01.011
82. Abd-Elsalam WH, El-Zahaby SA, Al-Mahallawi AM (2018) Formulation and in vivo assessment of terconazole-loaded polymeric mixed micelles enriched with Cremophor EL as dual functioning mediator for augmenting physical stability and skin delivery. Drug Deliv 25:484–492. https://doi.org/10.1080/10717544.2018.1436098

83. Varshosaz J, Taymouri S, Minaiyan M, Rastegarnasab F, Baradaran A (2018) Development and in vitro/in vivo evaluation of HPMC/chitosan gel containing simvastatin loaded self-assembled nanomicelles as a potent wound healing agent. Drug Dev Ind Pharm 44:276–288. https://doi.org/10.1080/03639045.2017.1391832
84. Zhou J, Zhang W, Pang J, Wang X, Zheng Z, Li Y, Yang F, Yang W (2020) Optimized preparation of vinpocetine micelles and in vivo evaluation of its pharmacokinetics in rats. Pharm Dev Technol 25:464–471. https://doi.org/10.1080/10837450.2019.1709501
85. El-Say KM, El-Sawy HS (2017) Polymeric nanoparticles: promising platform for drug delivery. Int J Pharm 528:675–691. https://doi.org/10.1016/j.ijpharm.2017.06.052
86. Zhang Z, Tsai PC, Ramezanli T, Michniak-Kohn BB (2013) Polymeric nanoparticles-based topical delivery systems for the treatment of dermatological diseases. Wiley Interdiscip Rev Nanomed Nanobiotechnol 5:205–218. https://doi.org/10.1002/wnan.1211
87. Gonzalez-Pizarro R, Silva-Abreu M, Calpena AC, Egea MA, Espina M, García ML (2018) Development of fluorometholone-loaded PLGA nanoparticles for treatment of inflammatory disorders of anterior and posterior segments of the eye. Int J Pharm 547:338–346. https://doi.org/10.1016/j.ijpharm.2018.05.050
88. Deshmukh AS, Chauhan PN, Noolvi MN, Chaturvedi K, Ganguly K, Shukla SS, Nadagouda MN, Aminabhavi TM (2017) Polymeric micelles: basic research to clinical practice. Int J Pharm 532:249–268. https://doi.org/10.1016/j.ijpharm.2017.09.005