DEVELOPMENT AND EVALUATION OF DESOXIMETASONE LOADED NIOSOMES FOR TOPICAL DRUG DELIVERY USING QUALITY BY DESIGN (QbD) APPROACH

By

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ABSTRACT OF THE DISSERTATION

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Drug delivery via the transdermal and topical routes offers many advantages, including improved patient acceptance, targeted local delivery, reduced systemic toxicity/side-effects, avoidance of the hepatic first-pass metabolism, and enablement of sustained or controlled drug release. However, skin functions as a barrier which poses a major challenge to the delivery of therapeutically significant amounts of drug into and across the skin. This barrier property of the upper skin layer (stratum corneum) also affects the deposition of a drug within the lower skin layers. Therefore, it is critical to select a suitable carrier and approach which can enhance drug deposition in the skin. The approaches include among others, the use of chemical permeation enhancers and vesicle based drug delivery. One example of the latter is niosomal topical formulations.

This research work was focused on the systematic development of non-ionic surfactant based niosomal formulations using Quality by Design (QbD) principles. The research was subdivided into three phases as described below.

In the first phase of the study, niosomes were prepared by considering various Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs). Organic solvent selection, drug concentration, surfactant concentration, cholesterol concentration,

and type of lipids used in the formulation were considered as CMAs. Systematically, experiments were performed by changing the concentrations of drug, surfactant, and cholesterol to evaluate the impact of these changes on the final drug product. Mixing parameters (speed and time), internal and external phase volume, external phase temperature, and organic phase addition rate were considered as CPPs. Various formulations were manufactured by changing process parameters to evaluate their impact on the final drug product. Each formulation was evaluated for entrapment efficiency, particle size, polydispersity index (PDI), and zeta potential. The result from these initial trial formulations showed that the concentration of surfactant and cholesterol, mixing parameters (time and speed), and organic phase addition rate have a significant impact on the final niosomal formulations produced.

In the second phase of the study, parameters impacted in the first phase were exhaustively studied by a 2^5 full factorial design using JMP® statistical software. Experiments were performed as per the formulation combination proposed by the design of the experiments. The formulations were evaluated for niosomal entrapment efficiency, particle size, polydispersity index (PDI), and zeta potential. All the data were added into the JMP® study design and the profile predictor was generated based on the experimental data. The ideal formulation profile was predicted using the profile predictor features from JMP® statistical software. The suggested ideal formulation was manufactured to validate the study design. The test results obtained from the formulation were in-line with the predicted results, which represent an accuracy of design of experiments (DoE) study. An additional batch was manufactured by changing the grade of surfactant to verify the

impact of surfactant (principal component) chemistry on the niosomal formulation characteristics.

In the final phase of the study, topical gels were manufactured with niosomal dispersion using various synthetic or natural gelling agents. Various topical gels were manufactured by changing the concentration or grade of the Carbomer or changing the gelling agent. Different gelling agents were used in identical concentrations to examine the impact of various gelling material on the final product. In these preparations, Carbomer 980 was used as the reference gelling agent since it was used in the marketed drug product which is used as a reference listed drug (RLD) in this study. Various concentrations of Carbomer 980 were also evaluated to match the physical characteristics of the gel with the reference drug product. Topical gel formulations were evaluated for chemistry parameters (assay, content uniformity, pH), physical parameters (yield stress, viscosity, rheology, flow curves, specific gravity, spreadability), and organoleptic properties.

Finally, studies were performed for delivery of desoximetasone (anti-inflammatory glucocorticoid) to human cadaver skin using a topical gel dosage form prepared with niosomal microstructures and compared to the RLD. Topicort® Gel (desoximetasone) USP, 0.05% marketed drug product manufactured by Taro Pharmaceuticals was used as the reference drug product for comparison of the niosome based topical gel product (the FDA has listed Topicort® Gel (desoximetasone) USP, 0.05% as a reference drug in the Orange Book). Topical gel formulations were prepared and evaluated in such a way as to match with the reference product. Based on their performance, results showed that topical gel formulations with 0.7% Carbomer 980 were

in complete concordance with the reference product. The results also showed that the rheological testing provided additional advantages over conventional methods for being sensitive, accurate, and versatile. Additionally, permeation experiments on human cadaver skin were conducted in order to compare the niosome containing topical gel and the reference product. Dermal delivery of desoximetasone in the niosome-containing topical gel formulation was found to be slower in comparison with the reference product. The result also showed a statistically significant increase in the amount of drug retained in the skin in the niosome-containing topical gel formulation, which showed a sustained drug release pattern. The stability study of the niosomal dispersion and niosome-containing topical gel shows that the niosome vesicle dispersion and topical gel products were stable at room temperature (20-25°C) and at accelerated temperature (40°C) conditions with no significant change occurring in the final drug product. Results from the present study highlight that optimized ideal niosome formulations can efficiently deliver a hydrophilic/hydrophobic drug to the target site with improved stability. Also, the niosomal topical gel was able to have a reduced drug concentration and dose frequency, and this was achieved by controlling drug release from the niosome matrix to gain maximum therapeutic effectiveness.

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1.1. Background

Improved patient compliance and the effectiveness of the pharmacological treatment are the integrated outcomes of new drug delivery systems [1]. There are many non-invasive routes which can be used to treat patients. Among these choices, the transdermal route has become increasingly more acceptable and accounted for about 4200 million applications through the global market in 2016. By 2024, North America is expected to account for 35% of the transdermal delivery market in terms of revenue [2].

1.2. Topical drug delivery systems

Topical drug delivery can be defined as the application of drug formulation to the skin to treat cutaneous disorders such as psoriasis, acne, eczema, redness, swelling, atopic dermatitis and others. The topical application of the drug shows pharmacological or other effects of the drug on the surface of the skin or within the skin. Various semisolid formulations such as gels, creams, lotions, and ointments are the most popular formulations used for topical delivery, along with foams, sprays, medicated powders, solutions, and medicated adhesive systems. Drug molecules with low doses delivered through the topical route effectively are limited to a smaller area [3].

Advantages of topical drug delivery systems:

1. Avoidance of first pass liver metabolism.
2. Convenient to use and easy to apply.

3. Results in better patient compliance.
4. Self-medication is possible.
5. Drugs are delivered selectively to a targeted site.
6. Easy to terminate the medication.
7. Ideal for drug candidates with short biological half-lives and narrow therapeutic windows.
8. Avoids fluctuation in drug levels.
9. Provides effectiveness in low doses and continuous drug delivery.
10. Many locations of body and skin areas for application are available.

Disadvantages of topical drug delivery systems:

1. Skin irritation or allergic reaction may occur at the site of application.
2. Contact dermatitis may occur due to some drugs.
3. Drugs with poor permeability or low lipid content have difficulty in penetrating through the dermal route.
4. Drugs with larger particle sizes have difficulty in penetrating through the dermal route.

Some examples of topical drug delivery systems are provided here:

Creams, lotions, gels, ointments, pastes, foams, sprays, solutions, suspensions, liniments, and powders are common dosage forms for topical drug delivery. More details on each dosage form is provided in section 1.10.

1.3. Barrier properties of the skin

Delivery of therapeutically effective amounts of a drug through the skin layers is one of the biggest challenges for the formulator of the dermal drug delivery system. Both active and passive drug delivery into and across the skin plays an essential role in topical and transdermal care applications [4]. Skin is the challenging barrier for the topical and transdermal delivery of the drug. The outermost layer of the epidermis is the stratum corneum, which is wholly composed of dead, enucleated keratinocytes in multi-layered brick and mortar like structure. The thickness of this layer is about 15-20 µm [5]. The other layers in the descending order, are stratum lucidum, stratum granulosum, stratum spinosum, and the stratum basale [6]. The stratum corneum presents itself as the outermost protective layer of the body against pathogenic invaders (Figure 1.1). The water concentration gradient reduced from 75% in the viable epidermis layer to below 30% on the surface.

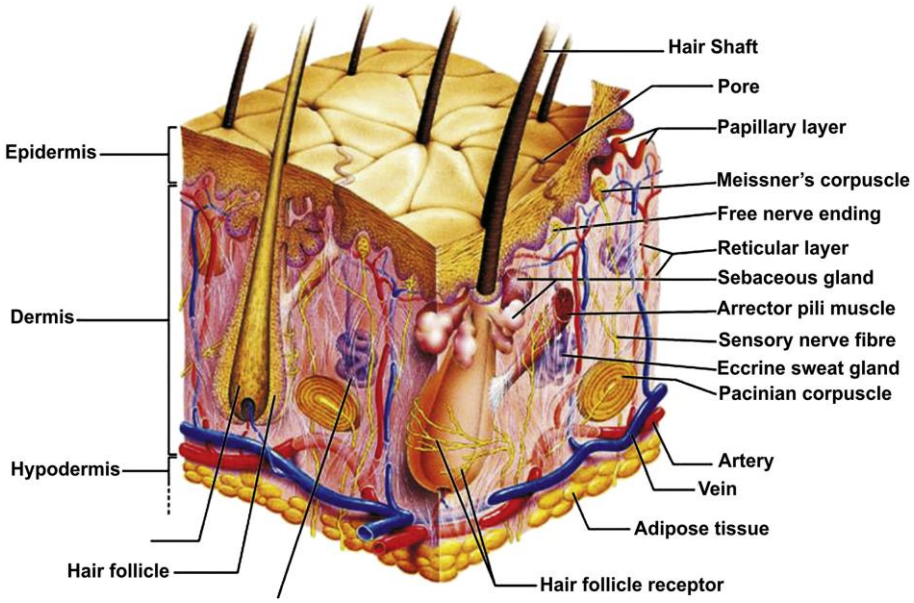

Figure 1.1. Diagrammatic representation of skin structure [7].

The hydrophilic (as well as lipophilic) pathways help in transporting drugs. Thus drug penetration through the skin becomes a challenge for poorly soluble drugs. Molecule size also plays a crucial role for the drug permeation through the skin. Small and lipophilic molecules permeate the skin relatively easily compare to the large and hydrophilic molecules which are hindered due to the low permeability in stratum corneum, while a significant fraction of currently marketed topical drug products is hydrophilic in nature [8].

1.4. Mechanism and routes of penetration

Topically applied drug molecules following potential three pathways to penetrate the skin layers or pass into the skin layers: i) transappendageal route, ii) intercellular route or iii) intracellular route [9, 10].

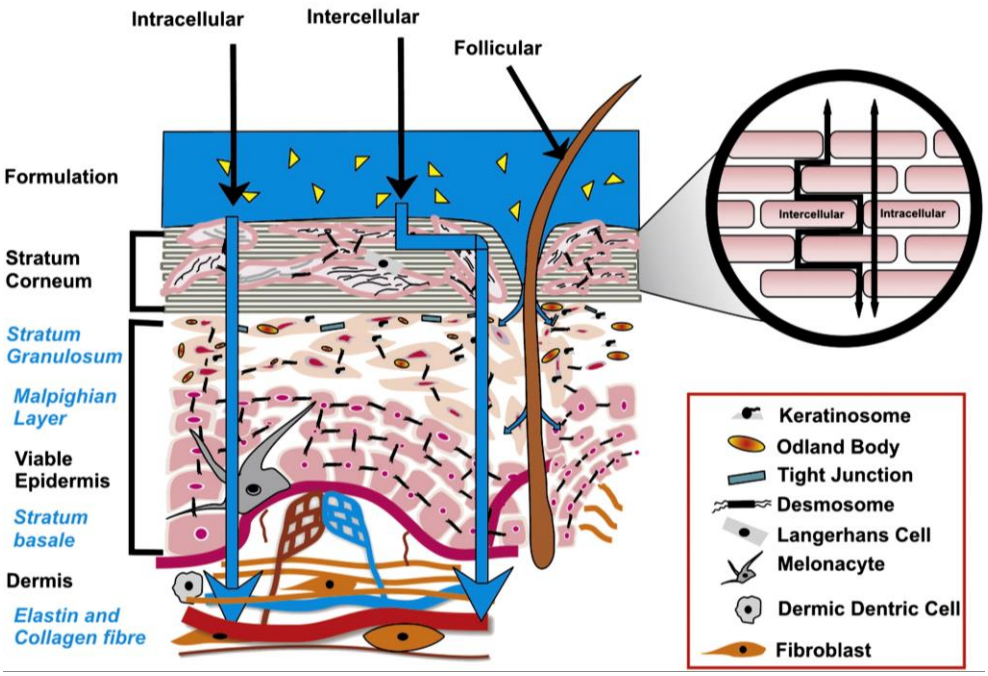

Figure 1.2. Diagrammatic representation of the routes of drug penetration pathways [7].

Figure 1.2 shows details on all three routes of drug penetration into the stratum corneum (the topmost layer of the skin). The penetration of the drug molecule from the formulation through the skin is influenced by potentially three factors: i) physicochemical properties of the drug molecule, ii) the type of formulation, and iii) delivery method of the drug molecule.

1.5. Chemical permeation enhancers

Penetration enhancers may act by one or more of the potential following three mechanisms: i) disruption of the highly ordered structure of the stratum corneum, ii) interaction with intercellular protein and/or iii) improved partition of the drug, co-enhancer, or solvent into the outer layer of the skin stratum corneum.

Chemical enhancers are pharmacologically inactive compounds that partition and diffuse into the skin and interact with stratum corneum components. They usually are considered safe excipients however, at high concentrations some can be irritant to the skin [9]. Many published manuscripts describe chemical penetration enhancers such as sulfoxides and similar chemicals, azones, pyrrolidones, water, fatty acids, ureas, oxazolidinones, surfactants and essential oil/terpenes/terpenoids [10].

Drug – chemical enhancer (CE) – skin interactions

Once a topical formulation is applied on the skin surface, drug-CE, CE-skin, and drug-skin interactions may occur. Drug-CE interactions play a significant role in the rate of drug release from the solvent system. CE-skin interactions control the drug penetration rate by impacting on the penetration of a drug across the skin [11]. If the drug molecule

has a more “chemical” tendency towards the chemical enhancer, than it may remain preferentially with the CE and shows low permeation of the drug. Physicochemical properties such as molecular weight, log P, melting point, and solubility parameters of the drug influence drug-skin interactions. Drug-solvent-skin interactions may be explained by the 'push-pull' effect as shown in Figure 1.3 [9]. There are two types of ‘push’ effects. In one scenario, the solubility parameter difference between the drug and CE is higher compared to the attraction between the drug and CE. In this case, the drug will easily escape from the CE and pass from the skin. In the other case, the drug shows a higher affinity for the CE then the CE will hold the drug and this will impact the drug penetration through the stratum corneum. The 'pull' effect shows that chemical enhancers change the stratum corneum by structural transformation and, therefore, increase the solubility of the drug into the SC or pull the drug while diffusing through the skin [12, 13].

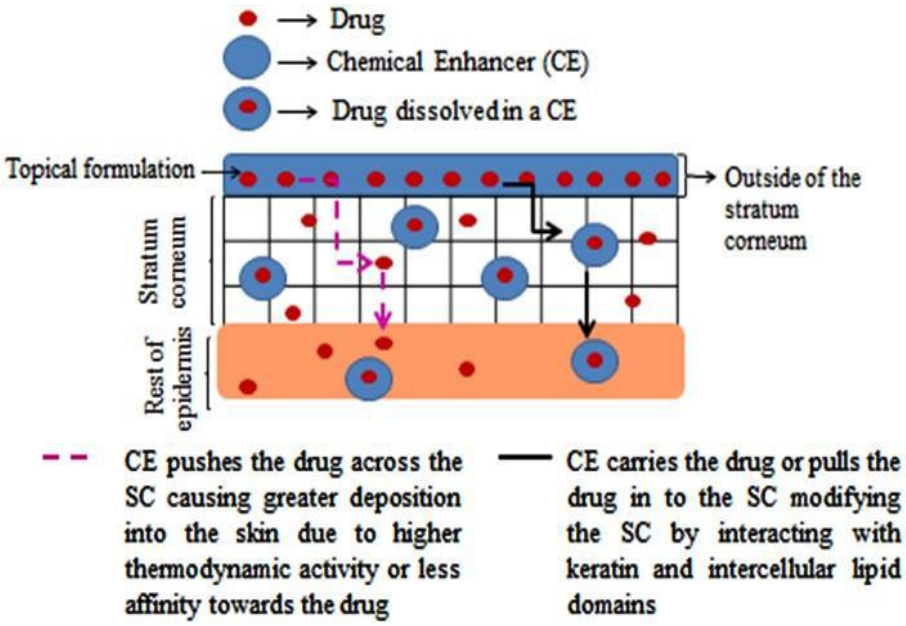

Figure 1.3. Diagrammatic representation of chemical enhancer interaction with SC (push-pull effect) [9].

Ideal characteristics of chemical penetration enhancers

Ideally, penetration enhancers reversibly reduce the barrier resistance of the SC without impacting on the viable cells. Few more desirable properties for the penetration enhancers acting within the skin are listed here:

- Nontoxic, nonirritating, and nonallergenic.
- Non-damaging to viable cells.
- Ideally, work rapidly; the activity and duration of the effect should be predictable and reproducible.
- No pharmacological activity within the body.
- Work is unidirectional as it should allow therapeutic agents into the body while preventing the loss of endogenous materials from the body.
- As the chemical enhancer is removed from the skin, the barrier properties of the stratum corneum should rapidly return to the original state.
- Physically and chemically compatible with drugs and excipients in the dosage form.
- Cosmetically acceptable with an appropriate skin feeling.

1.6. Physical penetration enhancement techniques

Various physical penetration enhancer techniques have been developed to bypass or modulate the barrier function of the skin and to allow easier administration of drugs. Some of the techniques are discussed below.

a) Iontophoresis

Iontophoresis as a technique is used to increase transdermal drug delivery through the skin by application of a low-level electric current, either directly to the skin or indirectly via the dosage form. Iontophoresis increases the permeation of charged and neutral compounds using electromigration and electro-osmosis processes. It is useful in the treatment of various skin disorders such as skin cancer, psoriasis, dermatitis, and hypertrophic scars. Iontophor ® (Life Tech, Inc,) utilizes iontophoresis in the lidocaine delivery device [14-16].

b) Electroporation

Electroporation is an electrical enhancement method that involves the application of short (microsecond or millisecond), high voltage (50-1000 volts) pulses to the skin. Other electrical parameters that affect delivery include pulse properties such as waveform, rate, and number. The increase in skin permeation is directly related to the generation of transient pores during electroporation. Larger molecules which are not suitable for iontophoresis have been delivered using electroporation and examples include insulin, vaccines, oligonucleotides, and microparticles [17, 18]. Investigational device exemption (AngioDynamic, Inc.) for NanoKnife Irreversible Electroporation utilizes this technique for “Direct IRE Cancer Treatment”.

c) Microporation

Microporation involves the use of microneedles which as an example, maybe 10 to 200 µm in height and 10 to 50 µm in width. In the microporation technique,

microneedles are applied to the skin and pierce only stratum corneum and hence increase skin permeability by delivering such actives as insulin and vaccines by infusion [19]. MicronJet™ is a FDA cleared microneedle based device for the intradermal drug delivery of suitable drug molecules [20].

d) Ultrasound

Ultrasound, which is frequently referred to as sonophoresis, involves the use of ultrasonic energy to enhance the delivery of a solute either simultaneously or through pre-treatment during drug delivery. The main principle behind the increase in skin permeability using the ultrasound technique is attributed to the formation of gaseous cavities within the intercellular lipids, resulting in the disruption of the stratum corneum. Ultrasound parameters such as treatment duration, intensity, pulse length, and frequency are usually varied to achieve various levels and duration of drug delivery. Among these, frequency is the most significant impacting parameter for drug delivery [21, 22]. SonoPrep device (Sontra Medical Co.) used this technique to deliver local anesthetics and insulin.

e) Laser radiation and photomechanical waves

Laser radiation damages target cancer cells but have a minimum effect on the healthy tissues and cells over a short frame of time during skin application. Such direct and controlled exposure of the skin to laser radiation during the ablation of the stratum corneum usually does not produce significant damage to the layers of skin below. Removal of stratum corneum using this technique has been shown to enhance the delivery of both lipophilic and hydrophilic drugs [23, 24]. The combination of

phototherapy with topical products has been used from a long time for the treatment of plaque psoriasis.

f) Magnetophoresis

In this technique, particles migrate in a magnetic field. This technique can be further categorized as positive and negative magnetophoresis. In positive magnetophoresis, magnetic particles migrate in a diamagnetic medium whereas in negative magnetophoresis magnetic particles migrate in a magnetic medium. Magnetophoresis is the most commonly used activation method on a chip device for fluid screening, cell sorting and pathogen detection [25].

g) Thermophoresis

Heat improves the applied drug permeability in the skin by increasing body fluid circulation, blood vessel walls permeability, rate-limiting membrane permeability, and drug solubility. Due to the application of heat, the kinetic energy of the drug molecules is known to increase in the cell membrane [26]. Controlled heat-aided drug delivery (CHADD™) technology is used to deliver local analgesia Synera® containing lidocaine and the tetracaine patch (Glen US Incorporated).

h) Radiofrequency

Exposure of the skin to high radiofrequency results in the formation of the heat-induced microchannel in the membrane in an identical way as when laser radiation is applied. The drug delivery rate is controlled by the number and depth of the microchannel formed by the device, which depends on the properties of the

microelectrodes in contact with the skin during treatment [27]. Radiofrequency ablation (RFA) applicator is used in electro surgery for intraoperative coagulation and soft tissue ablation.

i) Needless injection

This is a pain-free method for administering drugs to the skin. This dermal delivery is achieved by firing the liquid or solid particles at supersonic speeds through the stratum corneum by using a suitable energy source. The mechanism involves forcing compressed gas such as helium or nitrogen through the nozzle with the resultant drug particles entrained with the jet flow, reportedly traveling at sufficient speed for skin penetration [28, 29]. The PharmaJet Stratis® Needle-free Injection System (PharmaJet Inc.) is used to deliver 'Afluria' influenza virus vaccine.

1.7. Nanotechnology for dermal drug delivery

Recently, more effort has been focused in the field of nanotechnology for drug delivery. Nanocarriers are micron-sized or sub-micron-sized colloidal vesicles in which drug molecules can be entrapped within their lipid/polymeric matrix or adsorbed/conjugated them to their surface [30]. Currently, the U.S. Food and Drug Administration (FDA) has no specified guidelines in the nanotechnology area. However, the agency has suggested two primary criteria for evaluating the use of nanotechnology in FDA regulated products. These criteria are the following: 1) the materials must have at least one dimension in the nanoscale (1-100 nm) range, and 2) the material should exhibit specific physicochemical-biological properties that are attributable to their small sizes

[31]. There are many benefits to using nanoparticles such as 1) increases in the solubility of lipophilic agents for intravascular delivery and improving oral bioavailability, 2) controlling and sustaining release in drug delivery, 3) chemical and physical protection of the therapeutic agents and enhancing their stability in the formulation, 4) target specific drug delivery, 5) improve permeation and retention effect which results in higher drug localization for example, in tumours.

Vesicle based drug delivery system can be described as highly ordered assemblies consisting of one or several concentric bilayers formed as a result of the self-assembling of amphiphilic building blocks in the presence of water [32, 33]. Different vesicle based dermal drug delivery carriers for effective drug delivery are listed in Figure 1.4 and are discussed in more detail below.

a) Liposomes

Liposomes are microscopic vesicles composed of phospholipids and cholesterol in an aqueous medium resulting in closed bilayer arrangement. Liposomes consist of an aqueous core. In dispersion, both the inner core of the vesicles and the continuous phase are aqueous, and the two aqueous compartments are separated by a lipid bilayer. In the liposome system, lipophilic drugs can be entrapped in the bilayer membrane, whereas hydrophilic drug substances can be encapsulated in the aqueous core of the liposome vesicles. This structure of the vesicles enables liposomes to deliver both lipophilic and hydrophilic drugs to the targeted site of action. Phospholipids are the major part of the liposome composition, which are also the main building blocks of cell membranes, therefore, the biocompatibility of the drug molecules and formulation is improved [34]. The size of liposomes can range from 50 nm to several hundred nanometers or

micrometers. Liposomes are classified based on the size or number of lipid bilayers. Based on the size, liposomes can be divided into two categories: small unilamellar vesicles (SUV) and giant unilamellar vesicles (LUV). Based on the number of lipid bilayers, liposomes can be divided into two categories as well: multilamellar vesicles (MLV) and unilamellar vesicles (ULV). Different techniques are used to manufacture liposomes such as lipid film hydration, freeze-thaw process, solvent injection, and emulsification. High shear mixing is one of the most common technique used. The unloaded drug from the preparations is removed by centrifugation, filtration, or dialysis. Stability of liposomes is the biggest challenge in liposome formulation because it can undergo lipid oxidation, hydrolysis, aggregation, and fusion [35, 36]. Marqibo® Vincristine sulfate liposomes injection (Hana Biosciences) and DaunoXome® Daunorubicin citrate-liposome injection (NeXstar Pharmaceuticals) are currently approved and marketed liposomal formulations.

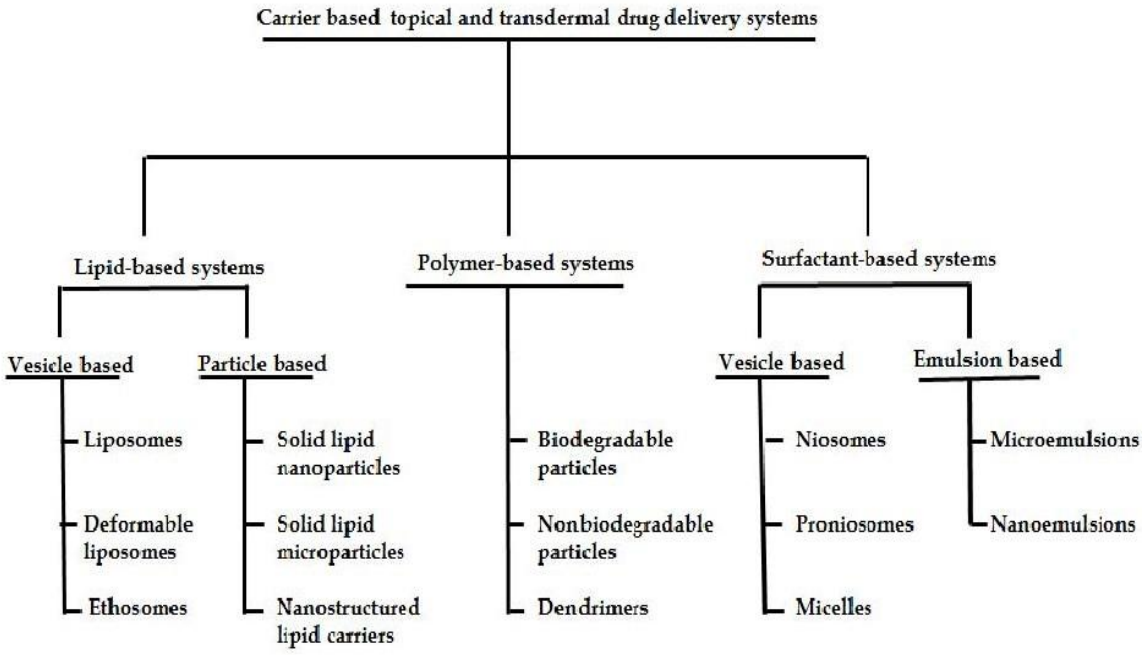

Figure 1.4. Nanocarriers used for dermal drug delivery [37].

b) Niosomes

Niosomes are relatively new drug carriers in which the drug is encapsulated in a non-ionic surfactant vesicle. Niosomes consist of small lamellar structures (range 10 – 1000 nm) that can be unilamellar or multilamellar, spherical and polyhedral vesicles surrounded by an aqueous medium. Niosomes are made up of non-ionic surfactant and cholesterol. In the dispersion system, the inner core of the niosomes and the outer continuous phase is aqueous, and these two aqueous layers are separated by a surfactant monomer bilayer. In this system, hydrophilic drug substances can be encapsulated in the aqueous core, whereas lipophilic drugs can be entrapped in the bilayer membrane of the niosome vesicles. Various non-ionic surfactants have been used to prepare the vesicles such as polyglycerol alkyl ethers, glycosyl dialkyl ethers, crown ethers, ester-linked surfactants, polyoxyethylene alkyl ethers as well as Spans (Sorbitan esters) and Tweens (Polyethoxylated sorbitan esters) [38-40].

c) Transferosomes

Transferosomes are a unique vesicular drug delivery system since they are structures which have the ability of ultra-deformation allowing them to overcome the skin barrier by squeezing in between the stratum corneum cells. Transferosomes are a special type of liposomes, containing of phosphatidylcholine and an edge activator. Transferosomes can deform and pass through narrow channels of the skin without measurable loss. This high deformability gives better penetration of intact vesicles. Deformability (flexibility) is the key to this vesicle system, which can be achieved by utilizing optimum concentrations of the suitable surface active agent mixture.

Transferosomes consist of at least one inner aqueous layer, which is covered by lipid bilayers with specially designed properties because of the incorporation of 'edge activators' into the transferosomes membrane. Various surfactants such as Span 80 (Sorbitan Oleate), Tween 80 (Polysorbate 80) can be used as edge activators in the formation of transferosomes [41-43].

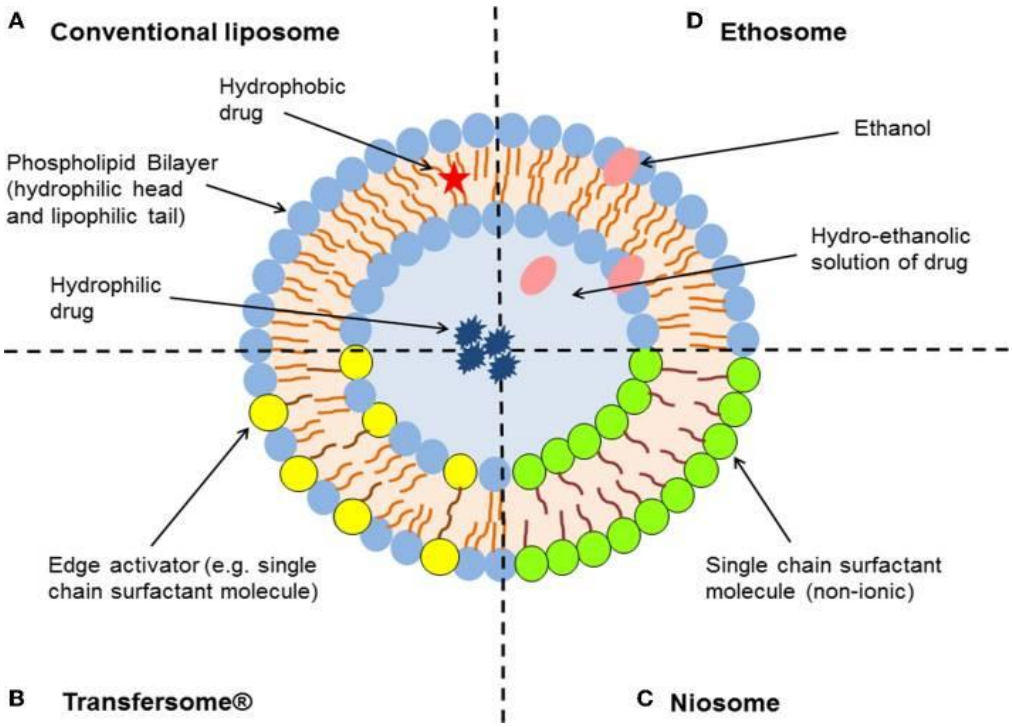

Figure 1.5. Schematic representation of the different types of lipid-based vesicular systems. (A) liposomes, (B) transferosomes, (C) niosomes and (D) ethosomes [44].

d) Ethosomes

Ethosomes have been developed for delivering various kinds of drugs with low skin penetration capacity. Ethosomes vesicle systems are soft lipid vesicles with size range from nanometers to micrometers containing phospholipids, alcohol (ethanol and

isopropyl alcohol) in high concentration, and water. Ethanol is the main ingredient (20-50%) which acts as a penetration enhancer and fluidizes the ethosomal lipids and stratum corneum layer, thus allowing the vesicles to diffuse through the disorganized lipid layer. Ethosomal vesicle size is smaller because of the net negative surface charge of ethanol. Due to smaller size ethosomes shows better penetration capability through the intercellular space. Hence, vesicle size can be optimized by changing the ethanol concentration [45-47]. The schematic representation of different types of lipid-based vesicular systems is provided in Figure 1.5.

e) Virosomes

Virosomes are spherical shaped unilamellar vesicles with an average vesicle size of 150 nm. Virosomes are reconstituted viral envelopes structured as lipid bilayers in which viral glycoproteins are inserted (obtained from various enveloped viruses). In simple words, virosomes are defined as liposomes with for example, influenza virus hemagglutinin (HA) or neuraminidase (NA) spikes on their surface. Characteristics of the virosomes vesicle depend on bilayer components used for the preparation. Modification of the virosomes content can be used to optimize virosomes that achieve the efficient incorporation of selected drugs. Various ligands such as cytokines, peptides, and monoclonal antibodies can be inserted on the virosomal surface [48, 49].

f) Pharmacosomes

Pharmacosomes are an amphiphilic lipoidal colloidal dispersion system of drugs, conveniently bound to lipids with the potential to enhance the bioavailability of hydrophilic and lipophilic drugs. Drugs with a free carboxyl group or an active hydrogen

atom like amino, hydroxyl groups, is esterified with the help of the hydroxyl group of the lipid, thus generating an amphiphilic prodrug. The prodrug shows amphiphilic characteristics and reduces interfacial tension, due to the reduced interfacial tension, the contact area increases, which increases the bioavailability of the drug. They assist the transport through the cell membrane, cell wall, and into the tissues. The three critical components of the pharmacosomes are drug, solvent, and lipid. Phosphatidylcholine is the most commonly used lipid for pharmacosomes preparation [50, 51].

g) Sphingosomes

Sphingosomes are more stable and show less oxidation, hydrolysis, degradation, leaching, sedimentation and drug aggregation in comparison with liposomes. Sphingosomes are much more stable to acid hydrolysis and have better drug retention characteristics. The only difference in the components of a sphingosomes as compared to a liposome is that phospholipids are replaced with sphingolipids in this vesicle system. The sphingosomes are more stable because of the following reasons: a) they are made up of only amide and ether linkages. These are more stable than the ester linkage of commonly use lecithin, b) they contain less amount of double bonds then lecithin and so are less susceptible to rancidity. Sphingolipid and cholesterol are the critical elements of the sphingosomes. There are numerous mechanisms by which sphingosomal vesicles interact with cells like adsorption, endocytosis, fusion, lipid transfer, etc. [52, 53]. Though sphingosomes are similar to liposomes, there are many advantages due to their structural components. Advantages of the sphingosomes are better drug retention characteristics, better stability via encapsulation, reduced toxicity of the encapsulated

drug, improvement of the pharmacokinetics of the encapsulated drug by increasing the circulation time and many others.

h) Solid lipid nanoparticles (SLN) or Nanostructured lipid carriers (NLC)

SLN and NLC are the first two generations of the nanoparticle field of application. The formation of SLN is performed by replacing liquid lipids from an oil/water emulsion with a specific lipid that is solid at body temperature and has a perfect crystalline lattice. The solid lipid content can vary from 0.1% to 30% w/w and can be stabilized with a surfactant. The size of SLNs can vary from 40 to 1000 nm [54]. High-pressure homogenization (most common and widely used) and microemulsion formation are the two common techniques to manufacture SLNs. NLCs are produced with a blend of solid and liquid lipids in a ratio ranging from 70.0:30.0 to 99.99:0.01. Both SLNs and NLCs show positive and negative charges. The significant advantage of NLC over SLN is that NLCs show a higher loading capacity for drugs and a lower water content with lower expulsion of drugs during storage [55]. For example, Pople et al. published in 2014 investigate novel particulate carrier system solid lipid nanoparticles (SLN) for topical application of vitamin A palmitate via gel formulation [56].

i) Polymeric microparticles and nanoparticles

Polymeric particles are in the nano or micro range size with a positive or negative charge on the particles. The nanoparticles targeting capacity depends on many factors such as particle surface charge, size, surface modification and hydrophobicity of the particle, etc. In most cases, these particles are not able to pass through the stratum corneum intact. A recent study demonstrates after 24 hours of application on the skin the

particles were found to be present in all different layers of the stratum corneum, in the area from stratum corneum and stratum granulosum, and hair follicles but did not cross the length of the skin [57]. Onpattro (patisiran) lipid complex injection is currently approved marketed polymeric nanoparticle formulation [58].

j) Nanocrystal technology applications

Nanocrystal technology is a widely accepted method to improve the bioavailability of poorly soluble drugs. The basic concept of this method is to reduce the drug particle size to the sub-micron range that increases the surface area of the drug, which proportionally improves drug solubility. Nanocrystals are nanoparticles with crystalline characters which makes them different from other nanosized particles. Additionally, the drug's saturation solubility also increases with the reduction of the drug substance's particle size. The principle of this system is that the nanocrystals improve the transport of drugs across a barrier/membrane. Since a poorly soluble drug has more lipophilicity, and therefore it shows better penetration ability, especially when the solubility and low-dissolution concerns are overcome by the nanocrystal system approach.

It is very crucial to select the correct stabilizer during the preparation of nanocrystal to stabilize the nanoparticles by preventing aggregation due to the attractive force between the particles. Generally, during the formation of nanocrystals, due to the modification of Gibbs free energy, a thermodynamically unstable system is formed, which results in agglomeration or crystal growth because of Ostwald ripening. Nanocrystals can be produced by two simple techniques, such as the bottom-up approach and the top-down approach. In the bottom-up approach, nanoparticles are produced using

precipitation principle wherein a top-down approach; nanocrystals are constructed by media milling or high-pressure homogenization technique or combination of both methods. In this case of nanocrystals, nanoparticles are dispersed in a liquid, and the system is termed a "nanosuspension" [59]. Rapamune® (Wyeth Pharmaceuticals) uses this technology to deliver sirolimus as an active drug. Emend® (Merck) and Tricor® (Abbott Laboratories) are other approved marketed products using nanocrystal technology for drug delivery [60].

1.8. Niosomes

Niosomes are first developed and reported in the cosmetic industry in the 70s. Niosomes (nanosized) are vesicles in which the medication is entrapped in vesicle bilayers. Niosomes are expected to be better drug carrier systems because of factors such as physical stability, entrapment efficiency, bioavailability, cost, and so forth (see further section for a more complete list).

1.8.1. Salient features of niosomes

- Niosomes can entrap drugs like other such carriers such as liposomes.
- Niosomes are osmotically active and more stable than liposomes.
- Niosomes contain the hydrophilic head and hydrophobic tails which entrap the drug molecules with a wide range of solubility.
- Niosomal structure is flexible and can be designed according to the conditions in which they are required.
- Niosomes can act as a drug performance enhancer.

- Niosomes protect drugs from the biological environment and deliver drugs at the targeted site.
- Niosomes surfactants are biodegradable, biocompatible and non-immunogenic [61].

1.8.2. Structural arrangement of the niosome

Two essential components required for the preparation of the niosomes are lipid compounds such as cholesterol and non-ionic surfactants. The amount of cholesterol in the niosome formulation plays a crucial role in entrapment efficiency, the rigidity of the vesicle, and the drug release profile. The selection of surfactant compound is important in the development of niosomes because of high transition temperature and the longer alkyl chain of the surfactant plays a crucial role in the entrapment efficiency capacity of the niosomes. Niosomes are manufactured using a hydration process in which a non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class is combined with cholesterol [62]. The bilayer in the niosomes is prepared by using a non-ionic surfactant that has its hydrophilic ends from the outer and inner side of the vesicle facing each other and hydrophobic tails are within the bilayer. The schematic representation of the niosome structure is provided in Figure 1.6. The non-ionic surfactants form a closed bilayer vesicle structure in aqueous media based on their amphiphilic nature using external energy such as heat and physical mixing during the formation of these niosomes [63].

Non-ionic Surfactant Selection

Non-ionic surfactants are the critical elements for the preparation of niosomes multilamellar vesicles. The drug entrapment efficiency directly depends on the

hydrophilic head group and a hydrophobic tail group of the non-ionic surfactant. The HLB value of the surfactant plays an essential role in influencing the size of the niosomes and as the HLB value increases the niosome size increases. Due to this behavior, non-ionic surfactants with HLB values in the range of 14-17 are not ideal for niosomes formation [64]. Additionally, the surfactant structure plays a critical role in the stability of the niosome formulation by preventing aggregation between the niosome particles due to the repulsion of steric or electrostatic forces [65]. Various nonionic surfactants used to manufacture niosomes are Spans (Span 20 (sorbitan monolaurate), Span 40 (sorbitan monopalmitate), Span 60 (sorbitan monostearate), Span 80 (sorbitan Oleate), Span 85 (sorbitan trioleate), and), Tweens (Tween 20 (polysorbate 20), Tween 40 (polyoxyethylene sorbitan monopalmitate), Tween 60 (polyoxyethylene sorbitan monostearate) and Tween 80 (polysorbate 80)), and Brij™ (Brij™ 30) (Polyoxyethylene (4) lauryl ether), Brij™ 35 ((Polyoxyethylene (23) lauryl ether), Brij™ 52 (Polyoxyethylene (3) cetyl ether), Brij™ 58 (Polyoxyethylene (20) cetyl ether Brij™ 72 (Polyoxyethylene (2) stearyl ether) and Brij™ 76 (Polyethylene glycol monooctadecyl ether) [63, 66].

Cholesterol

The amount of cholesterol in the niosome formulation plays an essential role in entrapment efficiency, the rigidity of the vesicle, and the drug release profile. In the niosome vesicle structures, cholesterol (an amphiphilic compound) can mix with non-ionic surfactants to form hydrogen bonding between hydroxyl groups of cholesterol and the hydrophilic head of the surfactant. The result of this process shows improvement in

the mechanical rigidity of vesicles and membrane cohesion and the leakiness of membrane.

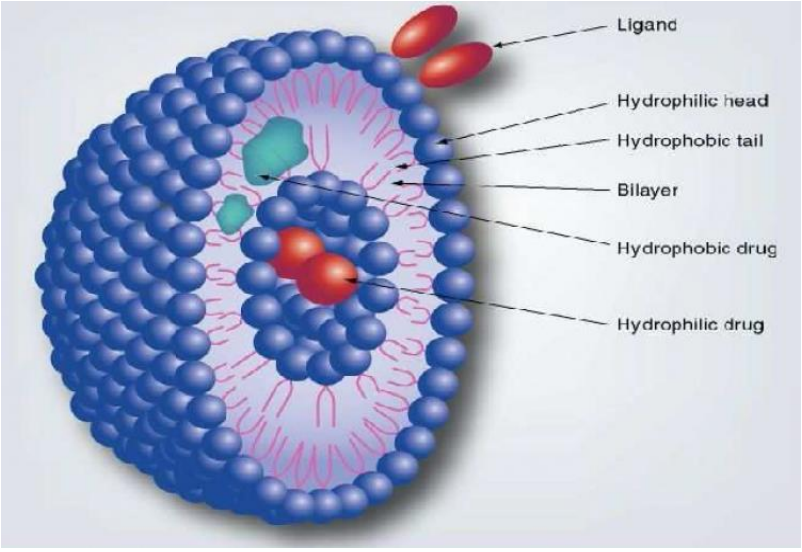

Figure 1.6. Schematic representation of a niosome structure [67].

Charge-Inducing Molecules

Charge-inducing molecules are added to the niosome to increase the stability of the niosome vesicle through electrostatic repulsion, which avoids aggregation and coalescence. The most common negative charge inducing agents such as stearic acid, diacetyl phosphate, and phosphoric acid, and positive charge inducing agents such as stearylamine and stearyl pyridinium chloride. The concentration of the charge inducing agent should be optimum as high concentration can prevent the formation of the niosomes.

1.8.3. Mechanisms of niosomal skin delivery

1) Niosomes in their entirety can diffuse from the stratum corneum layer of the skin to available at the target site.

2) Niosomes can break up and form new smaller vesicles in the skin. This mechanism may assist in their permeation where the space between the lipid lamellar spaces of the stratum corneum is smaller than that of the niosome vesicles [68].
3) Niosomes interact with the stratum corneum with aggregation, fusion, and adhesion to the cell surface, which causes a high thermodynamic activity gradient of the drug at the vesicle- stratum corneum surface. This is the leading force for the penetration of lipophilic drugs across the barrier or across the cells. [69].
4) Niosomes may alter the stratum corneum structure of the skin which makes the intercellular lipid barrier of stratum corneum looser and makes it more permeable. [70].
5) Non-ionic surfactant acts as a permeation enhancer and may partly contribute to the improvement in drug permeation from the niosomes [71].

1.8.4. Method of niosome preparation

The method of niosome preparation predominantly influences the size, size distribution, and the number of bilayers, entrapment efficiency, and the membrane permeability of the vesicles.

1) Handshaking or thin-film hydration method

At the beginning of this process, surfactant and cholesterol are dissolved in an organic solvent such as diethyl ether, chloroform, methanol, or a combination of solvents. Using the rotary flash evaporator, the organic solvent is evaporated at room temperature

at 20°C, which leaves a thin layer of the solid mixture on the wall of the flask. This thin layer is rehydrated using the aqueous solution with gentle agitation.

2) Ether injection method

In this method, the ether solution with a surfactant is injected at a pre-determined rate through a suitable needle into a preheated aqueous solution. Single layered needle shape niosomes are formed due to the vaporization of the organic phase [72]. The size of vesicles varies from 50 to 1000 nm based on the manufacturing conditions.

3) Micro fluidization method

This method manufacture unilamellar niosomes of desired size distribution, uniformity, and reproducibility. The basic principle behind this method is the submerged jet principle. The drug and the surfactant fluidized streams interact with each other at ultra-high velocities, into the microchannel within the interaction chamber. The impingement and the energy involved lead to the formation of niosomes [73].

4) Multiple membrane extrusion method

This is the ideal method for controlling the size of the niosome vesicles. The surfactant, cholesterol, and diacetyl phosphate mixture in chloroform is made into a thin film by the evaporation. In most cases, the thin film is rehydrated with the aqueous drug solution and the resultant suspension extruded through polycarbonate membranes, which are arranged in a series for up to eight passages [74].

5) Reverse phase evaporation method

In this technique, cholesterol and surfactant in the equal ratio are dissolved in the organic solvent mixture of ether and chloroform. Then, the aqueous drug solution is added to the mix. The two phases are sonicated at low temperatures (4-5°C). Small amounts of phosphate-buffered saline are added to the clear gel and the mixture is sonicated again. The organic phase is removed at 40°C and under lower pressure. Large unilamellar niosomes are formed during the organic solvent evaporation process [75].

6) Sonication method

An aliquot of the buffer solution containing the drug is added to the premixed surfactant and cholesterol mixture in a suitable container. After this, the mixture is sonicated at 60°C for 3 minutes with a titanium probe to manufacture a niosomal suspension [76].

7) Transmembrane pH gradient drug uptake method

Surfactant and cholesterol are dissolved in chloroform solvent in a specialized round bottom flask. A thin film of the mixture is generated on the wall of the flask by the evaporation process at reduced pressure. The film is then hydrated with 300 mM citric acid (pH 4.0) by vortex mixing to obtain the multilamellar vesicles. Then the mixture is placed in a freeze and thaw for three cycles followed by sonication to obtain the niosomal suspension. To obtain a pH between 7.0-7.2, 1M disodium phosphate buffer added to the mixture which is then vortexed to mix well. Then the final mixture is heated at 60°C for 10 minutes to yield the desired multilamellar vesicles [77].

8) The bubble method

The bubble method is a one-step preparation of niosomes without using any organic solvents. Surfactant, additives and the buffer are added into a bubbling unit. The bubbling unit has a round-bottomed flask with three necks positioned in a water bath to control the temperature. In the first neck there is water-cooled reflux, in the second a thermometer to record temperature and nitrogen is supplied through the third neck. Niosome components are dispersed in a pH of 7.4 buffer at 70°C and dispersion are mixed with homogenizer. After that, immediately the flask is placed in a water bath followed by the bubbling of nitrogen gas at 70°C [78].

9) Formation of niosomes from pro-niosomes

This technique includes the coating of a water-soluble carrier such as sorbitol and mannitol with surfactant. The coating process ends up in the formation of a dry formulation. This dry formulation is termed the “proniosomal” formulation which requires to be hydrated before being used. In this simple technique, water or saline at 80°C is added into a special screw-capped vial, which is prefilled with proniosomal powder. Then it is mixed by vortexing and followed by agitation for 2 minutes. The end of this process is the formation of niosomal suspension [79].

10) Emulsion method

Oil in water emulsion is prepared by adding an organic solution of surfactant, cholesterol, and the aqueous solution of the drug. The organic solvent is then evaporated leaving niosomes dispersed in the aqueous phase [80].

11) Lipid injection method

In this process, the mixture of lipids and surfactants is first melted and then injected into a highly agitated and heated aqueous phase containing the dissolved drug. Alternatively, the drug can be dissolved in the molten lipid and mixture and then it is injected into an agitated heated aqueous phase containing the surfactant. Mixing is continued for a certain period and depends on the experiment. The end of this process is the formation of the niosomal suspension [81].

1.9. Quality by design systematic approach

The fundamental concept of QbD is that "The quality cannot be tested in the product, but it should be built into the product." QbD is defined in the ICH Q8 Guidelines as a systematic risk-based, proactive approach to pharmaceutical development that begins with predefined objectives and emphasizes product and process understanding and process control based on advanced science and quality risk management.

Benefits of QbD [82]

- QbD is good for pharmaceutical businesses since it improves overall product quality.
- QbD process restricts the occurrence of batch failures.
- QbD improves product quality and minimizes out of specification deviations and costly investigations for the drug product.
- Avoids regulatory compliance problems.

- QbD is a new advanced concept and adopting QbD is the future investment for any company.
- QbD is a scientific concept that helps to minimize product failure.
- QbD provides robust data for the formulation development that helps for better development decisions.

1.9.1. Elements of QbD

The most widely accepted QbD elements consist of the quality target product profile (QTPP), critical quality attributes (CQAs), critical material attributes (CMAs), and critical process parameters (CPPs) [83, 84].

- Quality Target Product Profile (QTPP): QTPP includes dosage form, delivery systems, dosage strength(s), etc. It is a targeted summary of quality characteristics of a drug product to be achieved, dosage strength(s), and container closure system of the drug product. These quality characteristics are essential to verify that the final product meets the required standard of quality.
- Critical Quality Attributes (CQAs): CQAs including chemical, physical, biological, or microbiological properties or characteristics of an output material including finished drug product. CQAs represent the attributes that are important for the product's safety and efficacy. Targeted drug product CQAs derived from the prior knowledge and QTPP used to guide the final product and prove a development. They should be within the prefixed limit, range, or distribution to ensure the desired acceptable product quality.
- Critical Material Attributes (CMAs): CMAs include chemical, physical, biological, or microbiological properties of input materials. CMAs should be

within an acceptable limit, range, or distribution to ensure the targeted quality of the drug substance, excipient, or in-process material.

- Critical Process Parameters (CPPs): CPPs monitored before or throughout the process that influences the description, appearance, impurity, assay, and yield of the final product significantly. In other words, the process parameter whose variability has an impact on a critical quality attribute should be monitored and controlled to ensure the process produces the desired quality.

1.9.2. Steps for pharmaceuticals QbD implementation

The implementation of QbD in the development of new pharmaceutical products can go through the following steps [85].

1) Define the desired performances of the product and identify the QTPPs;
2) Identify CQAs;
3) Identify possible CMAs and CPPs;
4) Setup and execution of DoE to link CMAs and CPPs to CQAs and detail on how these parameters impact QTPP. After that, a process design space should be defined, leading to an end product with desired QTPP;
5) Identify and control the sources of variability from the excipients and the manufacturing process;
6) Continually monitor and improve the manufacturing process to verify consistent product quality.

1.9.3. Tools of QbD

QbD concept has two major components – the science underlying the design and the science of manufacturing. Upon understanding the elements of QbD and the steps for QbD implementation, it is essential to be familiar with the essential tools of the QbD, including risk assessment, design of experiment (DoE), and process analytical technology (PAT) [86].

1) Risk assessment

It is a systematic process of arranging available information to support a risk decision to be made within a risk management process. The key focus of this process is the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards. Risk assessment is the preliminary step in the sequence of quality risk management process followed by risk control and risk review. The goal of risk control is to reduce the risk to an acceptable level. In the last stage, the output/results of the risk management process should be reviewed to take into account new knowledge and experience.

Risk identification, risk analysis, and risk evaluation are three components of risk assessment. Risk analysis is the estimation of risk associated with the identified hazards, and risk evaluation is the comparison of the estimated risk of given risk criteria to determine the significant change of the risk.

2) Design of experiments (DOE)

A systematic approach to identify the relationship between factors affecting a process and the output of that process is known as 'Design of Experiments" (DoE). This method allows pharmaceutical scientists to manipulate factors according to a pre-specified design systematically. Sound design is based on sound cognition of product and effective management of the whole process during manufacturing. It is an efficient way to determine the connection between the inputs and outputs of a process. It is a crucial step to identify optimal conditions, CMAs, CPPs, and final design space.

3) Process analytical technology (PAT)

PAT is defined as "tools and systems that utilize real-time measurements, rapid measurements during processing, of evolving quality and performance attributes of in-process materials to provide information to ensure optimal processing to produce the final product that consistently conforms to established quality and performance standards" [87]. Based on the PAT concept, a process is considered well managed when: i) all critical sources of variability are identified and explained ii) variability is governed by the process, and iii) product quality attributes can be accurately and reliably predicted.

1.9.4. QbD approaches for the development of topical dermatological products

QbD approach in the development of topical dermatological products is highly recommended to develop a good quality product. Define QTPP and identifying the CQAs are critical parameters in designing a quality-based product. The risk assessment is an essential step to identify the CMAs and CPPs during the formulation and process design.

A general QbD approach to developing topical dermatological products is shown in Figure 1.7.

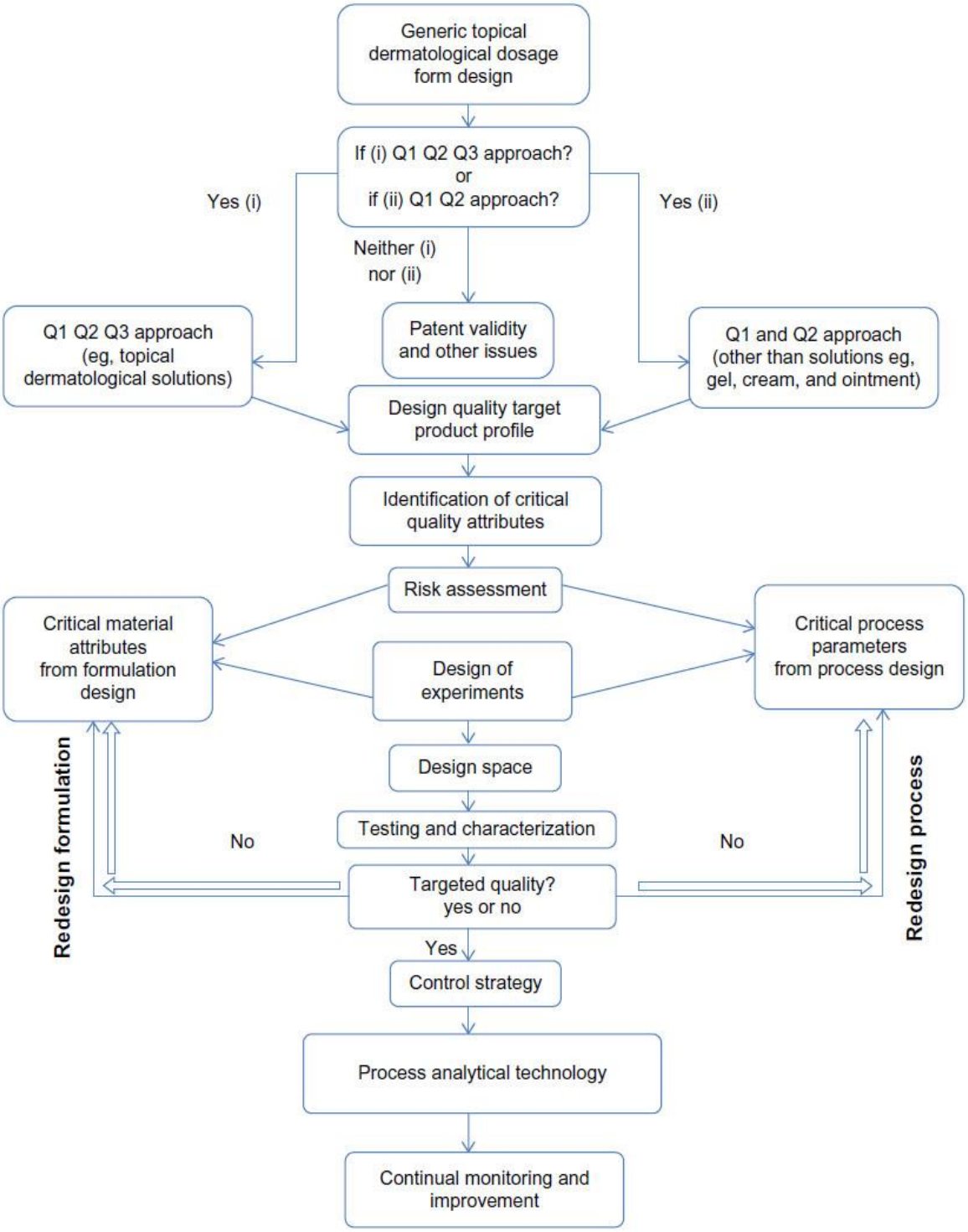

Figure 1.7. QbD approach for development of the topical dermatological products [88].

1.10. Various dosage forms and their impact on dermal drug delivery

Creams, ointments, gels, lotions, pastes, and foams are examples of semisolid formulations used topically and collodions, solutions and suspensions are examples of liquid formulations use for dermal drug delivery.

1) Creams

Creams are semisolid dosage forms that contain one or more drug substances dissolved or dispersed in a suitable emulsified base. Creams are thermodynamically unstable two-phase systems consisting of at least two immiscible oil and water, one of which is dispersed in the form of tiny droplets throughout the continuous phase. The phase present in the form of small droplets is known as the internal phase, and the continuous phase is known as the external phase. Creams are available in the form of either oil in water emulsions or water in oil emulsions. Creams typically contain > 20% water and volatiles and < 50% of hydrocarbons, waxes, or polyethylene glycols [89, 90]. A few examples of pharmaceutical creams are listed here.

- Antiviral cream: Denavir® 1% cream (for cold sores)
- Antihistamine cream: Benadryl® 2% itch cream
- Antifungal cream: Ketoconazole 2% cream
- Anesthetic cream: Pliaglis® 7% cream

2) Ointments

Ointments are greasy, viscous, semisolid preparations containing dissolved or suspended ingredients for external application to the skin. They usually contain < 20%

water and volatiles and more than 50% hydrocarbons, waxes, and polyols as the vehicle. Ointment bases recognized for use as vehicles fall into four major types: hydrocarbon base, absorption base, water-removable base, and water-soluble base. The ratio and grades of the ointment base components are specific to give the desired finished product viscosity/spreadability [89, 90]. A few examples of pharmaceutical ointments are listed here.

- Anti-inflammatory ointments Temovate® (clobetasol propionate) 0.05% ointment
- Anti-inflammatory/Anti-pruritic ointment: Diprolene® (betamethasone dipropionate) 0.05% ointment

3) Lotions

Lotions are thermodynamically unstable two-phase systems consisting of at least two immiscible liquid oil and water. Lotions are low to medium viscous product for application to the skin. Most lotions are oil in water emulsions in which tiny oil droplets are dispersed in the continuous water phase [89, 90]. A few examples of pharmaceutical lotion are listed here.

- Anti-inflammatory lotion: Clobex® (clobetasol propionate) 0.05% lotion
- Anti-inflammatory lotion: Diprolene® (augmented betamethasone dipropionate) lotion, 0.05%

4) Foams

Foams are emulsified systems packaged in pressurized containers or special dispensing devices. Foams are continuous one phase or two-phase system filled in a container with compressed hydrocarbon gas or combination of gases. Due to compression

in the container, when gas is dispensed, it is fluffy with semisolid consistency [89]. A few examples of pharmaceutical topical foams are listed here.

- Anti-inflammatory foams: Olux® (clobetasol propionate) 0.05% foam
- Anti-inflammatory foams: Luxiq® (betamethasone valerate) foam, 0.12%

5) Topical solutions

Water is the main ingredient of a topical solution, however often, other solvents such as alcohol and polyols that contain one or more dissolved chemical substances are included and are intended for topical application to the skin [89]. A few examples of topical pharmaceutical solutions are listed here.

- Anti-inflammatory acne solution: Cleocin T® (clindamycin phosphate) 1% solution
- Anti-inflammatory scalp solution: Temovate scalp application - clobetasol propionate solution 0.05%

6) Gels

A gel consists of suspended particles that form a “scaffold-like” structure in a dispersion medium. These particles, often called a gelling agent to undergo a high degree of cross-linking or association when hydrated, forming an interlaced three-dimensional structure that provides stiffness to the system. Gels show shear-thinning properties, meaning that they can spread easily on the skin when applied with pressure. Gels are transparent or translucent semisolid preparations of suitable hydrophilic or hydrophobic bases. Gels are prepared by various methods such as the fusion process or a particular procedure using gelling agents, humectants, and preservatives [91]. Gels can be water-

based or water-alcohol based system. The gel contains typical inactive ingredients include preservatives, antioxidants, pH-adjusting agents, and chelating agents. A few examples of pharmaceutical gels are listed here.

- Anti-inflammatory gel: Topicort® (desoximetasone) 0.05% gel
- Anti-inflammatory acne gel: Cleocin T® (clindamycin phosphate) 1% gel

7) Pastes

Pastes are semisolid dosage forms that contain a high concentration of finely dispersed solids with a stiff consistency intended for dermal application. Typically, two main classes of pastes are available in the market. One typical class is made from single-phase aqueous gels. In contrast, another class is the fatty pastes consisting of thick stiff ointments that normally do not flow or move at average body temperature and, therefore, serve as protective coatings. Usually, a paste is less greasy than an ointment [89, 90].

8) Topical suspensions

A suspension is a two-phase system consisting of finely divided solid materials dispersed in a continuous vehicle liquid. Suspending agents are added in the suspension to reduce the sedimentation rate of solid material by forming a film around the suspended particles and/or by increasing thickness of the suspension [89]. A few examples of topical pharmaceutical suspensions are listed here.

- Anti-fungal suspension: Loprox® (ciclopirox) 0.77% topical suspension
- Plaque psoriasis suspension: Taclonex® (calcipotriene hydrate and betamethasone dipropionate) topical suspension.

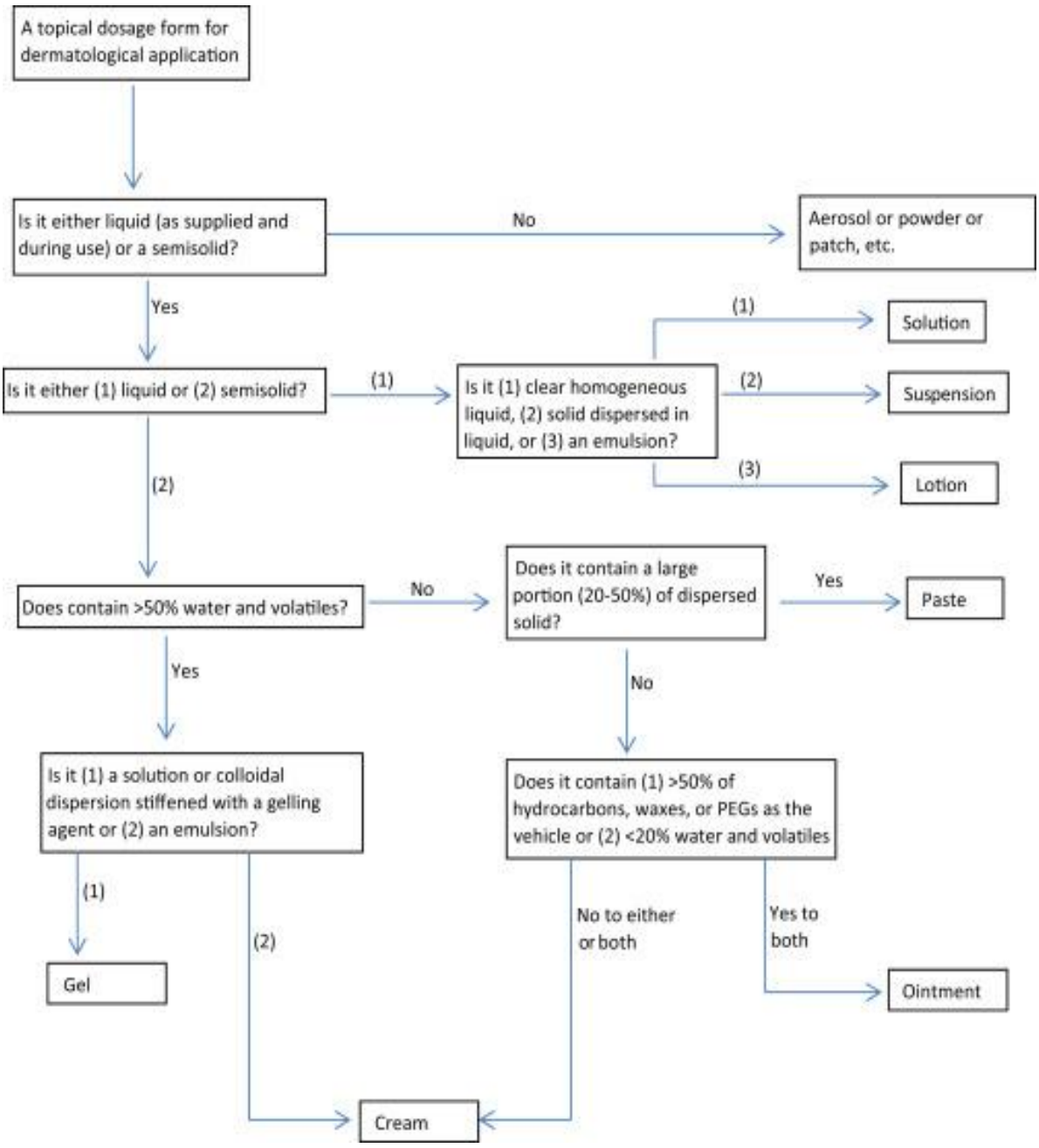

Figure 1.8. Classification of dermal drug delivery [92].

9) Liniments

The liniments are liquid or semisolid preparations use for topical application. Typically liniments act as rubefacient, soothing, or stimulants that contain alcohol, oil, or surfactant in the vehicle system [90].

10) Topical powders

Topical powders are solids or a mixture of solids in dry, finely divided insoluble states and are for external use only. Common ingredients used for the powder mixtures are talc, zinc oxide, or starch often used on the skin or wounds, especially for absorbing moisture, which discourages bacterial growth. Many are also used for their lubricant properties [89, 90].

11) Sprays

Sprays are very useful topical products formed by generating droplets of the solution containing the dissolved drug for dermal or mucous applications. A specially designed nozzle assembly is the most common technique to generate droplets. The metered-dose topical/ transdermal spray is the most common example in this class that delivers a controlled quantity of solution or suspension on each activation [89]. A few examples of topical pharmaceutical sprays are listed here:

- Anti-inflammatory spray: Clobex (clobetasol propionate) spray, 0.05%
- Anti-inflammatory scalp spray: Kenalog® spray (triamcinolone acetonide) topical aerosol, USP (0.147 mg/g).

1.11. Specific aims

In a dermal drug delivery system, achieving stable and prolonged effective permeation rates across the skin barrier is a challenge since the drug concentration in the formulation matrix has to be high to maintain a high concentration gradient of the drug within the skin. The goal of this study is to design an effective and stable topical gel

formulation for dermal delivery of anti-inflammatory glucocorticoids using a non-ionic surfactant system (niosomal) with a sustained release of the drug which will stay in the skin layers and reduce dosing frequency and/or have a reduced drug concentration to achieve the same therapeutic effectiveness.

Specific Aim 1: Developing, formulating and evaluating desoximetasone loaded niosomes using Quality by Design (QbD) elements.

This preliminary study was performed to provide data for various formulations designed by considering the Critical Material Attributes (CMAs) and Critical Processing Parameters (CPPs). The study was conducted using desoximetasone as a model drug. The drug was encapsulated in niosomes intended for topical drug delivery, and the formulation design focused on the impact of the various CMAs and CPPs such as drug concentration, surfactant, cholesterol, lipid concentration, internal and external phase volume, external phase temperature, mixing speed, mixing time and addition rate of the organic solvent solution. Each formulation was characterized for drug entrapment efficiency, particle size, polydispersity index, and zeta potential. Different analytical tools were utilized in this study as a screening tool to identify which CMAs and CPPs show the dominant effect on the final product and those we can consider for the detailed evaluation using the statistical tools.

Specific Aim 2: Quality by Design (QbD): A systematic approach using an advanced statistical tool to the optimization of niosomes preparation for the topical delivery.

Based on the preliminary experiments with 11 QbD elements such as organic solvent, drug concentration, surfactant cholesterol, cholesterol concentration, lipid type,

external phase temperature, external phase volume, internal phase volume, mixing speed, mixing time and addition rate along with the sound knowledge of Quality by Design, five elements such as 1) surfactant concentration 2) cholesterol concentration, 3) mixing speed, 4) mixing time, and 5) addition rate were considered as most impacting elements on niosomal formation. In this phase of the study, five impacting elements were used in two-level (high and low), and a detailed study was designed with full factorial model 2^5 using JMP® statistical software. The full factorial model suggested 32 additional formulation combinations and based on the study requirement, 32 experiments were executed. Each formulation was characterized for drug entrapment efficiency, particle size, polydispersity index, and zeta potential. Results from the experiments were imported into the full factorial model and based on the entered data the software generated a profile predictor. Using this profile predictor model, two different combination formulas were created to validate the model accuracy by comparing model-predicted data with actual experimental results. Based on the profile predictor best formulation combination for desoximetasone niosomes was selected. Additionally, a batch was made by replacing Span 60 surfactant with Span 20 to verify that selected surfactant Span 60 is the best compatible surfactant for the niosomal formulation.

Specific Aim 3: Development, formulation and evaluation of non-ionic surfactant vesicles (niosomes) based topical gel.

A semi-solid formulation is the most preferred way to deliver drugs topically, and hence a viscous gel of desoximetasone niosomes was developed for easy application to the skin. The various topical gels studied here were formulated with different Carbomer 980 concentrations: 0.62%, 0.70%, 1.00%, 1.50%, and 2.00% to evaluate the impact of

the viscosity agent concentration on the topical gel product. Additionally, more topical gel formulations were prepared by replacing Carbomer 980 with Carbomer 940, Carbomer 974p, Carbomer 981, Carbomer 1342, ethyl cellulose, hydroxyl propyl cellulose, hydroxypropyl methylcellulose, and xanthan gum to evaluate the impact of the various viscosity agents on the topical gels. All the generated gel formulations as well as Topicort® (Desoximetasone) gel USP, 0.05% reference marketed product were characterized for drug content, rheological properties, specific gravity, pH, spreadability, content uniformity, and organoleptic properties such as color, texture, homogeneity, phase separation, and description. The formulation with identical or similar rheological characteristics to Topicort® gel, 0.05% was chosen for the next series of studies. In-vitro permeation testing was performed in order to compare drug permeation from Topicort® gel 0.05% (reference marketed gel product) and selected desoximetasone niosomal topical gel formulations (test products) using human cadaver skin. In addition to amounts of drug permeated across the skin membranes, skin retention of the drug was also recorded.

Specific Aim 4: Stability evaluation of final desoximetasone niosomal dispersion and desoximetasone niosomal topical gel.

Stability studies are an integral part of successful formulation development. The ideal formulation should be stable at different stability conditions for the specified times. In this study, stability testing was performed with final optimized desoximetasone niosomal dispersion and the desoximetasone niosomal topical gel formulation simultaneously at room temperature and accelerated temperature (40°C). Stability study evaluation for desoximetasone niosomal dispersion and desoximetasone niosomal topical

gel was performed at 0 days, 15 days, one month, two months and three months at room temperature and 40°C. At each time point, desoximetasone niosomal dispersion was evaluated for drug content, entrapment efficiency, particle size, polydispersity index, and zeta potential and desoximetasone niosomal topical gel was evaluated for drug content, pH, spreadability, color, phase separation, texture, homogeneity, and description. Success was defined by a stable formulation with a controlled release of the drug from the formulation and optimized retention of the drug into the skin layers. Such formulation results in reducing dosing frequency and/or reducing drug concentration in the original formulation.

1.12. References

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2. CHAPTER - 2. DEVELOPING, FORMULATING AND EVALUATING DESOXIMETASONE LOADED NIOSOMES USING QUALITY BY DESIGN (QbD) ELEMENTS

2.1. Introduction

Drug delivery systems play a significant role in dermatological product development for creating efficient treatments for patients [1]. Sustained release drug delivery systems may be required to achieve modified drug release kinetics with targeted site-specific delivery, which shows improved therapeutic efficacy and patient compliance. The concept of site-specific drug delivery was introduced to the emerging pharmaceutical industry by Paul Elrich in 1909 [2]. The core purpose of the target site-specific drug delivery system is not limited to increasing the selectivity but also eliminating the systemic toxicity of the drug. An ideal drug delivery system should carry the therapeutic agent to the targeted site and adequately release it over a predetermined time [3].

Colloidal particulate carriers such as niosomes and liposomes have distinct advantages over conventional non-carrier topical dosage forms. The carriers can act as drug reservoirs from which the drug can be released at the targeted site, and the drug release can be tailored by changing the composition design of the carriers [4].

In recent times, niosome technologies are broadly studied as an alternative to solve the drawbacks of liposomes as carrier systems. Disadvantages with the liposome formulations are high formulation cost and limited shelf life, which eventually lead to the development of vesicular systems that may overcome significant concerns of the

liposomal formulations. Niosomes are non-ionic surfactant vesicles with alternatives that share identical properties and components when compared to the liposomes. Niosomes are highly organized microscopic drug-containing vesicles and commonly measure between 10 and 1000 nm in diameter. Niosomes can be unilamellar or multi-lamellar vesicles, where an aqueous solution is enclosed by a bilayer structure consisting of non-ionic surfactants, such as Span 20, Span 40, Span 60, Span 80, Tween 20, Tween 60, Tween 61, Tween 80, Brij^{TM} with or without cholesterol [5-7]. The hydrophilic drugs are entrapped in the hydrophilic core, and lipophilic drugs, which are distributed entirely around the bilayer. The drug with intermediate logP values is appropriately allocated between the hydrophilic and lipophilic phases (both in the bilayer and in the hydrophilic core). The fluidity of the niosomal bilayers is depended on the hydrophilic head group, alkyl chain length, and cholesterol content [8]. Niosomes are established carrier candidates that have been successfully used in target drug delivery to the skin, brain, liver, lung, and ocular system. In the past two decades, niosomes use is extended to drug delivery systems for anticancer, anti-inflammatory, antifungal, and dermal (topical/transdermal) applications [9-14].

Recently, niosomes have attracted attention for topical/transdermal drug delivery because of their key advantages, such as non-toxicity, biodegradability, non-immunogenicity, modulation of drug bioavailability, enhancement of drug penetration, provision of solubilizing matrix and local depot in deeper layers of skin for controlled drug release. Moreover, topical administration of niosomes can increase the residence time of the drug in the stratum corneum and epidermis, thereby increasing the skin deposition while reducing systemic absorption, since the outer layers act as rate-limiting

membranes [15, 16]. During the topical application, niosomes show the desired interaction with human skin by enhancing the stratum corneum characteristics due to a reduction in transepidermal water loss and an increase in smoothness via replenishing the lost skin lipids. Additionally, the adsorption and fusion of niosomes on the skin surface show a higher thermodynamic activity gradient of the drug at the site of the interface and, therefore, can influence the permeation of lipophilic drugs [17-19].

Common skin diseases such as atopic dermatitis, redness, itching, eczema, and psoriasis are usually treated with topical corticosteroids. Topical corticosteroids can be encapsulated in a suitable carrier system that can improve therapeutic efficacy and drug targeting efficiency by reducing adverse effects and improved patient compliance.

Desoximetasone is a model drug used to treat various skin diseases, including eczema and psoriasis, and acts by minimizing inflammation and relieving itching. Desoximetasone is a safe and effective topical corticosteroid which widely accepted by patients [20]. The molecular weight of 376.468 g/mol, logP of 2.35, and aqueous solubility of 0.031 mg/ml are the critical characteristics of the desoximetasone. Due to these characteristics, along with poor water solubility, desoximetasone is not an ideal drug for conventional dermal drug delivery. A niosomal vesicle based drug delivery system may be an ideal way to deliver a poorly water-soluble drug by improving water solubility and modifying drug penetration at the target site.

For the manufacturing of niosome vesicles, various material attributes and processing parameters can influence the final product. Thus, the study of CMAs and CPPs in niosome preparation provides valuable information about carrier drug delivery. The quality by design (QbD) systematic approach includes designing and developing a

final product in which manufacturing processes meet pre-designed product criteria [21]. QbD is a systematic approach to recommend that quality should be built into the process and product during the development process. The design of experiments can be conducted and the effect of several factors can be considered by varying them simultaneously and by carrying out an optimum number of experiments. Thus, using this advanced technique, the time and cost of drug development can be reduced significantly.

Moreover, it is useful to obtain the ideal possible combination for the formulation design and obtain a holistic understanding of product development [22, 23]. This advanced drug development approach is widely promoted by the Food and Drug Administration (FDA) and the International Conference on Harmonization (ICH). Moreover, QbD elements are necessary regulatory requirements for the new product submission to the FDA agency [24, 25].

In this research, we aimed to manufacture drug-loaded niosomes using the ether injection method (Figure 2.1) in order to find the CMAs and CPPs, which are critical impacting elements on the final formulation/product. Desoximetasone niosomal formulation was characterized by examining drug content, niosomal entrapment efficiency, niosomal particle size, polydispersity index, and zeta potential.

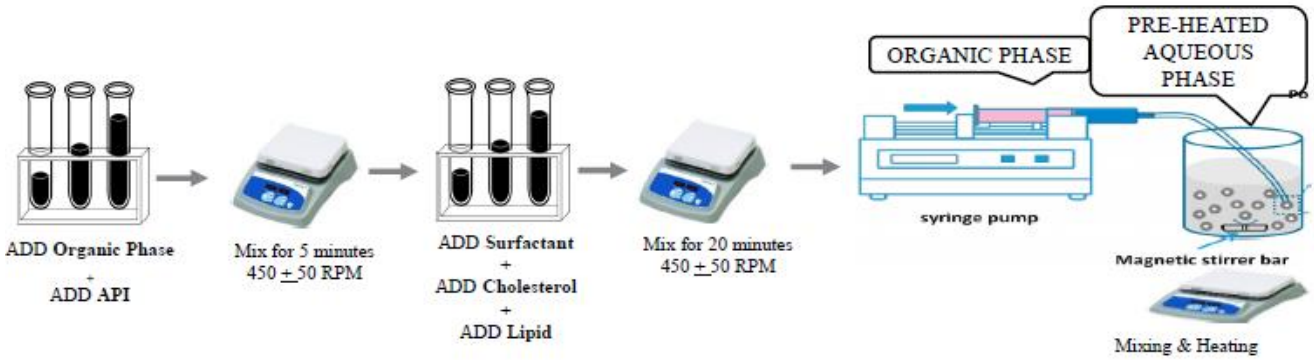


Figure 2.1. Schematic representation of the ether injection method.

2.2. Materials and Methods

2.2.1. Materials

Desoximetasone was gifted by Flavine, New Jersey, USA. Diethyl ether and Stearyl amine were purchased from Sigma-Aldrich, Saint Louis, MO, USA. Ethanol was procured from Decon Labs, Inc., King of Prussia, PA, USA. Acetone, methanol, and acetonitrile were purchased from BDH VWR Analytical, Radnor, PA, USA. Cholesterol and Span 60 (sorbitan monostearate) were gifted from Croda Inc., Mill Hall, PA, USA. Stearic acid was received from BASF Corporation, Edison, NJ, USA. HPLC water and chloroform were procured from Sigma-Aldrich, Saint Louis, MO, USA. Glacial acetic acid was purchased from Fisher Scientific, Fair Lawn, NJ, USA.

2.2.2. Niosome vesicle preparation

Initially, a pre-weighed amount of drug was placed into an organic phase and mixed thoroughly until it was completely dissolved. Next, surfactant, cholesterol, and lipid were added into the solution and mixed using a magnetic spin bar in a 20 mL glass scintillation vial. Purified water was heated in a separate 50 mL glass beaker at various predetermined temperatures using a hot plate with magnetic stirring. The temperature of the aqueous phase was selected based on the experimental design. The organic phase was then filled into a 10 mL syringe using a 26 G needle. The organic phase mixture was injected into the preheated water phase using predetermined parameters based on the study design. Mixing was carried out based on the values identified from the design of experiments. In the final step of the process, the batch was cooled down to room temperature, and the formulation was stored in a suitable glass storage container.

2.2.3. Desoximetasone

Desoximetasone is used to treat inflammation and relieve itching caused by various skin conditions such as allergic reactions, eczema, psoriasis, and pruritic manifestations of corticosteroid-responsive dermatoses. Desoximetasone is a synthetic glucocorticoid receptor agonist with metabolic, anti-inflammatory, and immunosuppressive activity. Desoximetasone activates specific intracellular receptors that bind to distinct sites on DNA to modify gene transcription. This results in an induction of the synthesis of anti-inflammatory proteins and suppression of the synthesis of inflammatory mediators. This leads to a decreased level of inflammation and autoimmune reactions [26].

2.2.3.1. Desoximetasone characteristics

- Mechanism of action: The mechanism of action of desoximetasone is a corticosteroid hormone receptor agonist
- Molecular formula: $C_{22}H_{29}FO_4$
- Molecular weight: 376.468 g/mol
- Physical form: Practically white powder
- LogP: 2.35
- Melting point: + 206°C to + 218°C
- CAS number: 382-67-2
- IUPAC name: (8S,9R,10S,11S,13S,14S,16R,17S)-9-fluoro-11-hydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-,8,11,12,14,15,16,17-octahydro-6H-cyclopenta[a]phenanthren-3-one

- Solubility: Insoluble in water, soluble in alcohol, chloroform and acetone
- Chemical structure:

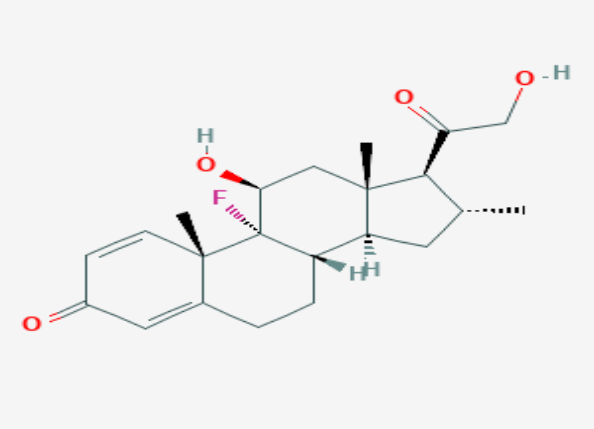


Figure 2.2. Desoximetasone chemical structure [26].

2.2.4. Desoximetasone analysis

A validated HPLC method was developed to quantify concentrations of desoximetasone (assay value) and amounts of an entrapped drug in niosomal vesicles (entrapment efficiency) in the formulation.

High performance liquid chromatography (HPLC) method

The mobile phase was prepared by mixing methanol, HPLC grade water, and glacial acetic acid in ratio 65:35:1 [27]. The diluent was prepared by mixing methanol and acetonitrile in ratio 50:50. The HPLC equipment used was Agilent 1100 series chromatograph (Agilent Technologies, CA, USA) coupled with a UV/vis detector (DAD) and HP ChemStation software V.32. [27]. The flow rate was 1.0 mL/min, and the injection volume was 10 µL. The sample run time was 10 min at room temperature and retention time for the drug peak was at approximately 5 minutes. Drug content quantification for desoximetasone was performed using HPLC coupled with UV analysis at a wavelength of λ_{max} 254 nm.

2.2.4.1. Standard solutions and calibration curve

A desoximetasone standard stock solution of 400 µg/mL was prepared by dissolving 10.0 mg of desoximetasone into 25.0 mL volumetric flask, and methanol was used to make the final volume 25.0 mL. 20 mL of the standard stock solution was transferred to a 100.0 mL volumetric flask, and the final volume was made with diluent (Methanol: Acetonitrile :: 50: 50) to give a final concentration of 80 µg/mL. The process was repeated with a suitable quantity of diluent to produce calibration standards of 40, 20, 10, 5, and 2.5 µg/mL.

2.2.4.2. Linearity and precision for HPLC analytical method

The precision of an analytical method is the similarity between a series of individual measurements repeatedly applied to numerous aliquots of the same sample. It can be calculated as a relative standard deviation (RSD).

The method was validated for linearity of the calibration curve. Intraday variability was determined by running HPLC measurement for the standard solutions three times a day. The linearity measurement was performed as per the ICH guideline with six serial dilutions ranging from 2.5 to 80.0 µg/mL with an R^2 value of 0.999. Drug quantification was determined using the pre-determined HPLC method, as mentioned in section 2.2.4. The limit of quantification is 7.9 µg/mL, and the limit of detection is 2.6 µg/mL.

2.2.5. Niosomal vesicle characterization and data analysis

2.2.5.1. Organoleptic properties

Niosomal dispersions were evaluated for visual appearance, color, and odor to confirm any residual solvent standard quality check.

2.2.5.2. Assay determination (Drug content)

The desoximetasone niosomal dispersion was carefully collected and placed into an intermediated solvent containing a mixture of chloroform: methanol (40:60) and then using a vortexing mixer was mixed until it completely dissolved at room temperature. In the final step, niosomal dispersion samples were further diluted with equal ratios of diluent. Drug quantification was determined using the pre-determined HPLC method, as mentioned in section 2.2.4.

2.2.5.3. Drug entrapment efficiency of niosome vesicles

The desoximetasone free drug was separated and determined from the entrapped drug in niosomal formulation. To separate the free drug from the formulation, ultracentrifugation at 14,000 rpm for 30 minutes using an ultracentrifuge (Branson Ultrasonics Corporation, CT, USA) at room temperature was performed. To validate the centrifugation method, centrifugation speed was evaluated using 3750 rpm and 14,000 rpm, and centrifugation time was evaluated between 30 min and 60 min. The supernatant containing the free drug was carefully collected and separated from the sediment of the sample without disturbing the formulation. The supernatant was further dissolved into chloroform: methanol (40:60) mixture using a vortex mixer. After mixing, the sample was further diluted with an equal amount of the diluent. Drug quantification was

determined using the pre-determined HPLC method, as mentioned in section 2.2.4. Drug % entrapment efficiency was calculated in triplicate by using the following formula [28].

$$\% \text{ Entrapment Efficiency} = \frac{Total\ amount\ of\ Drug - Free\ amount\ of\ Drug}{Total\ amount\ of\ Drug} \times 100 \quad [29]$$

2.2.5.4. Niosomes vesicle size and polydispersity index (PDI)

The mean vesicle size and distribution were evaluated at room temperature using a Delsa Nano S Particle Sizer (Beckman Coulter, CA, USA) in triplicate based on the light scattering spectroscopy principles.

2.2.5.5. Niosomes zeta potential

The zeta potential of the niosomal dispersion was measure in triplicate using a Malvern Particle Sizer 2000 (Malvern Technologies, Worcestershire, UK).

2.3. Systematic experimental design – optimization of drug loaded niosomes

Known elements were identified through a literature search and published manuscripts.

2.3.1. Detail about critical material attribute(s) (CMAs)

Based on the experimental design, 1) type of organic phase, 2) drug concentration, 3) surfactant concentration, 4) cholesterol concentration, and 5) types of lipids are considered as critical material attributes and studied in detail by the design of experiments.

Table 2.1. Critical material attributes and critical process parameters for the formulation design.

Variables: CMAs and CPPs	Parameters
Organic phase (Solvent)	1. Diethyl Ether 2. Diethyl Ether: Ethanol (80:20) 3. Diethyl Ether: Methanol (75:25) 4. Ethanol 5. Acetone
Drug concentration (mg)	1. 20 mg (0.2%) 2. 30 mg (0.3%) 3. 40 mg (0.4%)
Surfactant concentration (mg)	1. 20 mg (0.2%) 2. 30 mg (0.3%) 3. 40 mg (0.4%) 4. 50 mg (0.5%) 5. 60 mg (0.6%) 6. 70 mg (0.7%)
Cholesterol concentration (mg)	1. 20 mg (0.2%) 2. 30 mg (0.3%) 3. 40 mg (0.4%) 4. 50 mg (0.5%)
Selection of lipid	1. Stearic acid 2. Stearylamine 3. No lipids
External phase temperature (°C)	1. 55°C 2. 65°C 3. 75°C
External phase volume (mL)	1. 10 mL 2. 20 mL 3. 30 mL
Internal phase volume (mL)	1. 10 mL 2. 15 mL 3. 20 mL
Mixing speed (rpm)	1. 450 rpm 2. 550 rpm 3. 650 rpm
Mixing time (minutes)	1. 30 min 2. 45 min 3. 60 min
Addition Rate (mL/min)	1. 0.25 mL/min 2. 0.50 mL/min 3. 1.00 mL/min

Table 2.2. Niosome preliminary batch experimental design based on selected critical material attributes (CMAs) and critical process parameters (CPPs).

Batch Detail	Critical Material Attributes					Critical Process Parameters					
	Drug (mg)	Organic Phase Composition	Span 60 (mg)	Cholesterol (mg)	Stearic Acid (mg)	External Phase Temperature (°C)	External Phase Volume (mL)	Internal Phase Volume (mL)	Mixing speed (RPM)	Mixing time (Minutes)	Add. Rate (mL/Min)
DND-1	20	**diethyl ether**	40	20	5	65	20	10	650	60	1.00
DND-2	20	**diethyl ether : ethanol**	40	20	5	65	20	10	650	60	1.00
DND-3	20	**diethyl ether : methanol**	40	20	5	65	20	10	650	60	1.00
DND-4	20	**ethanol**	40	20	5	65	20	10	650	60	1.00
DND-5	20	**acetone**	40	20	5	65	20	10	650	60	1.00
DND-6	**30**	diethyl ether : methanol	40	20	5	65	20	10	650	60	1.00
DND-7	**40**	diethyl ether : methanol	40	20	5	65	20	10	650	60	1.00
DND-8	20	diethyl ether : methanol	**20**	20	5	65	20	10	650	60	1.00
DND-9	20	diethyl ether : methanol	**30**	20	5	65	20	10	650	60	1.00
DND-10	20	diethyl ether : methanol	**50**	20	5	65	20	10	650	60	1.00
DND-11	20	diethyl ether : methanol	**60**	20	5	65	20	10	650	60	1.00
DND-12	20	diethyl ether : methanol	**70**	20	5	65	20	10	650	60	1.00
DND-13	20	diethyl ether : methanol	40	**30**	5	65	20	10	650	60	1.00
DND-14	20	diethyl ether : methanol	40	**40**	5	65	20	10	650	60	1.00
DND-15	20	diethyl ether : methanol	40	**50**	5	65	20	10	650	60	1.00
DND-16	20	diethyl ether : methanol	40	20	**5 (SA)**	65	20	10	650	60	1.00
DND-17	20	diethyl ether : methanol	40	20	**N/A**	65	20	10	650	60	1.00
DND-18	20	diethyl ether : methanol	40	20	5	**55**	20	10	650	60	1.00
DND-19	20	diethyl ether : methanol	40	20	5	**75**	20	10	650	60	1.00
DND-20	20	diethyl ether : methanol	40	20	5	65	**10**	10	650	60	1.00
DND-21	20	diethyl ether : methanol	40	20	5	65	**30**	10	650	60	1.00
DND-22	20	diethyl ether : methanol	40	20	5	65	20	**15**	650	60	1.00
DND-23	20	diethyl ether : methanol	40	20	5	65	20	**20**	650	60	1.00
DND-24	20	diethyl ether : methanol	40	20	5	65	20	10	**450**	60	1.00
DND-25	20	diethyl ether : methanol	40	20	5	65	20	10	**550**	60	1.00
DND-26	20	diethyl ether : methanol	40	20	5	65	20	10	650	**30**	1.00
DND-27	20	diethyl ether : methanol	40	20	5	65	20	10	650	**45**	1.00
DND-28	20	diethyl ether : methanol	40	20	5	65	20	10	650	60	**0.25**
DND-29	20	diethyl ether : methanol	40	20	5	65	20	10	650	60	**0.50**

2.3.2. Critical process parameter(s) (CPPs)

Based on the experimental design 1) external phase temperature, 2) external phase volume, 3) internal phase volume, 4) mixing speed, 5) mixing time, and 6) organic phase addition rate were considered as critical process parameters and were studied in detail by using the design of experiments DOE.

2.3.3. Formulation matrix design based on CMAs and CPPs

Preliminary trials were undertaken to establish the effect of variables as described here.

2.3.3.1. Selection of organic phase

For the selection of the internal phase, various investigations were carried out using different organic phase as 1) diethyl ether, 2) diethyl ether: ethanol (80:20), 3) diethyl ether: methanol (75:25), 4) ethanol and 5) acetone while other variables were kept constant as mentioned in Table 2.2.

2.3.3.2. Selection of drug concentrations

The niosomal dispersions were manufactured with different drug concentrations as 1) 20 mg, 2) 30 mg, and 3) 40 mg, while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.3. Selection of surfactant concentrations

The niosomal dispersions were manufactured with different surfactant concentrations as 1) 20 mg, 2) 30 mg, 3) 40 mg, 4) 50 mg, 5) 60 mg, and 6) 70 mg, while other variables were kept constant as mentioned in Table 2.2.

2.3.3.4. Selection of cholesterol concentrations

The niosomal dispersions were manufactured with different cholesterol concentrations as 1) 20 mg, 2) 30 mg, 3) 40 mg, and 4) 50 mg, while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.5. Selection of lipid types

The niosomal dispersions were formulated with different types of lipid as 1) stearic acid, 2) stearylamine, and 3) no lipids, while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.6. Selection of internal phase volumes

The niosomal dispersions were manufactured with different internal phase volume as 1) 10 mL, 2) 15 mL, and 3) 20 mL while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.7. Selection of external phase volumes

The niosomal dispersions were formulated with different external phase volume of 1) 10 mL, 2) 20 mL, and 3) 30 mL while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.8. Selection of external phase temperatures

The niosomal dispersions were manufactured with various external phase temperature as 1) 55°C, 2) 65°C, and 3) 75°C while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.9. Selection of mixing speed

The niosomal dispersions were manufactured with various mixing speed of 1) 450 rpm, 2) 550 rpm, and 3) 650 rpm while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.10. Selection of mixing times

The niosomal dispersions were manufactured with a various mixing time of 1) 30 min, 2) 45 min, and 3) 60 min while other variables were kept constant, as mentioned in Table 2.2.

2.3.3.11. Selection of addition rates

The niosomal dispersions were manufactured with various addition rates of the internal phase of a) 0.25 mL/min, b) 0.50 mL/min, and c) 1.00 mL/min while other variables were kept constant, as mentioned in Table 2.2.

2.3.4. Risk assessment of critical quality attributes from preliminary phase batches to develop a QbD approach

Risk assessment has been performed carefully to identify CMAs and CPPs, which may affect product quality for CQAs by process characterization that defines satisfactory changes in CMAs and CPPs. Moreover, this can result in quality assurance by process design space to understand and develop a control strategy. The critical quality attributes are categorized into high, medium, and low-risk parameters based on knowledge space. Technically, high-risk parameters are considered necessary for the Design of Experiments as they are having more effect than medium or low-risk parameters and required to be accepted in multivariate ranges [30].

2.4. Results and Discussion

Niosome formulations may be influenced directly by surfactant chemistries, process parameters, physiochemical drug-specific attributes, and presence of membrane additives. To achieve better entrapment efficiency in the vesicle matrix, surfactants with alkyl-chain lengths with C12-C18 are the best suited for ensuring stable and robust niosomes, as demonstrated in previous literature [31]. Sorbitan monostearate (Span 60) is solely a surfactant used in the preparation of niosomal formulation because of its longer saturated alkyl chain characteristics, which shows higher drug loading capacity than other surfactants. Moreover, the higher phase transition temperature and lower HLB (4.7) of Span 60 are essential elements for its higher entrapment efficiency capacity [32].

Desoximetasone niosomes were prepared using the Span 60, cholesterol and lipid by considering type of organic solvent, drug concentration, surfactant concentration, cholesterol concentration, selection of lipid as CMAs and external phase temperature, external phase volume, internal phase volume, mixing time, mixing speed, and addition rate as CPPs. Niosomes were evaluated for entrapment efficiency, particle size, polydispersity index, and zeta potential.

2.4.1. HPLC method validation

Using the current HPLC method, the desoximetasone peak appeared around 4.9 min and showed in Figure 2.3. The peak's shape passed the requirement for symmetry and sharpness.

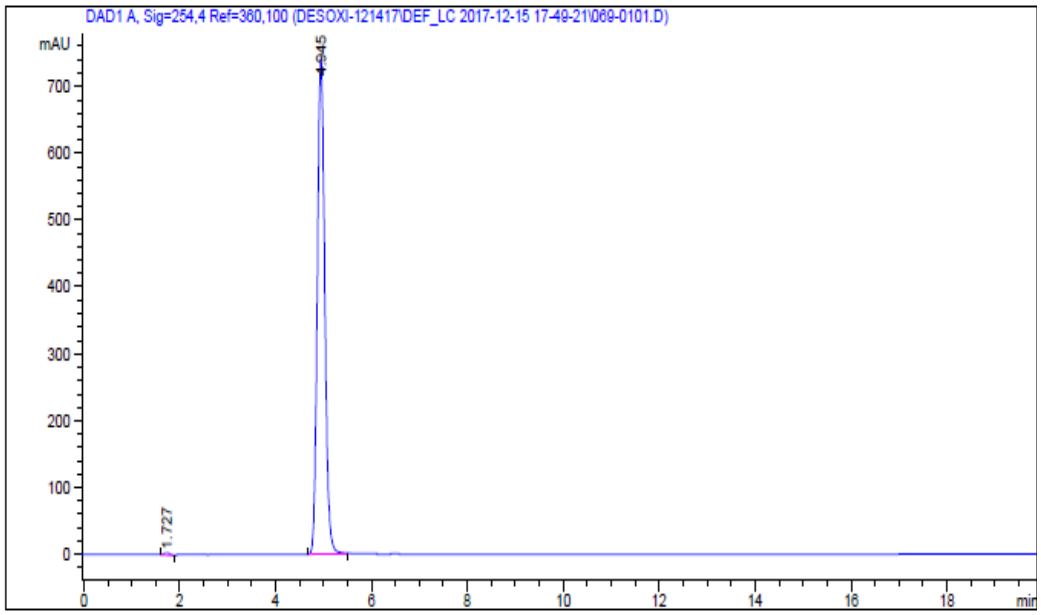


Figure 2.3. Desoximetasone chromatogram peak at the retention of 4.9 min.

The average standard calibration curve obtained from three separate injections for desoximetasone solution ranging from 2.5 - 80 µg/mL was shown in Figure 2.4, and data given in Table 2.3. The method was linear with an R^2 value of 0.999.

Table 2.3. Desoximetasone standard curve.

Concentration (µg/mL)	Average AUC (mAU*s)	SD	%RSD
2.5	47.69	0.142	0.299
5.0	95.08	0.192	0.202
10.0	186.56	0.414	0.222
20.0	363.00	0.038	0.010
40.0	732.06	0.923	0.126
80.0	1530.36	2.070	0.135

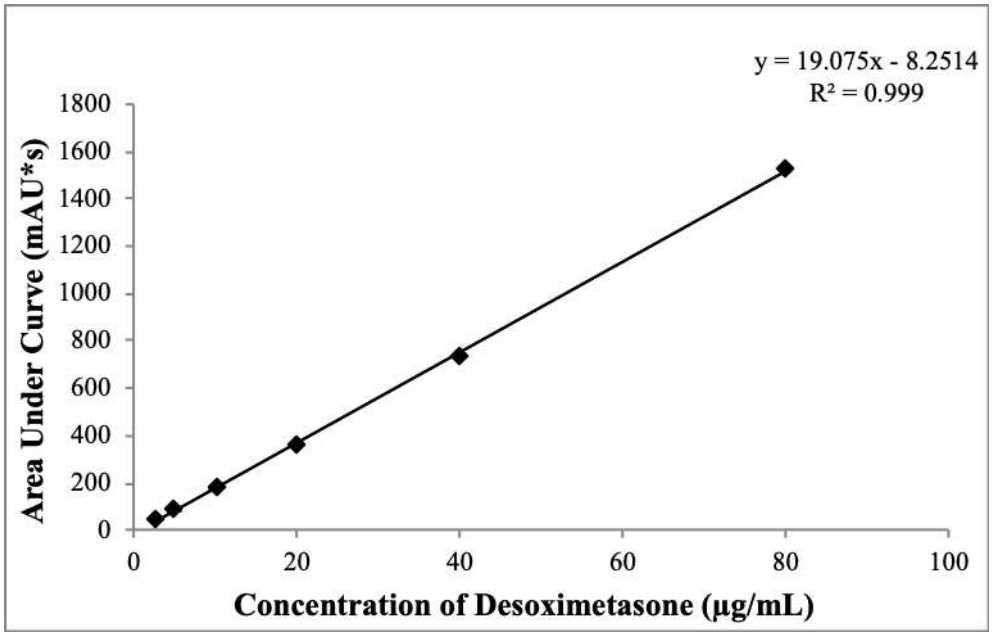


Figure 2.4. Desoximetasone standard curve for the HPLC assay method.

The intra-day and inter-day variations of the standards were determined by replicated analysis (n=3) of calibration samples of desoximetasone at concentrations within the range of calibration curve (10 – 40 µg/mL) in a single analytical run on the same day and in three different days, respectively. The results of intra-day and inter-day variability assessments are provided in Table 2.4 and 2.5 respectively. For intra-day and inter-day precision, the % RSD for the slop of the best fit was calculated as 0.12% and 0.77% respectively. These values are lower than the requirement % RSD value of 2%

Table 2.4 Intra-day variability of Desoximetasone standard solutions of three separate runs in one day.

Conc. (ug/mL)	Average AUC (mAU*s)	SD	% RSD
10	211.32	0.42	0.20%
20	426.10	0.49	0.12%
40	796.75	0.99	0.12%

Table 2.5 Inter-day variability of Desoximetasone standard solutions of three separate runs in three days.

Conc. (ug/mL)	Average AUC (mAU*s)	SD	% RSD
10	195.22	2.60	1.33%
20	396.19	7.39	1.87%
40	807.03	6.20	0.77%

2.4.2. Organoleptic Properties

Niosomal formulations are described as being milky white, odorless dispersions (Table 2.6) with a fluid-like consistency. Except in formulation DND-1, niosomal dispersions were obtained in all the formulations. Niosomal dispersion was not obtained in formulation DND-1 due to drug insolubility in diethyl ether [33].

Table 2.6. Preliminary design of experiments results summary.

Batch Detail	RESULTS (NIOSOMAL DISPERSION)					
	Dispersion formed or not	Organoleptic properties	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
DND-1	No	N/A	N/A	N/A	N/A	N/A
DND-2	Yes	White Milky	81.48 ± 0.07	423.27 ± 16.48	0.294 ± 0.04	− 75.63 ± 0.61
DND-3	Yes	White Milky	93.69 ± 0.05	374.80 ± 9.48	0.289 ± 0.01	− 63.83 ± 4.26
DND-4	Yes	White Milky	75.30 ± 0.06	154.40 ± 1.47	0.144 ± 0.02	− 54.13 ± 1.16
DND-5	Yes	White Milky	84.79 ± 0.06	161.87 ± 10.83	0.330 ± 0.03	− 52.10 ± 1.51
DND-6	Yes	White Milky	89.38 ± 0.03	411.20 ± 22.53	0.235 ± 0.03	− 43.03 ± 0.59
DND-7	Yes	White Milky	91.43 ± 0.01	655.07 ± 46.64	0.276 ± 0.02	− 46.30 ± 0.87
DND-8	Yes	White Milky	83.06 ± 0.04	759.87 ± 16.66	0.316 ± 0.01	− 51.03 ± 0.15
DND-9	Yes	White Milky	84.69 ± 0.03	377.13 ± 10.90	0.322 ± 0.02	− 69.53 ± 0.40
DND-10	Yes	White Milky	80.48 ± 0.03	346.13 ± 6.03	0.303 ± 0.01	− 58.30 ± 0.66

Batch Detail	RESULTS (NIOSOMAL DISPERSION)					
	Dispersion formed or not	Organoleptic properties	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
DND-11	Yes	White Milky	82.54 ± 0.05	874.77 ± 109.42	0.394 ± 0.01	− 57.53 ± 1.86
DND-12	Yes	White Milky	70.38 ± 0.20	457.10 ± 12.21	0.328 ± 0.03	− 38.50 ± 2.14
DND-13	Yes	White Milky	89.33 ± 0.11	709.60 ± 24.44	0.345 ± 0.01	− 81.67 ± 2.30
DND-14	Yes	White Milky	84.64 ± 0.03	919.87 ± 64.56	0.371 ± 0.01	− 54.67 ± 1.20
DND-15	Yes	White Milky	83.93 ± 1.42	794.73 ± 9.07	0.324 ± 0.01	− 57.60 ± 2.01
DND-16	Yes	White Milky	82.71 ± 0.11	56237.83 ± N/A	N/Av	11.43 ± 0.76
DND-17	Yes	White Milky	63.90 ± 0.12	616.33 ± 7.02	0.348 ± 0.01	− 41.53 ± 0.72
DND-18	Yes	White Milky	80.75 ± 0.02	435.77 ± 27.99	0.441 ± 0.05	− 40.23 ± 1.30
DND-19	Yes	White Milky	70.41 ± 0.05	460.95 ± 12.07	0.320 ± 0.02	− 60.07 ± 0.78
DND-20	Yes	White Milky	95.58 ± 0.03	355.30 ± 8.11	0.282 ± 0.01	− 58.53 ± 1.33
DND-21	Yes	White Milky	74.56 ± 0.06	823.77 ± 19.59	0.334 ± 0.01	− 53.77 ± 0.95
DND-22	Yes	White Milky	77.68 ± 0.06	444.37 ± 0.11	0.318 ± 0.01	− 51.17 ± 0.81
DND-23	Yes	White Milky	76.48 ± 0.10	439.70 ± 56.28	0.273 ± 0.03	− 47.23 ± 1.75
DND-24	Yes	White Milky	80.41 ± 0.09	680.00 ± 15.57	0.292 ± 0.01	− 34.13 ± 1.17
DND-25	Yes	White Milky	83.04 ± 0.06	505.13 ± 20.78	0.261 ± 0.03	− 48.73 ± 1.10
DND-26	Yes	White Milky	82.36 ± 0.03	487.03 ± 30.69	0.304 ± 0.01	− 48.73 ± 0.81
DND-27	Yes	White Milky	91.59 ± 0.84	479.60 ± 7.79	0.310 ± 0.02	− 49.53 ± 2.48
DND-28	Yes	White Milky	86.12 ± 0.78	521.80 ± 23.80	0.310 ± 0.00	− 54.17 ± 1.25
DND-29	Yes	White Milky	92.34 ± 0.68	480.70 ± 24.61	0.302 ± 0.03	− 58.60 ± 0.44

N/A = Not applicable, N/Av = Not available

2.4.3. Optimization of niosomes by Design of Experiments (DoE)

Niosome formulations were prepared by utilizing a design of experiments approach. The effect of various CMAs and CPPs were considered in designing the study. The combination of independent elemental variables resulted in an observable change in entrapment efficiency, particle size, polydispersity index, and zeta potential.

2.4.4. Optimization of critical material attributes

2.4.4.1. Effect of organic phase system on formulations

In the different solvent systems, it was found that niosomes did not form when diethyl ether was used as a solvent system. Drug entrapment efficiency was comparatively lower when acetone or ethanol was used as a solvent system. Higher drug entrapment efficiency was observed when the solvent system was switched from a combination of diethyl ether: ethanol to diethyl ether: methanol. These data sets are given in Table 2.6, and the impact of the organic phase solvent system on crucial elements such as entrapment efficiency and particle sizes is illustrated in Figure 2.5.

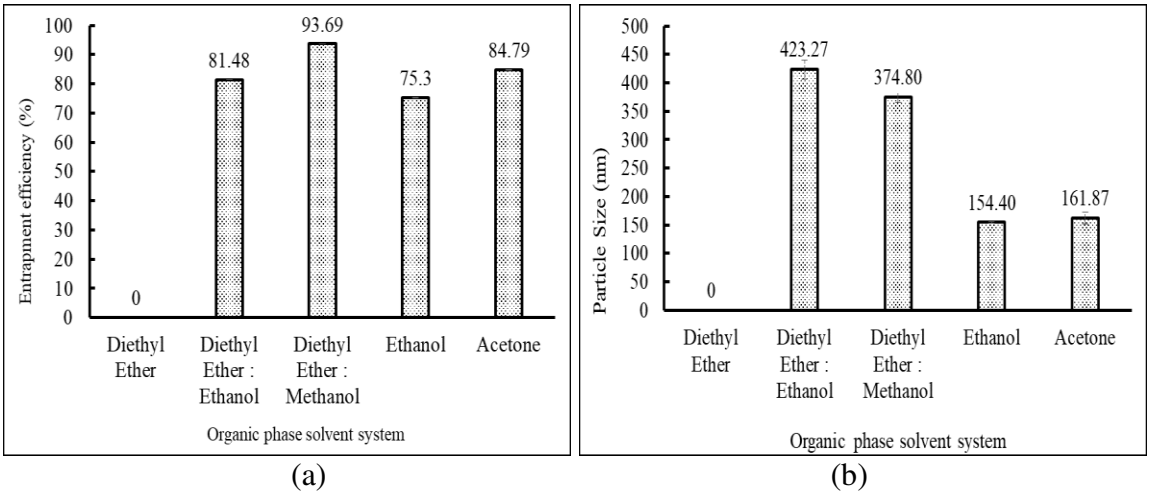
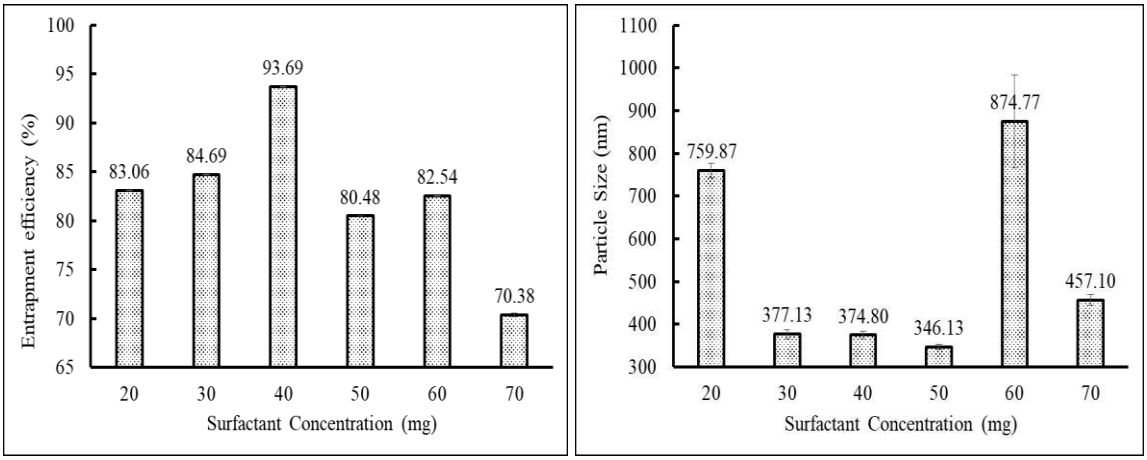


Figure 2.5. Effect of organic phase system on (a) entrapment efficiency (%) and (b) particle size (nm) of niosomes (n=3, mean ± SD).

2.4.4.2. Effect of drug, surfactant and cholesterol concentrations on formulations

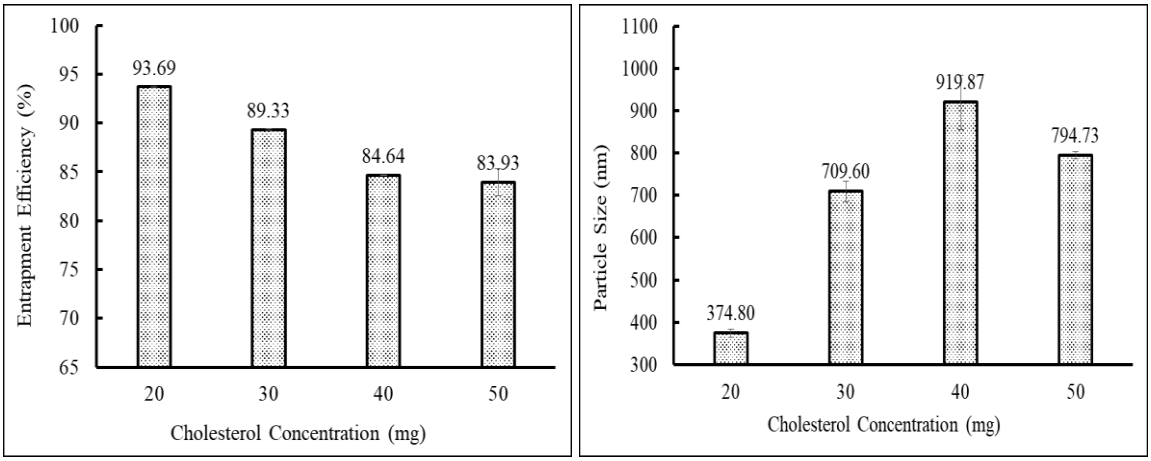
The formulations containing 2:4:2, 4:4:2, 2:4:3 ratios of drug: surfactant: cholesterol prepared using the diethyl ether: methanol solvent system showed better entrapment efficiency while comparing with the other formulations containing 3:4:2, 2:2:2, 2:3:2, 2:5:2, 2:6:2, 2:7:2, 2:4:4 and 2:4:5 ratios of drug: surfactant: cholesterol.

Results indicate that the relative amount of Span 60 and cholesterol played a crucial role in determining the drug loading capacity into the niosomal matrix. It was observed that entrapment efficiency increased with an increase in surfactant concentrations, up to and including a 2:4:2 ratio of drug: surfactant: cholesterol. Beyond this point, an increase in cholesterol concentration showed a decrease in entrapment efficiency along with an increase in particle size. There may be several explanations for this behavior: (i) higher cholesterol content increases the bilayer hydrophobicity and stability and decreases bilayer permeability, which may lead to efficient hydrophobic drug entrapment in bilayers as the vesicle matric formed [34], (ii) higher cholesterol content directly impacts on the niosome structure. It makes it more rigid and increases the particle size while competing with the drug particles for packing space within the bilayer. A similar trend was reported in various research work [35, 36]. These data sets entrapment efficiency, particle size, PDI, and zeta potential are given in Table 2.6. The impact of the surfactant and cholesterol concentrations on crucial elements such as entrapment efficiency and particle sizes are illustrated in Figure 2.6 and Figure 2.7 respectively.



(a) (b)

Figure 2.6 Effect of surfactant concentration (mg) on (a) entrapment efficiency (%) and (b) particle size (nm) of niosomes (n=3, mean ± SD).



(a) (b)

Figure 2.7. Effect of cholesterol concentration (mg) on (a) entrapment efficiency (%) and (b) particle size (nm) of niosomes (n=3, mean ± SD).

2.4.4.3. Impact of electrostatic charge on formulations

In this study, niosomes were formulated with a variety of charged surfactant materials. Control niosomes were also prepared without charged materials to evaluate their impact on the formulation. Intentionally added of charge inducing agent in the lipid layer prevents the aggregation and fusion of vesicles, and maintains by repulsive effect and maintain their integrity and uniformity [36]. The optimum charge-inducing agent was selected based on the entrapment efficiency and particle size of the niosomes. Stearylamine, which has a positive charge, produced niosome with large particle sizes, about 56,000 nm that was not acceptable, and the PDI could not even be measured. The reason behind this behavior is that stearylamine probably failed to create enough repulsion force between the niosome particles. The study showed that 5 mg stearic acid was sufficient to produce uniformly dispersed niosomes with higher drug entrapment efficiency, particle size, and homogeneous distribution, as shown in Table 2.6.

2.4.5. Optimization of critical process parameters

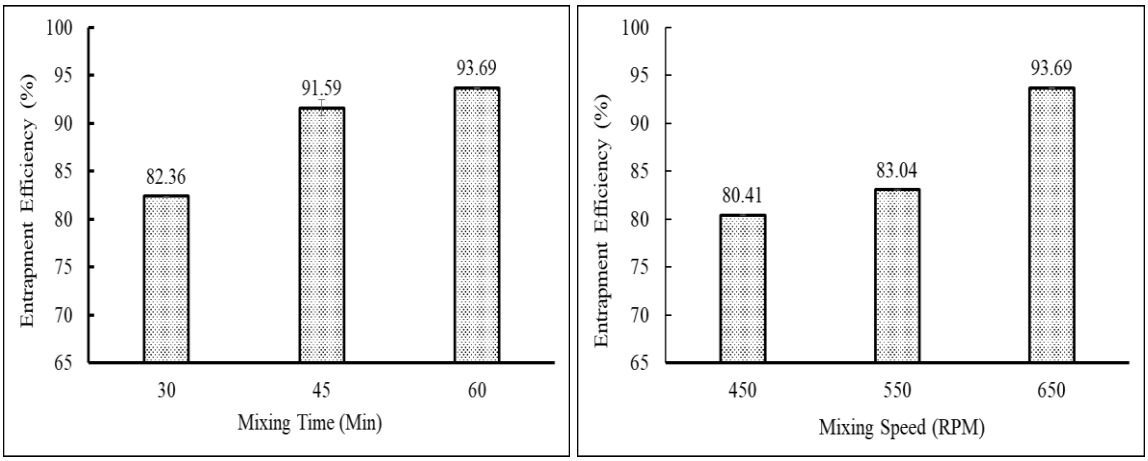
2.4.5.1. Effect of external phase volume and temperature

We observed the impact of external phase volume and temperature on niosomal formulations containing drug: surfactant: cholesterol with the ideal ratio of 2:4:2 with the diethyl ether: methanol solvent system and stearic acid as the charge-inducing agent. Various external phase temperature values (55°C, 65°C, 75°C) and external volumes (10 mL, 20 mL, 30 mL) were also investigated. The resulting trend demonstrates that external phase temperature had no discriminative impact on particle size and entrapment efficiency of the niosome formulations. The formulation, which was manufactured with 75°C, shows lower entrapment efficiency. This behavior may have been caused by higher temperature interacting with the drug molecule stability. The formulation containing 10 mL and 20 mL external phase shows similar particle size and entrapment efficiency. It was concluded from the data that entrapment efficiency was decreasing, and the particle size trend was increasing with an increase in external phase volume above 20 mL. This behavior can be explained by the possibility that increased hydration volume may have increased drug leakage from the niosomes matrix, leading to a decrease in entrapment efficiency [37].

2.4.5.2. Effect of mixing time (min) and speed (rpm)

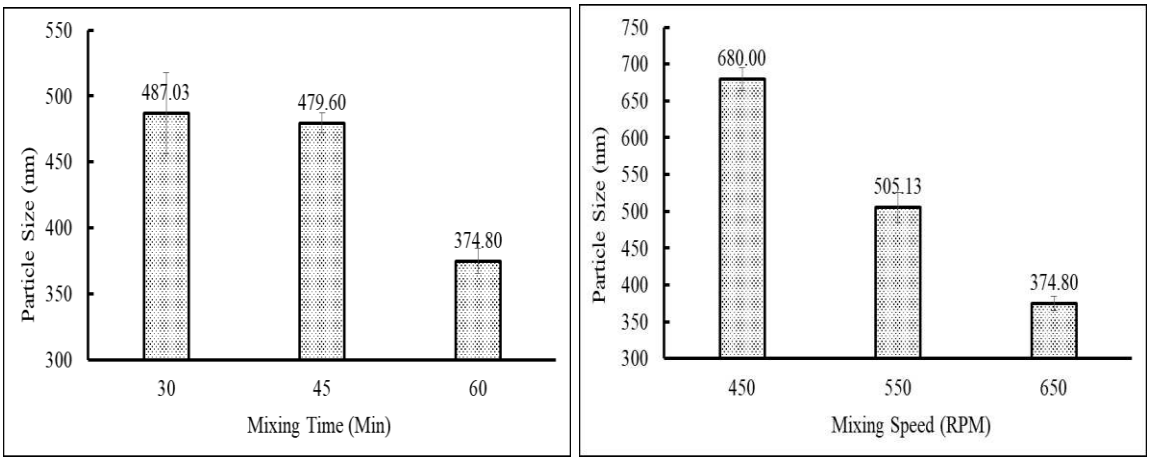
The niosomal formulations containing a drug: surfactant: cholesterol ratio of 2:4:2 with the diethyl ether: methanol solvent system and stearic acid as a charge inducing agent were subjected to various mixing time (30, 45, 60 min) and mixing speeds (450, 550, 650 rpm). The experimental data demonstrate that increasing mixing speeds from

450 to 650 rpm and mixing times from 30 to 60 min resulted in an increasing trend in entrapment efficiency and a decreasing trend in particle size. This behavior may be explained as that higher mixing speed tends to faster evaporation of the organic solvent system, which in turn resulted in uniform niosomes with higher entrapment efficiencies.



(a) (b)

Figure 2.8. Effect of (a) mixing time (minutes) and (b) mixing speed (rpm) on entrapment efficiency (%) of niosomes (n=3, mean ± SD).



(a) (b)

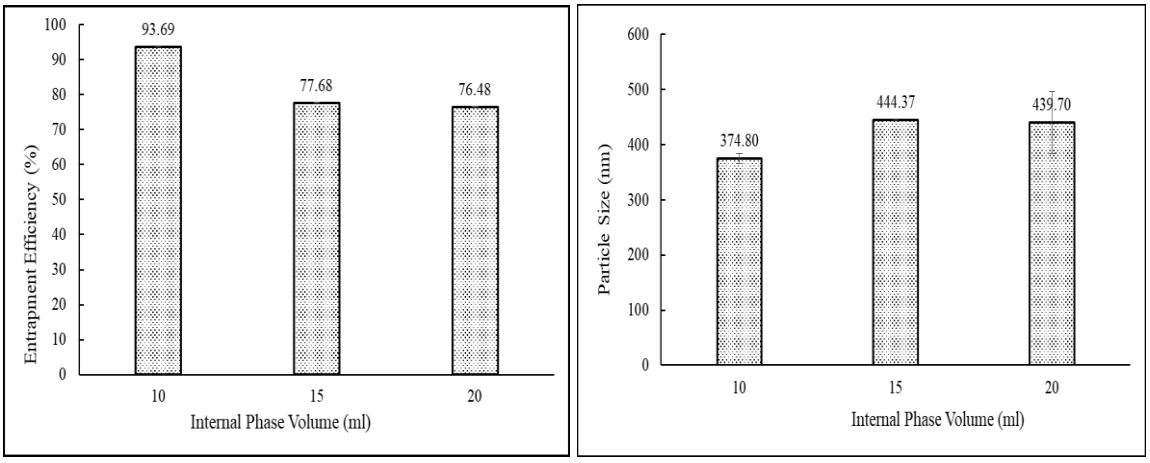
Figure 2.9. Effect of (a) mixing time (minutes) and (b) mixing speed (rpm) on particle size (nm) of niosomes (n=3, mean ± SD).

The result demonstrates that lower mixing time is not sufficient to form a complete niosome matrix that leads to the more free drug in the formulation. Data for

these experiments are given in Table 2.6. Comparisons of mixing time and mixing speeds and their effect on entrapment efficiency and particle size are illustrated in Figure 2.8 and Figure 2.9, respectively.

2.4.5.3. Effect of internal phase volume

We examined the effect of internal phase volume on niosomal formulations containing drug: surfactant: cholesterol with ratio 2:4:2 with diethyl ether: methanol solvent system and stearic acid as the charge inducing agent. Various internal phase volumes (10 mL, 15 mL and 20 mL) were tried. The results demonstrate that internal phase volume had no discriminative impact on particle size and drug entrapment efficiency of the niosomal formulations. Data for these experiments are given in Table 2.6. Comparisons of internal phase volume effect on entrapment efficiency and particle size are illustrated in Figure 2.10.

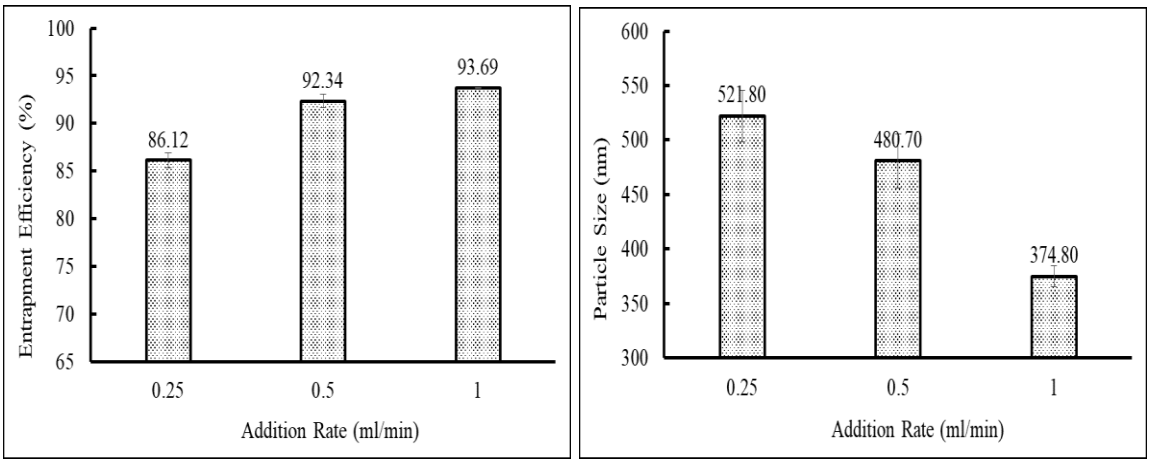


(a) (b)

Figure 2.10. Effect of internal phase volume (mL) on (a) entrapment efficiency (%) and (b) particle size (nm) of niosomes (n=3, mean ± SD).

2.4.5.4. Effect of addition rate

Results show that there is a direct relationship with drug entrapment efficiency and particle sizes of the niosomes. The data demonstrated that entrapment efficiency shows a direct relationship, as shown by increasing trend, where particle size. It shows the inverse relationship with a particle size as it has shown a decreasing trend with increasing addition rate from 0.25 to 1.00 mL/min. The effect of the addition rate on entrapment efficiency and particle size is shown in Figure 2.11.



(a) (b)

Figure 2.11. Effect of addition rate (mL/min) on (a) entrapment efficiency (%) and (b) particle size (nm) of niosomes (n=3, mean ± SD).

2.5. Conclusion

Desoximetasone-loaded niosomes with targeted particle size and entrapment efficiencies for topical application may be obtained by carefully selecting the correct combination of CMAs and CPPs. The critical material attributes (surfactant, cholesterol amounts) and critical process parameters (mixing time, mixing speed, and addition rate)

were identified and used to understand the critical quality attributes of topical niosomal formulations. A summary of fixed and impacting parameters are given in Table 2.7.

Table 2.7. Fixed and impacting parameters for desoximetasone loaded niosomes.

Fixed parameters	Impacting parameters
Method of preparation (Diethyl ether : methanol :: 75 : 25)	Surfactant concentration
Drug concentration (20 mg)	Cholesterol concentration
Lipid (Stearic Acid – 5 mg)	Mixing speed
External phase temperature (65°C)	Mixing time
External phase volume (20 mL)	Addition rate
Internal phase volume (10 mL)	

2.5.1. Entrapment efficiency (%)

A higher drug entrapment efficiency is preferable to provide higher drug delivery to the targeted site in the patient. Niosomal entrapment efficiency is a crucial parameter that conveys the stability of the vesicles. It depends on the multiple factors and combinations used to manufacture the niosomal dispersion. The entrapment efficiencies of the various formulations used in the preliminary study are provided in Table 2.6. Entrapment efficiency results demonstrate that it depends on multiple factors. In the preliminary research, the entrapment efficiency of niosomal formulations ranged from 63.90% to 95.85%.

Data suggests that the entrapment efficiency of niosomes formed from diethyl ether: methanol (75:25) was found to be high compared to other solvent systems. Changes in drug concentrations had no noticeable impacts on the entrapment efficiency capacity of the niosomes. The formulation with 2:4:2, 4:4:2, and 2:4:3 ratios of the drug: surfactant: cholesterol showed high entrapment efficiency compared to other

combinations. External phase temperature, external phase volume, and internal phase volume did not show any conclusive impact on the entrapment efficiency of niosomes. For mixing parameters, entrapment efficiency was directly correlated and following identical trends as mixing properties and showed increasing entrapment efficiencies with increasing addition rates.

2.5.2. Vesicle sizes and polydispersity index

Particle size and polydispersity index of niosomal formulations ranged from 154.40 to 919.87 nm and 0.144 to 0.441, respectively. As per the data in Table 2.6, the particle size of DND-3 used an organic phase containing diethyl ether: methanol (75:25) and was found to be an optimum that could be used for topical dermal applications. Niosomal particle size was increased when access amounts of cholesterol and desoximetasone were used. This behavior can be explained by understanding the interactions that both the drug and cholesterol have on the niosome vesicle bilayer [35, 36]. The data shows the increasing trend of particle size with increasing cholesterol content. This behavior may be described by the fact that higher cholesterol content impacts rigidity to the niosomal matrix and accumulates in the bilayer, which increases the hydrodynamic diameter and particle size of the niosomal vesicles [38]. The cholesterol mechanism of action is twofold: (a) cholesterol increases the chain order liquid state bilayers, and (b) cholesterol reduces the chain order of gel state bilayers. The gel state is gradually transformed into a liquid-ordered phase with increasing cholesterol concentration. With an increase in cholesterol concentration, bilayers decrease the release rate of entrapped drugs and, therefore, increase the rigidity of the bilayer [39]. External phase parameters (temperature and volume) and internal phase volume did not show any

conclusive impact on the niosomal particle size. Particle size trend shows inverse relation with mixing parameters, as mixing time and mixing speed increases shows a decreasing trend in particle size. The organic phase addition rate also follows an identical trend as mixing parameters, which shows a decreasing trend in particle size with increasing organic phase addition rate.

2.5.3. Risk assessment of critical quality attributes from preliminary batches to establish a QbD approach

The critical quality attributes are categorized into high, medium, and low-risk parameters based on the knowledge space and scientific knowledge. High-risk parameters having more impact on the formulation; therefore, high-risk parameters considered necessary for the Design of Experiments and need to be in accepting multivariate ranges. The summary of critical quality attributes are categorized, as shown in Table 2.8. The critical parameters and critical quality attributes for the selection of optimum formulation are shown in Table 2.8 [40].

Table 2.8. Risk assessment to identify variables affecting drug product quality.

Drug Product CQAs	Entrapment Efficiency	Particle Size	Polydispersity Index	Zeta Potential
Organic solvent selection	Low	Medium	Medium	Low
Drug concentration	Medium	Low	Low	Low
Surfactant concentration	High	Medium	Medium	Low
Cholesterol concentration	High	High	High	Low
Lipid selection	Low	Low	Low	Medium
External phase temperature	Medium	Low	Low	Low
External phase volume	Medium	Medium	Low	Low

Drug Product CQAs	Entrapment Efficiency	Particle Size	Polydispersity Index	Zeta Potential
Internal phase volume	Low	Low	Low	Low
Mixing speed	High	High	High	Low
Mixing time	High	High	Medium	Low
Addition rate	High	High	Medium	Low

This work establishes the fundamental foundations which may be further explored to develop a robust manufacturing process by optimizing QbD elements. This extensive study provides a detailed understanding of drug product design.

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3. CHAPTER – 3. QUALITY BY DESIGN (QbD): A SYSTEMATIC APPROACH USING AN ADVANCED STATISTICAL TOOL TO OPTIMIZATION OF NIOSOMES PREPARATION FOR THE TOPICAL DELIVERY

3.1. Introduction

In the early 1950s, the topical delivery field expanded when medical professionals successfully began treating various skin conditions more easily using steroid therapy [1, 2]. Atopic dermatitis (AD) common skin disease is an itchy and chronic inflammatory skin condition with a frequently remitting and relapsing course [3]. In the treatment of AD compromised the use of emollients in semisolid formulations [4]. Psoriasis and eczema are commonly diagnosed with skin disease in today's world. Psoriasis is an autoimmune skin condition in which the skin epidermal cells multiply up to 10 times faster than the average multiplication rate. As a consequence, the top layer of the epidermis appears as thick, red, and white hues of scaly patches that are dry and itchy and may give rest to pain and swelling [5, 6].

Several studies reporting on topical corticosteroid use reported that fluticasone propionate and mometasone furoate used once daily led to reduced systemic absorption and showed identical efficacy when compared to twice daily dosing [7, 8]. In this scenario, reduced dosing frequency will reduce the side effects and lead to improved patient compliance [9]. Topical corticosteroids are commonly used in atopic dermatitis and have shown definite impacts in treating atrophy, perioral dermatitis, acne, and rosacea [10, 11]. Desoximetasone (DM) is a topical corticosteroid that is well accepted by patients with good safety and efficacy [12]. It has been available in the market in the

form of creams, gel, spray and ointments; however, there is no extended-release formulation available containing desoximetasone [13-15].

Since multiple factors play an essential role in the properties of niosomes, the traditional one-factor-at-a-time (OFAT) experimental design would be tedious and utilize a lot of material to identify the factors that have an impact on the final products [16]. Design of Experiments (DoE) is a more scientific and logical multivariate statistical approach that allows the simultaneous investigation of multiple factors, not limited to single ones, but also their multiple interactions [17]. The full-factorial design is an advanced and most robust design that allows us to study the responses of different factors and the relationship between factors and interactions among them [18]. JMP® by SAS Institute is a powerful advanced statistical software that can be utilized for complex customized design of experiments and detailed study of the results. Utilizing advanced analytical tools such as fit modeling or profile predictor, it can be used to get an extensive understanding of the results obtained in a Quality by Design (QbD) experimental approach [19].

In past years, the pharmaceutical industry has spent a lot of time and effort to ensure product quality, to achieve regulatory compliance, and to yield pharmaceuticals as economically as possible. Therefore, advanced technologies and processes that require operational complexity and science are being applied [20]. QbD is an advanced systematic risk-based approach implemented for pharmaceutical products to develop formulations with predefined objectives and increase product and process understanding with improvements in safety, efficacy, and product stability [21]. This holistic approach to QbD starts with a predefined list of quality requirements and involves a quality target

product profile (QTPP) and subsequent application of various principles and tools in different steps of the process. Some tools such as quantified risk assessment (QRA), risk analysis, fishbone plots, and failure mode and effects analysis (FMEA) are used to predefine CQAs and CPPs. In the next step, previously identified CPPs are utilized to establish an experimental design and select those with a significant impact on the process or final product. Therefore, quality is developed throughout the manufacturing process rather than set up for the final product [22-24].

U.S. Food and Drug Administration (FDA) is appealing to pharmaceutical companies to adopt risk-based approaches and QbD principles in product development from the beginning phase of research and development to the end of the cycle manufacturing process [25]. Pharmaceutical QbD has expanded with the issuance of ICH Q8 (R2) (Pharmaceutical Development), ICH Q9 (Quality Risk Management), and ICH Q10 (Pharmaceutical Quality System) [26-28]. This makes the product likely to follow regulatory requirements and avoid product failure during the manufacturing cycles.

The work in this chapter is aimed at utilizing a JMP®-enabled DoE approach to develop desoximetasone loaded niosomal formulations. As per the basic principles of QbD, the methodology began with setting the quality targeted product profile and identifying the critical quality attributes needed to meet that objective. The critical process parameters and critical material attributes were evaluated to determine their impact on the critical quality attributes. DOE is used in this study for risk evaluation and optimization and to evaluate different variables impact on the responses. Experimental trials were defined by the use of full factorial designs to accomplish the optimized formulation, and the process was finally validated and verified for robustness.

3.2. Materials and Methods

3.2.1. Materials

Desoximetasone was gifted by Flavine, New Jersey, USA. HPLC water, Diethyl ether, Stearylamine, and Chloroform were purchased from Sigma-Aldrich, Saint Louis, MO, USA. Methanol, Acetone, and Acetonitrile were purchased from BDH VWR Analytical, Radnor, PA, USA. Ethanol was procured from Decon Labs, Inc., King of Prussia, PA, USA. Span 20 (sorbitan laurate), Span 60 (sorbitan monostearate) and Cholesterol were gifted from Croda Inc. Mill Hall, PA, USA. Stearic Acid was gifted from BASF Corporation, Edison, NJ, USA. Glacial Acetic Acid was purchased from Fisher Scientific, Fair Lawn, NJ, USA.

3.2.2 Niosomal Vesicle Preparation

The niosomes were prepared by an ether injection method with the drug dissolved in 10 mL diethyl ether: methanol (75:25) organic mixture until completely dissolved. Next, sorbitan monostearate, cholesterol, and stearic acid were added into the mixture and mixed with a suitable magnetic stir bar in a 20 mL glass scintillation vial. In a separate 50 mL glass beaker, 20 mL purified water was preheated at 65°C temperature using a hot plate with magnetic stirring. The organic phase was filled into a 10 mL syringe with a 26 G needle. The organic phase mixture was injected into the preheated aqueous phase using prefixed parameters based on the experimental study design. Mixing was continued based on the values identified from the design of experiments (DoE). In the final step of the process, the batch formulation was cooled down at room temperature, and the formulation was stored in a suitable glass storage container.

3.2.3. HPLC Methods

The mobile phase was prepared by mixing methanol, HPLC grade water, and glacial acetic acid (65:35:1). The diluent was prepared by mixing methanol and acetonitrile (50:50). The desoximetasone was measured using a Discovery C18 column (Sigma-Aldrich, Saint Louis, MO, USA) of 150 mm x 4.6 mm with 5 µm particle size, and UV absorbance set to 254 nm. The injection volume was 10 µL, and the flow rate was selected at 1.0 mL/min. The sample run time was 10 minutes at room temperature, and retention time for the drug elution peak was at approximately 5 min. The HPLC instrument used was Agilent 1100 series instrumentation (Agilent Technologies, CA, USA) coupled with UV detection (DAD) and HP ChemStation software V. 32.

3.2.4. Niosomal vesicle characterization

3.2.4.1. Organoleptic properties

Niosomal dispersions were characterized for visual appearance, color, and odor and tested to confirm the presence of any residual solvent as a standard quality verification.

3.2.4.2. Drug content (Assay determination)

The desoximetasone niosomal dispersion (100 mg) was carefully collected and placed into chloroform: methanol (40:60) and then mixed until it was dissolved entirely at room temperature. Upon mixing, niosomal dispersion samples were further diluted with the same quantity of diluent. Drug content qualification for desoximetasone was performed using HPLC (Agilent 1100 – ChemStation software) method mentioned in section 3.2.3. coupled with UV analysis at a wavelength λ_{max} 254 nm.

3.2.4.3. Drug entrapment efficiency of niosomal vesicles

Desoximetasone free drug was determined from the entrapped drug by ultracentrifugation at 14,000 rpm for 30 min using an ultracentrifuge (Branson Ultrasonic Corporation, CT, USA) at room temperature. The supernatant containing the free available drug was carefully collected without disturbing the sediment, and 200 mg of collected supernatant was mixed and dissolved into chloroform: methanol (40:60) mixture using a vortex mixer. After mixing, the sample was further diluted with an equal amount of the diluent. Drug quantification was determined using the predetermined HPLC method as mentioned in section 3.2.3. Drug % entrapment efficiency was calculated in triplicate by using the following formula [29].

$$\% \text{ Entrapment Efficiency} = \frac{Total\ amount\ of\ drug - Free\ amount\ of\ drug}{Total\ amount\ of\ drug} \times 100 \qquad [30]$$

3.2.4.4. Niosomal vesicle size and zeta potential

The mean particle size and its distribution were determined at room temperature using a Delsa Nano S Particle Sizer (Beckman Coulter, CA, USA) in triplicate based on the light scattering spectroscopy principles. The zeta potential of the niosomal dispersion was measure in triplicate using Malvern Particles Sizer 2000 (Malvern Technologies, Worcestershire, UK).

3.2.5. Fourier Transform Infrared (FTIR) analysis

FTIR spectra of samples were taken on Thermo Scientific (Model Nicolet iS10) instrument to investigate the possible interaction between the drug and excipients. FTIR

spectra of pure drug, excipients, physical mixture of drug and excipients in formulation concentration, were scanned in the range between 4000-400 cm^{-1}.

3.3. Advanced quality by design for niosomes

The present study was undertaken to utilize quality by design approach to develop the desoximetasone-loaded niosomes and understand the effect of the interaction of formulation variables and physicochemical properties in enhancing topical desoximetasone permeation in a defined design space.

3.3.1. Identification of critical material attributes (CMAs) and critical process parameters (CPPs)

Parameters were identified for testing based on our prior experience, scientific knowledge about vesicular drug delivery systems, an extensive literature review of patents, research articles, scientific peer review journals, and expert opinions. Selected investigation parameters were a type of organic phase, drug concentration, surfactant concentration, cholesterol concentration, and type of lipids as CMAs and internal and external phase volume, external phase temperature, mixing time, mixing speed, and addition rate as the CPPs. Preliminary experiments defined that diethyl ether: methanol (75: 25), 20 mg desoximetasone, stearic acid, 65°C external phase temperature, 20 mL external phase volume, and 10 mL internal phase volume were acceptable parameters to spontaneously form niosomal formulations [31].

3.3.2. 2^5 full factorial design using JMP® statistical software

At the end of the preliminary studies, surfactant concentration and cholesterol concentration were selected as impacting CMAs and mixing speed, mixing time, and addition rate were selected as impacting CPPs [31].

The effect of surfactant concentration (X_1), cholesterol concentration (X_2), mixing speed (X_3), mixing time (X_4), and addition rate (X_5) were considered as independent variables. The dependent variables measured were entrapment efficiency (Y_1), particle size (Y_2), and polydispersity index (Y_3). The factors were studies at two levels (-1, +1) for surfactant concentration 40 and 60 mg, cholesterol concentration 20 and 40 mg, mixing speed 450 and 650 rpm, mixing time 45 and 60 min, and addition rate 0.5 and 1.0 mL/min as described in Table 3.1.

Table 3.1. CMAs and CPPs for the design of formulation.

Variables	Levels	
	-1	**+1**
Independent variables		
X_1 = Span 60 (mg)	40	60
X_2 = Cholesterol (mg)	20	40
X_3 = Mixing Speed (RPM)	450	650
X_4 = Mixing Time (min)	45	60
X_5 = Addition Rate (mL/min)	0.5	1
Dependent variables		
Y_1 = Particle Size (nm)		
Y_2 = Entrapment Efficiency (%)		
Y_3 = Polydispersity Index		

Batches were manufactured using a 2^5 randomized full factorial design, as described in Table 3.2, and the effect of independent variables was evaluated. The factorial experimental design was developed using JMP® statistical software from SAS (version 11.0.0), and the responses found were input back to the software. The best-fitting model was selected based on the results from previous experimental data and using the response profile predictor by JMP® [31]. These tools help to formulate with relatively better accuracy using mathematical modeling.

Table 3.2. Full factorial design guiding the formulation of niosomal dispersion batches obtained using the QbD approach.

Batch Detail	Pattern	Span 60	Cholesterol	Mixing Speed	Mixing Time	Addition Rate
		X_1	X_2	X_3	X_4	X_5
DND-30	+ − − + −	+1	-1	-1	+1	-1
DND-31	− − + − +	-1	-1	+1	-1	+1
DND-32	+ + + − −	+1	+1	+1	-1	-1
DND-33	− + + − +	-1	+1	+1	-1	+1
DND-34	+ − − + +	+1	-1	-1	+1	+1
DND-35	− − − + +	-1	-1	-1	+1	+1
DND-36	− + − + −	-1	+1	-1	+1	-1
DND-37	+ − + − +	+1	-1	+1	-1	+1
DND-38	− + − − +	-1	+1	-1	-1	+1
DND-39	− − − − −	-1	-1	-1	-1	-1
DND-40	− + − + +	-1	+1	-1	+1	+1
DND-41	+ + − − +	+1	+1	-1	-1	+1
DND-42	+ + + − +	+1	+1	+1	-1	+1
DND-43	+ − + − −	+1	-1	+1	-1	-1
DND-44	− − + + +	-1	-1	+1	+1	+1
DND-45	− − − − +	-1	-1	-1	-1	+1
DND-46	+ − − − +	+1	-1	-1	-1	+1
DND-47	− − − + −	-1	-1	-1	+1	-1
DND-48	+ + − + −	+1	+1	-1	+1	-1

Batch Detail	Pattern	Span 60	Cholesterol	Mixing Speed	Mixing Time	Addition Rate
		X_1	X_2	X_3	X_4	X_5
DND-49	− + + − −	-1	+1	+1	-1	-1
DND-50	+ + − − −	+1	+1	-1	-1	-1
DND-51	+ − − − −	+1	-1	-1	-1	-1
DND-52	+ + + + −	+1	+1	+1	+1	-1
DND-53	− − + + −	-1	-1	+1	+1	-1
DND-54	+ + − + +	+1	+1	-1	+1	+1
DND-55	+ − + + −	+1	-1	+1	+1	-1
DND-56	− + + + +	-1	+1	+1	+1	+1
DND-57	− + − − −	-1	+1	-1	-1	-1
DND-58	+ + + + +	+1	+1	+1	+1	+1
DND-59	− − + − −	-1	-1	+1	-1	-1
DND-60	+ − + + +	+1	-1	+1	+1	+1
DND-61	− + + + −	-1	+1	+1	+1	-1

3.4. Validation of profile predictor by checkpoint formulations

Formulations data for entrapment efficiency, particle size, and polydispersity index were captured and entered in full factorial design from JMP®. Based on the results from the study, using a fit model prediction profile was generated.

3.4.1. Target formulation with optimum parameters

Target niosomal dispersion formulation should have ideal characteristics such as better entrapment efficiency, optimize particle size, and lower polydispersity index. Using the profile predictor, the best suitable combination of surfactant concentration, cholesterol concentration, mixing speed, mixing time, and addition rate were selected to achieve ideal niosomal dispersion. Predicted results for entrapment efficiency, particle

size, and polydispersity index from the ideal combination were compared with actual executed formulation results for accuracy of profile predictor and selected fit model.

3.4.2. Formulation to verify profile predictor "best-fit" model

To verify the accuracy of the fit model and profile predictor, one additional combination was generated and predicted data from the profile predictor for entrapment efficiency, particle size, and polydispersity index were compared with actual executed formulation results.

3.5. Selection of ideal surfactant for the niosome formulation

Surfactants play a crucial role in the niosome formulation; therefore, the selection of the ideal surfactant is very crucial. In this research, throughout niosome study was executed with a Span 60 (sorbitan monostearate) surfactant. Therefore, it is essential to use different surfactants to evaluate the impact of surfactants on the final product. A new formulation with Span 20 (sorbitan laurate) was designed to assess a surfactant impact on the product.

3.6. Results and Discussion

Based on the observed experimental data, this study demonstrates the impact on the niosome formulations caused by the CPPs, surfactant concentration, presence of membrane additives, and drug-specific physicochemical attributes. Surfactants containing an alkyl-chain length ranging from C_{12}-C_{18} are typically preferred due to favorable skin compatibility [32]. The results from this study reveal that alkyl chain length was a key

element suitable for effective drug encapsulation. Span 60 (sorbitan monostearate) was sufficient to be used as the primary surfactant in the preparation of niosomes due to the stability from longer saturated alkyl chains. Higher drug entrapment efficiency was targeted by selecting the surfactant with longer chain lengths. The physicochemical properties associated with these findings may be linked to a higher phase transition temperature and lower HLB (4.7) of Span 60, which resulted in higher entrapment efficiency [33].

Critical material attributes and critical process parameters were considered to have the most substantial impact on the various performance aspects of niosomes containing desoximetasone. Various quantitative measurements were used to evaluate their effects on entrapment efficiency (%), particle size (nm), polydispersity index (PDI), and zeta potential (mV).

3.6.1. Organoleptic Properties

All the niosomal formulation obtained were evaluated by their physical state as milky white, odorless dispersions with a fluid-like consistency.

3.6.2. Fourier Transform Infrared (FTIR) Spectroscopic studies

The FTIR analysis was employed to study the compatibility of the drug with the excipients used in the formulation. The samples were scanned in the region of 4000-400 cm^{-1}. The IR spectra analysis of pure desoximetasone showed that the major peak was observed at wavenumbers 3324.87 (alcohol O-H stretch), 2922.57 (C-H stretching of an aliphatic group), 1714.83 (ketone C=O stretch), 1450.05, 1364.83 (C-H methyl rock), 1059.07 (C-F), and 885.44, confirming the purity of the drug as shown in Figure 3.1. In

the IR spectra of the physical mixture of desoximetasone and other excipients in Figure 3.1. shows in the original concentration ratio as the proposed formula, the major peaks of desoximetasone were observed at 3353.46, 2916.97, 1714.73, 1450.18, 1364.71, 1058.80, and 885.77. Infrared spectra of the physical mixture of desoximetasone and excipients showed all the characteristic peaks indicating the absence of any possible interaction between the drug and excipients. Therefore, it can be stated that the drug and excipients are compatible and can be formulated into the drug-loaded niosomes. The characteristic peaks of the drug can also be seen in a mixture of desoximetasone with individual excipient in Figure 3.1.

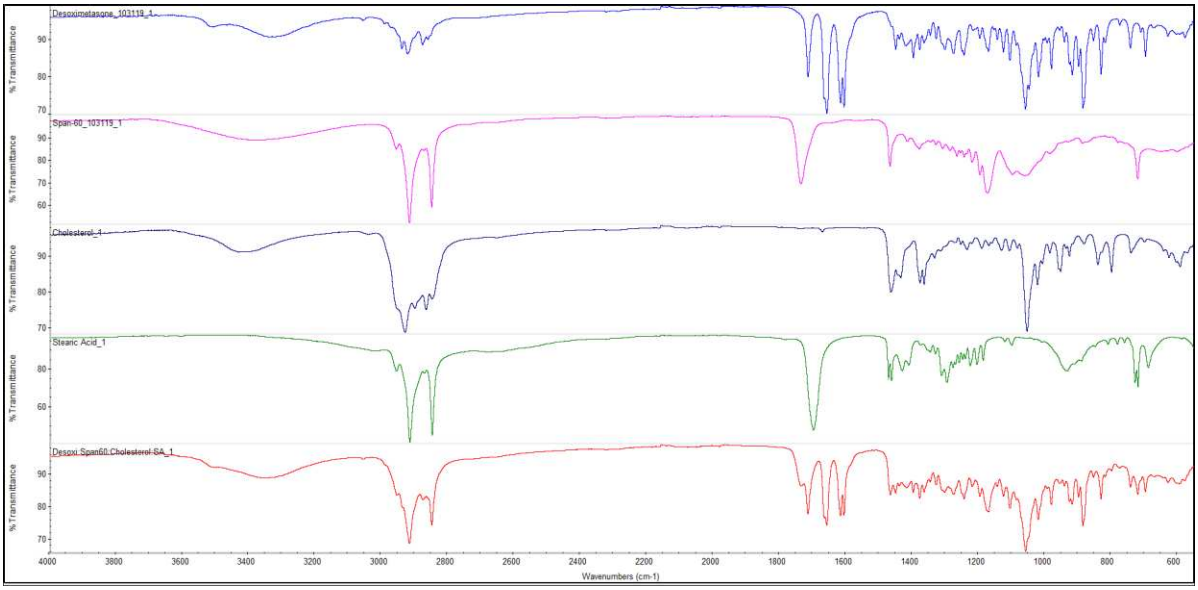


Figure 3.1. FTIR spectrum of desoximetasone, Span 60, cholesterol, stearic-acid, physical mixture of drug and excipients for compatibility evaluation.

3.6.3. Experimental Design for Optimizing Niosomes

Niosomal formulations were manufactured following using the full-factorial design of the experiment to measure the associated effect of selected formulation variables. Variables measured were surfactant and cholesterol concentrations, mixing

time, mixing speed, and addition rate. Niosome input variables were chosen with the presumption that their use levels are acceptable for a range of vehicle interactions.

Details about formulations tested using an acceptable range of low and high values (+1, -1) are defined using input variables (X_1, X_2, X_3, X_4, X_5) in Table 3.3 with associated dependent variables (Y_1, Y_2, Y_3, Zeta potential) measurements. The entrapment efficiency was found in the range between 83.68 – 93.72%, whereas particle size and polydispersity index were found to be in the ranges of 326.53 – 1730.83 nm and 0.285 – 0.422 respectively.

Table 3.3. DOE observed responses for niosomes using a 2^5 full factorial design (n=3, mean ± SD).

Batch Detail	Design Input (Independent Variables)					Design Output (Dependent Variables)			
	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	
	Span 60	Cholesterol	Mixing Speed	Mixing Time	Addition Rate	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
DND-30	+1	-1	-1	+1	-1	92.06 ± 0.03	1076.57 ± 13.94	0.400 ± 0.00	-73.63 ± 1.89
DND-31	-1	-1	+1	-1	+1	92.06 ± 0.04	411.90 ± 9.07	0.350 ± 0.01	-77.37 ± 1.60
DND-32	+1	+1	+1	-1	-1	90.65 ± 0.02	632.63 ± 16.98	0.354 ± 0.02	-70.57 ± 3.41
DND-33	-1	+1	+1	-1	+1	90.13 ± 0.05	487.73 ± 12.50	0.330 ± 0.00	-77.20 ± 0.62
DND-34	+1	-1	-1	+1	+1	90.58 ± 0.01	888.37 ± 61.11	0.350 ± 0.02	-39.27 ± 3.33
DND-35	-1	-1	-1	+1	+1	88.37 ± 0.15	598.00 ± 38.84	0.302 ± 0.01	-57.27 ± 3.87
DND-36	-1	+1	-1	+1	-1	92.28 ± 0.06	965.20 ± 27.51	0.358 ± 0.01	-68.43 ± 1.21
DND-37	+1	-1	+1	-1	+1	90.49 ± 0.03	645.30 ± 27.72	0.315 ± 0.03	-69.87 ± 1.27
DND-38	-1	+1	-1	-1	+1	90.23 ± 0.04	1730.83 ± 100.45	0.422 ± 0.05	-54.10 ± 0.44
DND-39	-1	-1	-1	-1	-1	90.71 ± 0.04	748.67 ± 8.75	0.302 ± 0.01	-54.80 ± 0.95
DND-40	-1	+1	-1	+1	+1	89.06 ± 0.02	821.13 ± 4.20	0.331 ± 0.02	-37.60 ± 3.75
DND-41	+1	+1	-1	-1	+1	87.99 ± 0.02	739.57 ± 31.08	0.360 ± 0.03	-61.67 ± 1.52

Batch Detail	Design Input (Independent Variables)					Design Output (Dependent Variables)			
	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	
	Span 60	Cholesterol	Mixing Speed	Mixing Time	Addition Rate	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
DND-42	+1	+1	+1	-1	+1	88.14 ± 0.05	564.13 ± 4.92	0.338 ± 0.01	-36.77 ± 1.12
DND-43	+1	-1	+1	-1	-1	91.48 ± 0.01	380.83 ± 7.49	0.312 ± 0.01	-51.70 ± 2.23
DND-44	-1	-1	+1	+1	+1	89.96 ± 0.03	476.33 ± 7.19	0.322 ± 0.03	-31.73 ± 1.05
DND-45	-1	-1	-1	-1	+1	91.37 ± 0.02	480.70 ± 10.57	0.310 ± 0.01	-53.17 ± 0.40
DND-46	+1	-1	-1	-1	+1	83.68 ± 0.04	548.10 ± 18.71	0.298 ± 0.03	-57.63 ± 1.31
DND-47	-1	-1	-1	+1	-1	93.72 ± 0.01	678.43 ± 14.00	0.344 ± 0.01	-57.93 ± 2.08
DND-48	+1	+1	-1	+1	-1	91.34 ± 0.01	974.07 ± 52.01	0.383 ± 0.01	-45.27 ± 0.86
DND-49	-1	+1	+1	-1	-1	92.71 ± 0.06	912.47 ± 32.65	0.360 ± 0.01	-56.47 ± 1.11
DND-50	+1	+1	-1	-1	-1	89.87 ± 0.07	664.27 ± 7.72	0.356 ± 0.01	-70.60 ± 0.35
DND-51	+1	-1	-1	-1	-1	86.80 ± 0.01	621.93 ± 25.32	0.285 ± 0.02	-70.80 ± 0.17
DND-52	+1	+1	+1	+1	-1	91.77 ± 0.06	370.33 ± 7.70	0.326 ± 0.01	-76.70 ± 2.86
DND-53	-1	-1	+1	+1	-1	90.55 ± 0.04	492.23 ± 23.32	0.294 ± 0.03	-44.43 ± 1.72
DND-54	+1	+1	-1	+1	+1	90.42 ± 0.07	457.83 ± 20.34	0.298 ± 0.01	-43.43 ± 1.46
DND-55	+1	-1	+1	+1	-1	89.39 ± 0.01	765.73 ± 29.57	0.316 ± 0.01	-49.17 ± 1.69
DND-56	-1	+1	+1	+1	+1	89.11 ± 0.08	712.27 ± 6.35	0.350 ± 0.02	-47.27 ± 2.40
DND-57	-1	+1	-1	-1	-1	90.72 ± 0.04	750.00 ± 37.12	0.345 ± 0.02	-60.03 ± 2.50
DND-58	+1	+1	+1	+1	+1	91.01 ± 0.02	413.83 ± 12.60	0.326 ± 0.01	-63.00 ± 1.32
DND-59	-1	-1	+1	-1	-1	89.15 ± 0.05	552.63 ± 33.04	0.302 ± 0.02	-47.40 ± 2.00
DND-60	+1	-1	+1	+1	+1	87.29 ± 0.05	326.53 ± 8.13	0.303 ± 0.00	-47.67 ± 0.91
DND-61	-1	+1	+1	+1	-1	90.75 ± 0.02	599.93 ± 14.02	0.344 ± 0.01	-70.40 ± 0.95

3.6.4. Analysis of Responses

Responses obtained from 32 formulations using JMP® were utilized for evaluating potential significance differences using a full-factorial design study.

3.6.4.1. Response 1 (Y_1): Effect of formulation variables in entrapment efficiency

Vesicular entrapment efficiency is directly linked to the stability of the niosome vesicles. Various parameter combinations to manufacture a niosome dispersion were summarized in Table 3.3 associated with Y_1 results. A "best-fit" model approach was suitable to analyze further the R^2 value shown in Table 3.4 along with the regression equation without requiring further data transformation method. CMAs and CPPs variables had a significant impact on the entrapment efficiency of niosome.

Utilizing an appropriate response-variable relationship, orders of magnitude and mathematical signs of each coefficient resulted in a linear correlation factor of $R^2 = 0.60$ for input variables cholesterol concentration (X_2), mixing time (X_3) and mixing speed (X_4) and demonstrated combinations of X_1&X_2, X_1&X_3, X_1&X_4, X_3&X_5 variables had considerably favorable Y_1 due to desirable increase in entrapment efficiency. Additionally, X_2&X_3, X_2&X_4, X_3&X_4, X_1&X_5, X_2&X_5, X_4&X_5 resulted in unfavorable combinations for entrapment efficiency due to decreased entrapment efficiency. Input variables surfactant concentration (X_1) and addition rate (X_5) possess inverse proportionality with entrapment efficiency. No linear correlation was drawn between responses and factors at ranges used in selected formations.

Table 3.4. Summary of results of regression analysis for entrapment efficiency response Y_1.

Quadratic Model	R^2	Adjusted R^2
Response (Y_1) entrapment efficiency	0.600	0.22536

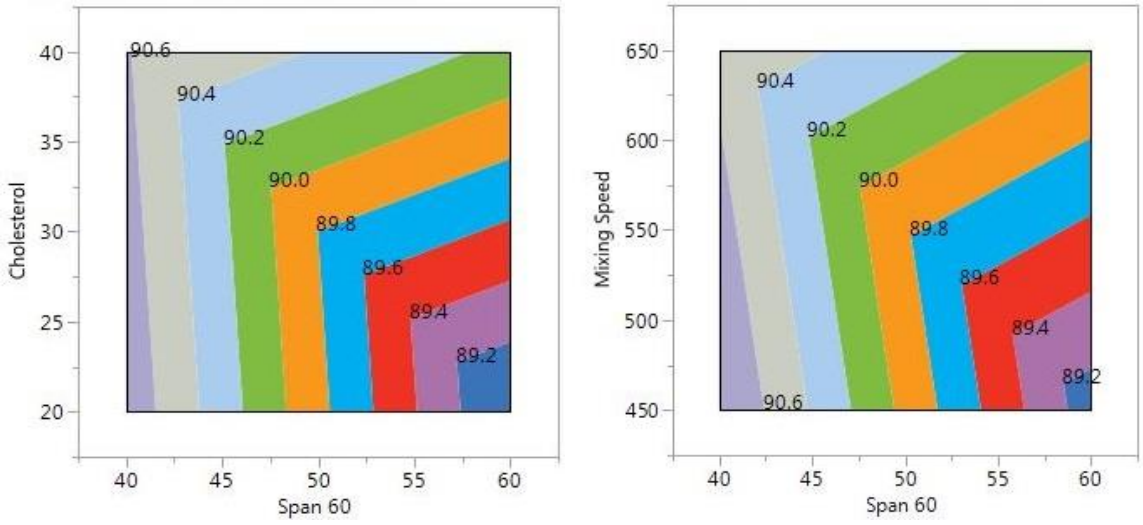
Regression analysis for the entrapment efficiency of the polynomial model was established using the described equation:

Niosomes entrapment efficiency (Y_1) = 90.12 – 0.56 (X_1) + 0.27 (X_2) + 0.17 (X_3) + 0.36 (X_4) – 0.75 (X_5) + 0.32 (X_1X_2) + 0.30 (X_1X_3) - 0.02 (X_2X_3) + 0.56 (X_1X_4) – 0.03 (X_2X_4) – 0.67 (X_3X_4) – 0.11 (X_1X_5) – 0.12 (X_2X_5) + 0.24 (X_3X_5) – 0.25 (X_4X_5).

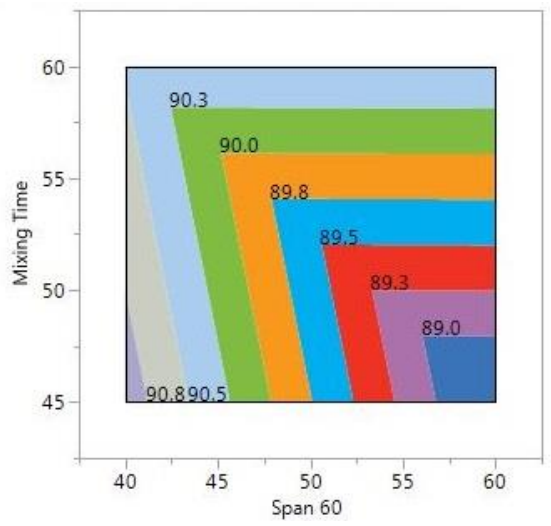
Where X_1 is Span 60 (mg), X_2 is cholesterol (mg), X_3 is mixing speed (rpm), X_4 is mixing time (minutes), and X_5 is the addition rate (mL/min.).

3.6.4.1.1. Contour model graph for entrapment efficiency

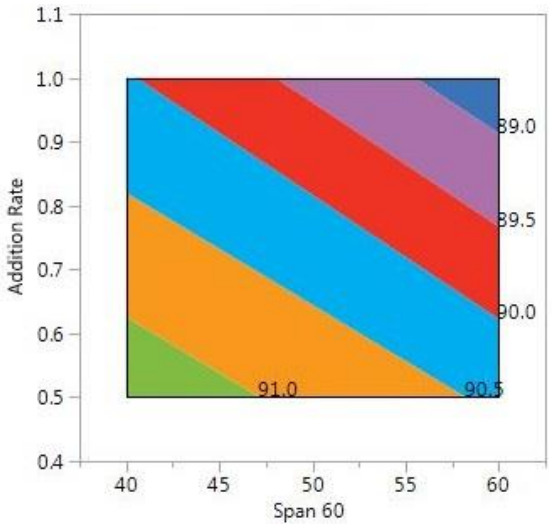
The two-dimensional contour plots compare the effects of two independent variables. The interactions between the values of two independent variables that showed no linear correlation, while the remaining three independent variables remained at constant to illustrate their respective response interactions shown in Figure 3.2 (a-j).



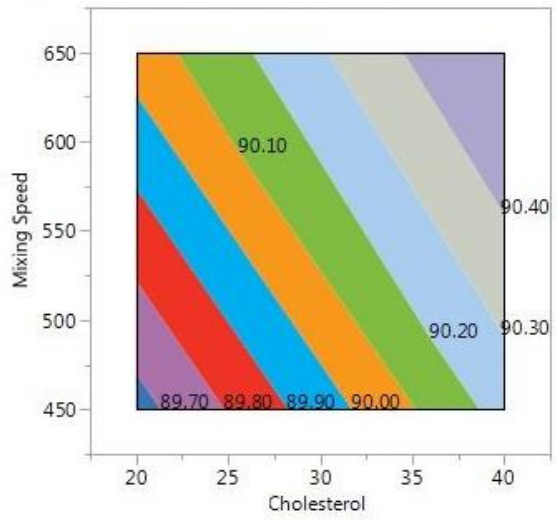
(a) **(b)**


Mixing Time
Span 60
90.3
90.0
89.8
89.5
89.3
89.0
90.8
90.5

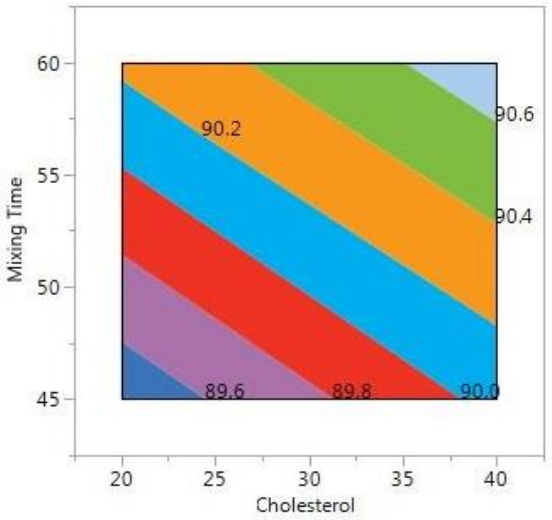
(c)


Addition Rate
Span 60
89.0
89.5
90.0
91.0
90.5

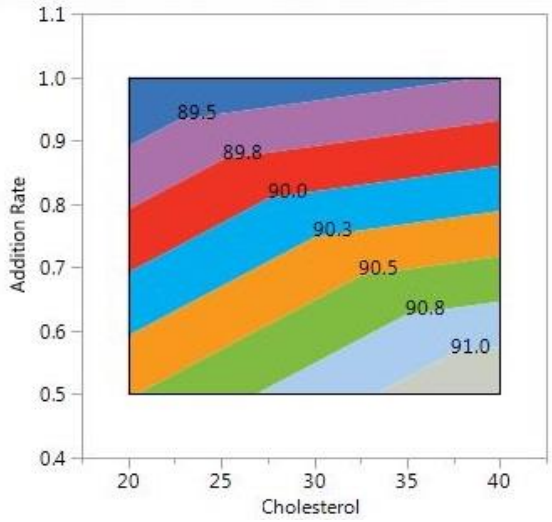
(d)


Mixing Speed
Cholesterol
90.10
90.40
90.20
90.30
89.70
89.80
89.90
90.00

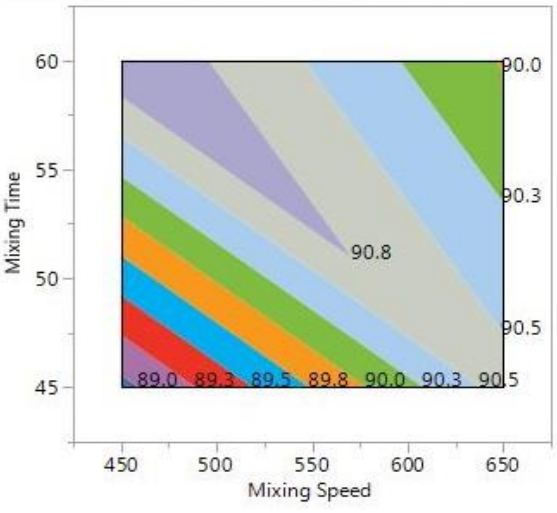
(e)


Mixing Time
Cholesterol
90.2
90.6
90.4
89.6
89.8
90.0

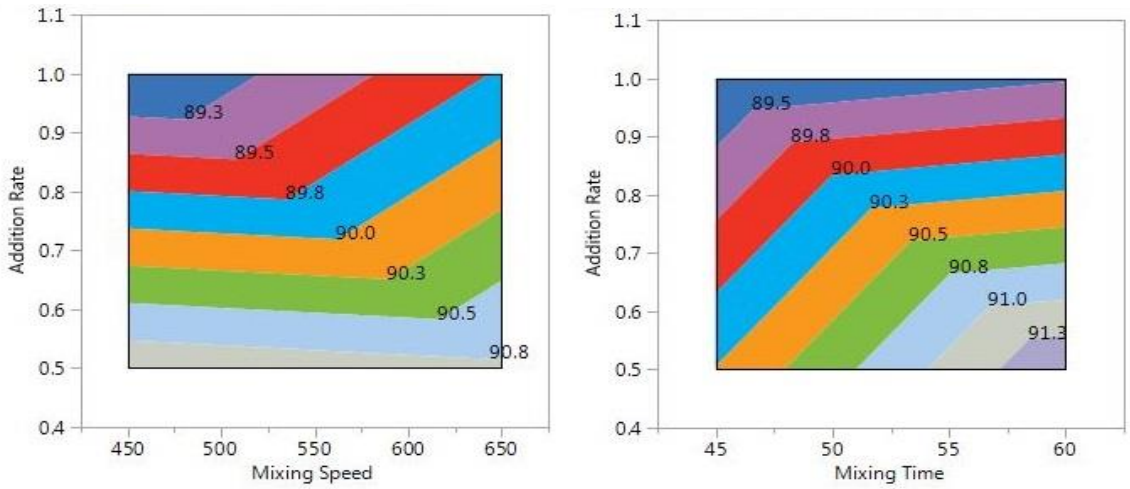
(f)


Addition Rate
Cholesterol
89.5
89.8
90.0
90.3
90.5
90.8
91.0

(g)


Mixing Time
Mixing Speed
90.0
90.3
90.8
90.5
89.0
89.3
89.5
89.8
90.0
90.3
90.5

(h)



(i) **(j)**

Figure 3.2. Contour model graph for entrapment efficiency (a) Span 60 (X_1) and cholesterol (X_2), (b) Span 60 (X_1) and mixing speed (X_3), (c) Span 60 (X_1) and mixing time (X_4), (d) Span 60 (X_1) and addition rate (X_5), (e) cholesterol (X_2) and mixing speed (X_3), (f) cholesterol (X_2) and mixing time (X_4), (g) cholesterol (X_2) and addition rate (X_5), (h) mixing speed (X_3) and mixing time (X_4), (i) mixing speed (X_3) and addition rate (X_5) and (j) mixing time (X_4) and addition rate (X_5).

3.6.4.1.2. Effect of Span 60 (surfactant) concentration on entrapment efficiency

Contour plot examination demonstrated that in the case of interaction between (a) Span 60 (X_1) and cholesterol (X_2) at other three variables constant, (b) Span 60 (X_1) and mixing speed (X_3) at other three variables constant, (c) Span 60 (X_1) and mixing time (X_4) at other three variables constant, (d) Span 60 (X_1) and addition rate (X_5) at other three variables constant, the response of surfactant was reasonably partial. With the gradient inclination of Span 60 concentration, the entrapment efficiency was gradually and inversely decreasing. The explanation is related to the compromised polymer availability for the small particles, which decreases flexibility at higher surfactant concentrations. With low flexibility, it was observed that membrane irregularities and

even vesicle rupture are quite high. These results were observed to be consistent with trends published in various literature [34].

3.6.4.1.3. Effect of cholesterol concentration on entrapment efficiency

Contour plot investigation showed that in the case of (a) Span 60 (X_1) and cholesterol (X_2) at other three variables constant, (e) cholesterol (X_2) and mixing speed (X_3) at other three variables constant, (f) cholesterol (X_2) and mixing time (X_4) at other three variables constant, (g) cholesterol (X_2) and addition rate (X_5) at other three variables constant, the response of cholesterol was reasonably partial. With the gradient inclination of cholesterol concentration, the entrapment efficiency was increasing gradually, except it showed an inverse trend with an increasing Span 60 concentration. Cholesterol is one of the essential ingredients for the niosome vesicle formation. Increasing cholesterol concentration increases membrane rigidity and thus increasing of percentage entrapment efficiency by the formation of stable and less leaky vesicles. A similar trend was also observed and reported in the previous publication [35].

3.6.4.1.4. Effect of mixing speed on entrapment efficiency

Contour plot analysis showed in the case of (b) Span 60 (X_1) and mixing speed (X_3) at other three variables constant, (e) cholesterol (X_2) and mixing speed (X_3) at other three variables constant, (h) mixing speed (X_3) and mixing time (X_4) at other three variables constant, (i) mixing speed (X_3) and addition rate (X_5) at other three variables constant, the response of mixing speed variations was observed to be quite arbitrary when evaluated against Span 60 and addition rate. Entrapment efficiency appears to be increasing with gradually increasing mixing speed along with cholesterol. At higher

mixing time and lower mixing speed, the entrapment efficiency was also seen to increase. As mixing speed increases gradually, it shows an opposite trend in entrapment efficiency.

3.6.4.1.5. Effect of mixing time on entrapment efficiency

Contour plot analysis showed in the case of (c) Span 60 (X_1) and mixing time (X_4) at other three variables constant, (f) cholesterol (X_2) and mixing time (X_4) at other three variables constant, (h) mixing speed (X_3) and mixing time (X_4) at other three variables constant, (j) mixing time (X_4) and addition rate (X_5) at other three variables constant, the response of mixing time was quite arbitrary when evaluated against Span 60. Entrapment efficiency shows an upward trend with periodically increasing mixing time together with cholesterol. At lower addition rate, entrapment efficiency is higher and shows gradually increasing trend with increasing mixing time. At higher mixing time and lower mixing speed, it was observed an increasing trend for entrapment efficiency. As mixing speed increases gradually, it shows a decreasing trend in entrapment efficiency. Overall, it is understandable that mixing time has a direct impact on entrapment efficiency. This behavior can be explained as longer mixing time provides adequate hydration time, which shows improved dispersibility and provides rigid and less leaky niosomes.

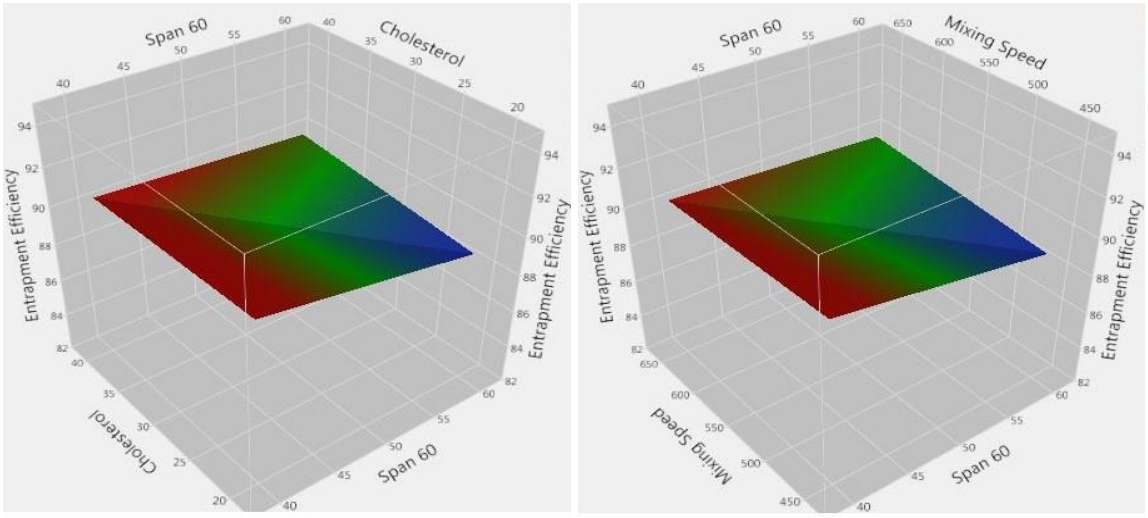
3.6.4.1.6. Effect of addition rate on entrapment efficiency

Contour plot inspection showed in the case of (d) Span 60 (X_1) and addition rate (X_5) at other three variables constant, (g) cholesterol (X_2) and addition rate (X_5) at other three variables constant, (i) mixing speed (X_3) and addition rate (X_5) at other three variables constant, (j) mixing time (X_4) and addition rate (X_5) at other three variables

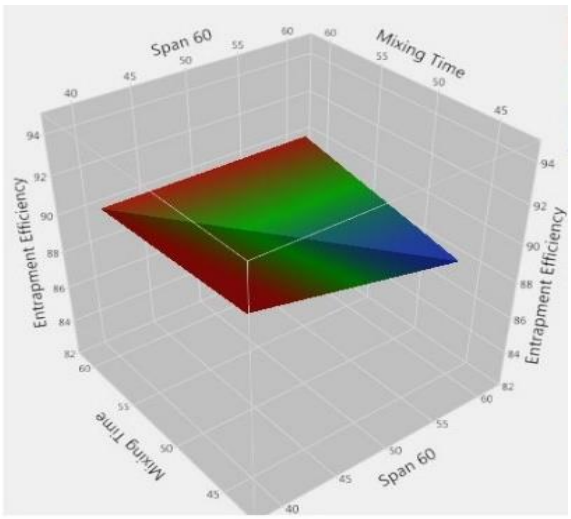
constant, the response of addition rate is quite constant and shows better entrapment efficiency at a slower addition rate regardless of other independent variables. This behavior can be explained as a slower addition rate in the end to increase total mixing time, which provides adequate hydration time with better dispersibility and shows less leaky and rigid niosomes.

3.6.4.1.7. Response surface analysis for entrapment efficiency

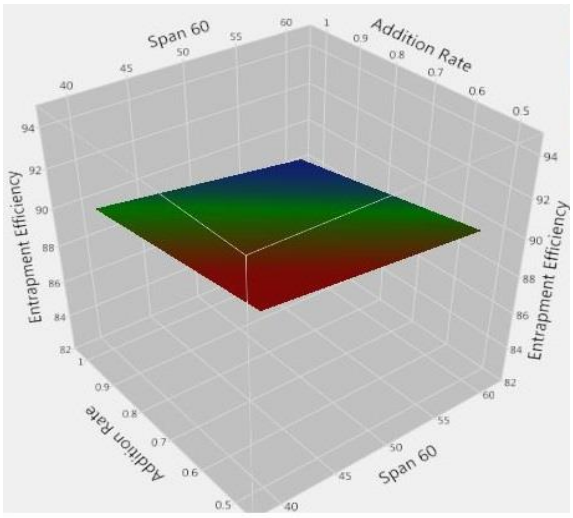
Response surface analysis further explained that in all probable cases of interactions, Span 60 (X_1) and cholesterol (X_2) with three other variables constant provided a controllable and favorable range of entrapment efficiency to the niosomes. The surface response curve in Figure 3.3 further justified this results. Based on contour 2D plots and response surface analysis conclusion suggested a specific combination of Span 60 (X_1) and cholesterol (X_2) while keeping the other three variables constant to achieve entrapment efficiency within a desirable range.



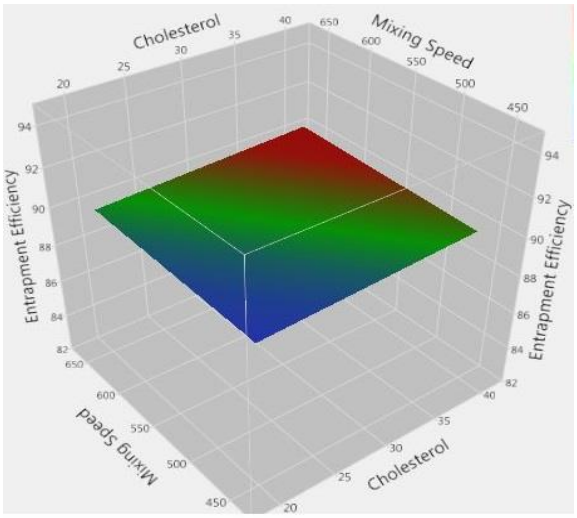
(a) **(b)**


Span 60
Mixing Time
Entrapment Efficiency
Mixing Time
Span 60
Entrapment Efficiency

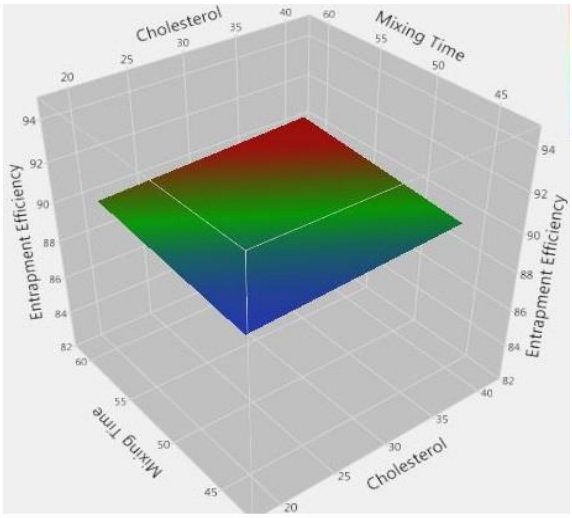
(c)


Span 60
Addition Rate
Entrapment Efficiency
Addition Rate
Span 60
Entrapment Efficiency

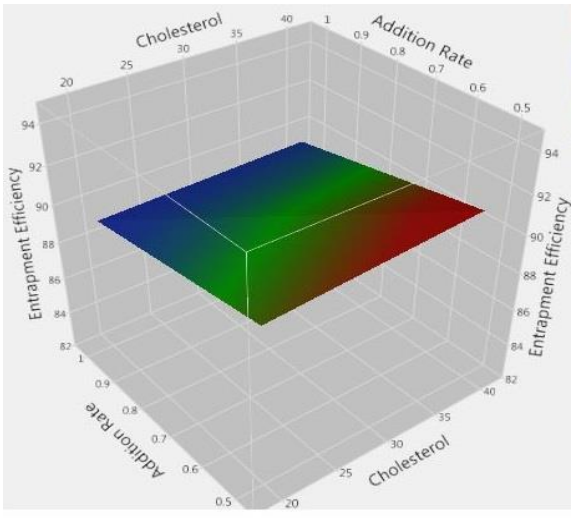
(d)


Cholesterol
Mixing Speed
Entrapment Efficiency
Mixing Speed
Cholesterol
Entrapment Efficiency

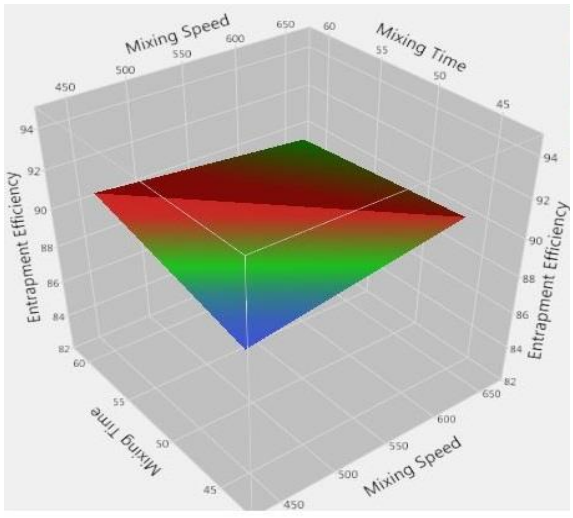
(e)


Cholesterol
Mixing Time
Entrapment Efficiency
Mixing Time
Cholesterol
Entrapment Efficiency

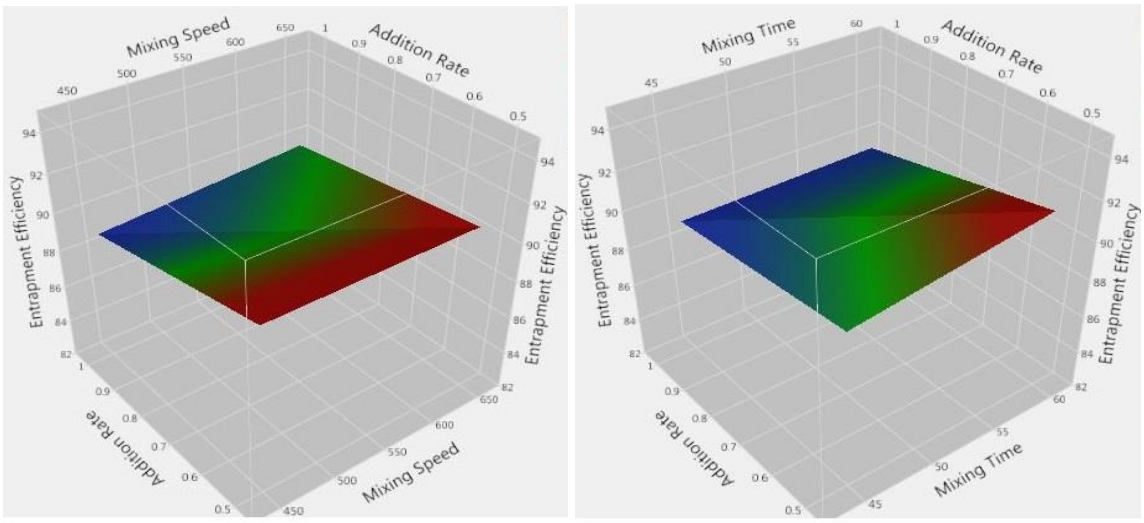
(f)


Cholesterol
Addition Rate
Entrapment Efficiency
Addition Rate
Cholesterol
Entrapment Efficiency

(g)


Mixing Speed
Mixing Time
Entrapment Efficiency
Mixing Time
Mixing Speed
Entrapment Efficiency

(h)



(i) **(j)**

Figure 3.3. 3D surface model graph for entrapment efficiency (a) (a) Span 60 (X_1) and cholesterol (X_2), (b) Span 60 (X_1) and mixing speed (X_3), (c) Span 60 (X_1) and mixing time (X_4), (d) Span 60 (X_1) and addition rate (X_5), (e) cholesterol (X_2) and mixing speed (X_3), (f) cholesterol (X_2) and mixing time (X_4), (g) cholesterol (X_2) and addition rate (X_5), (h) mixing speed (X_3) and mixing time (X_4), (i) mixing speed (X_3) and addition rate (X_5), and (j) mixing time (X_4) and addition rate (X_5). In the figure, red color indicates the highest entrapment efficiency, followed by green color and then blue color (lowest entrapment efficiency) achieved for specific combinations.

3.6.4.2. Response 2 (Y_2): Effect of formulation variables on particle size

Analyzing this response for particle size did not require further plot transformation. A best-fit model represented an R^2 value 0.59, which represented that all of the CMAs & CPPs had a significant impact on niosomal particle size. The allusion was drawn from the magnitude and mathematical sign of each coefficient.

The regression equations explain that the corresponding effect of surfactant concentration (X_1), cholesterol concentration (X_2), mixing time (X_3), mixing speed (X_4) and addition rate (X_5) as they all demonstrated an inverse relationship with niosome particle size. In some cases, the relationship between responses and factors were not

always linear and suggested that interactions existed between different variables. Combinations X_1&X_2, X_2&X_3, X_2&X_4, X_3&X_4, X_1&X_5, X_3&X_5, X_4&X_5 shows favorable interactions with decreased particle size, while X_1&X_3, X_1 &X_4, X_2&X_5 combinations are not favorable as they confirm increased particle size of Y_2 response.

Table 3.5. Summary of results of regression analysis for particle size response Y_2.

Quadratic Model	R^2	Adjusted R^2
Response (Y_2) particle size	0.591	0.209481

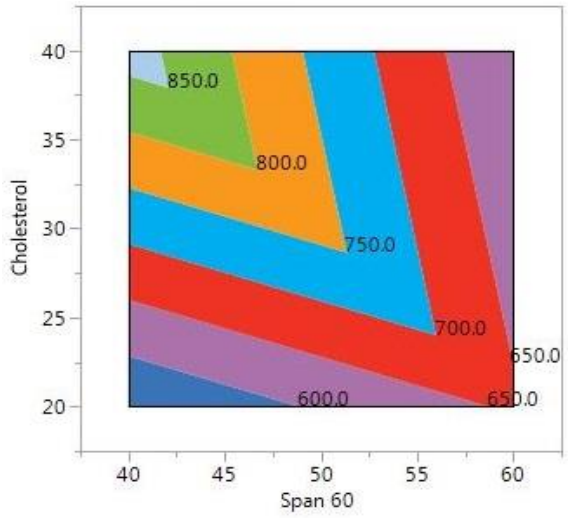
After a regression analysis for the particle size, the polynomial model was established using the following equation:

Niosomes particle size (Y_2) = $671.50 - 42.16(X_1) - 65.77(X_2) - 124.98(X_3) - 7.95(X_4) - 27.59(X_5) - 93.02(X_1X_2) + 7.98(X_1X_3) - 25.62(X_2X_3) + 37.77(X_1X_4) - 64.99(X_2X_4) - 18.92(X_3X_4) - 28.79(X_1X_5) + 31.24(X_2X_5) - 14.17(X_3X_5) - 49.18(X_4X_5)$.

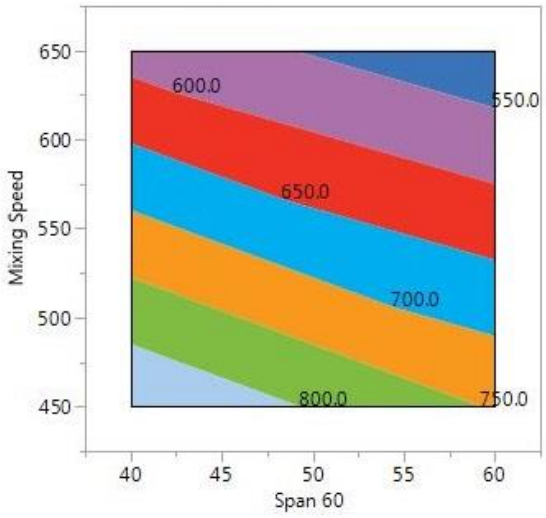
Where X_1 is Span 60 (mg), X_2 is cholesterol (mg), X_3 is mixing speed (rpm), X_4 is mixing time (min), and X_5 is addition rate (mL/min.).

3.6.4.2.1. Contour model graph for particle size

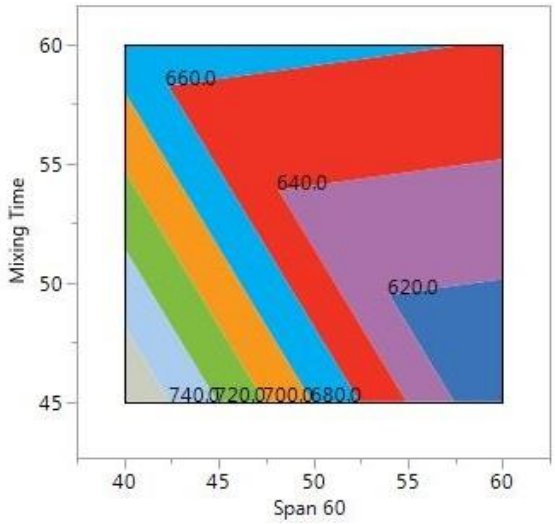
The interaction between two independent variables that showed no linear correlation, while the other three independent variables remained at constant to illustrate their respective response is shown in Figure 3.4 (a-j).


Cholesterol
Span 60
850.0
800.0
750.0
700.0
650.0
600.0

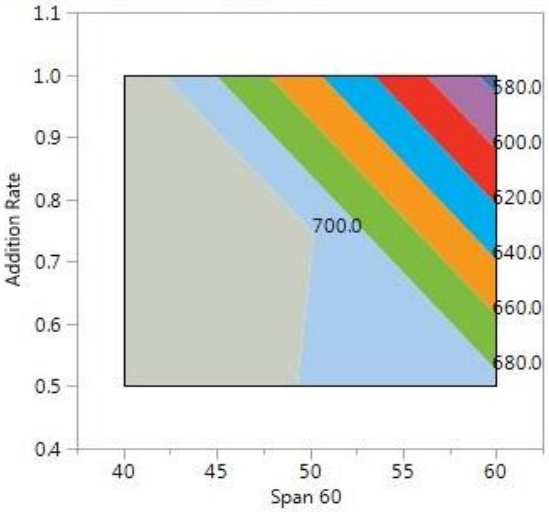
(a)


Mixing Speed
Span 60
600.0
650.0
700.0
800.0
550.0
750.0

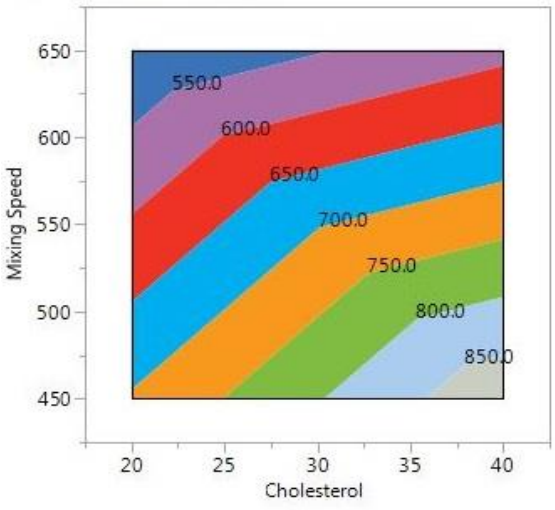
(b)


Mixing Time
Span 60
660.0
640.0
620.0
740.0
720.0
700.0
680.0

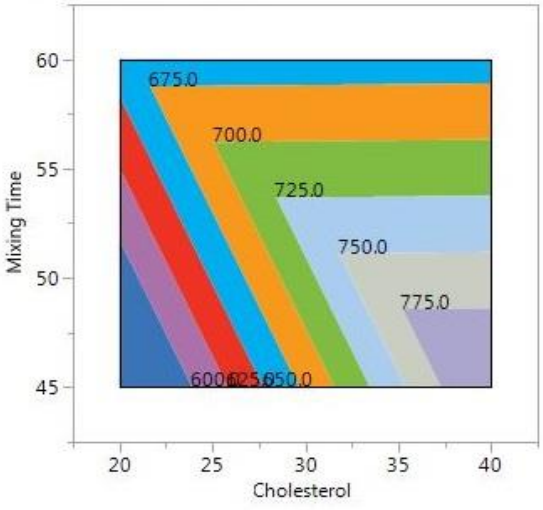
(c)


Addition Rate
Span 60
700.0
580.0
600.0
620.0
640.0
660.0
680.0

(d)


Mixing Speed
Cholesterol
550.0
600.0
650.0
700.0
750.0
800.0
850.0

(e)


Mixing Time
Cholesterol
675.0
700.0
725.0
750.0
775.0

(f)

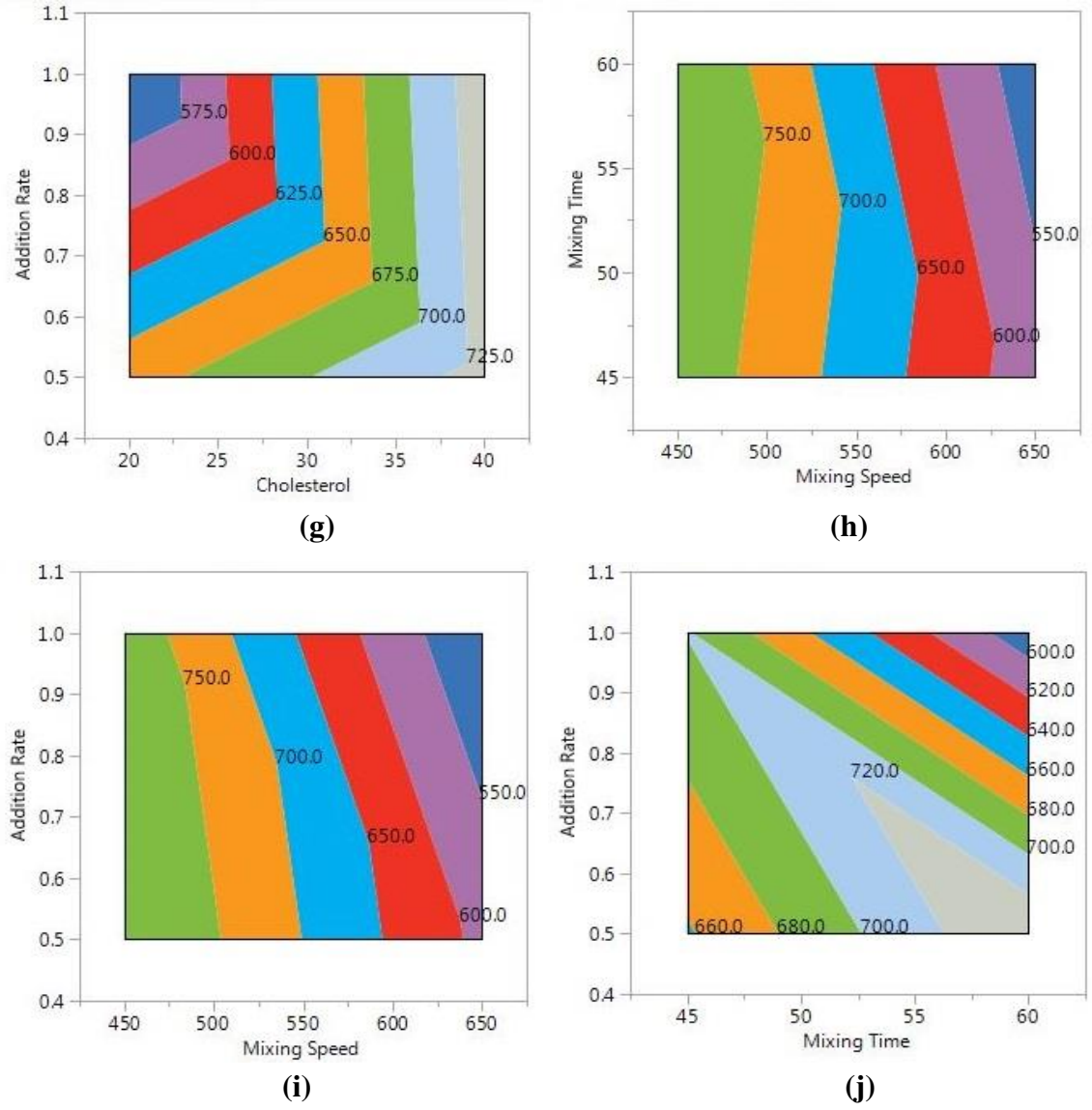


Figure 3.4. Contour model graph for particle size (a) Span 60 (X_1) and cholesterol (X_2), (b) Span 60 (X_1) and mixing speed (X_3), (c) Span 60 (X_1) and mixing time (X_4), (d) Span 60 (X_1) and addition rate (X_5), (e) cholesterol (X_2) and mixing speed (X_3), (f) cholesterol (X_2) and mixing time (X_4), (g) cholesterol (X_2) and addition rate (X_5), (h) mixing speed (X_3) and mixing time (X_4), (i) mixing speed (X_3) and addition rate (X_5) and (j) mixing time (X_4) and addition rate (X_5).

3.6.4.2.2. Effect of Span 60 (surfactant) concentration on particle size

Contour plot analysis showed in the case of (a) Span 60 (X_1) and cholesterol (X_2) at other three variables constant, (b) Span 60 (X_1) and mixing speed (X_3) at other three

variables constant, (c) Span 60 (X_1) and mixing time (X_4) at other three variables constant, (d) Span 60 (X_1) and addition rate (X_5) at the three other variables constant, the response of Span 60 concentration is quite arbitrary. It is trending proportionally like cholesterol concentration, mixing speed, mixing time, and addition rate cases, which decreases particle size with an increasing Span 60 concentration. This can be explained as high surfactant concentration in formulation decreases surface tension and stabilizes newly developed vesicle surfaces during the manufacturing and produce smaller particles. The observed change in particle size with the change in surfactant concentration was seen to be confirmed with the published in the prior article [36].

3.6.4.2.3. Effect of cholesterol concentration on particle size

Contour plot analysis showed that in the case of (a) Span 60 (X_1) and cholesterol (X_2) at other three variables constant, (e) cholesterol (X_2) and mixing speed (X_3) at other three variables constant, (f) cholesterol (X_2) and mixing time (X_4) at other three variables constant, (g) cholesterol (X_2) and addition rate (X_5) at other three variables constant, the cholesterol response was reasonably consistent. The particle size was seen to increase parallels in the presence of other independent variables with the gradient inclination of cholesterol concentration. It can be explained that cholesterol is a crucial element for the niosome structure. The observed change in mean vesicle size of desoximetasone niosomes with the addition of cholesterol was seen to be verified with the findings reported in previous literature [35, 37].

3.6.4.2.4. Effect of mixing speed on particle size

Contour plot inspection showed in the case of (b) Span 60 (X_1) and mixing speed (X_3) at other three variables constant, (e) cholesterol (X_2) and mixing speed (X_3) at other three variables constant, (h) mixing speed (X_3) and mixing time (X_4) at other three variables constant, (i) mixing speed (X_3) and addition rate (X_5) at other three variables constant, the response of mixing speed was reasonably consistent. A gradual decrease in the particle size of the niosomes was observed with the gradient increase of mixing speed. These results are supported by previous research works that reported the dependence of vesicle size on the preparation method, bilayer composition, and bio-component concentration [38, 39].

3.6.4.2.5. Effect of mixing time on particle size

Contour plot analysis showed in the case of (c) Span 60 (X_1) and mixing time (X_4) at other three variables constant, (f) cholesterol (X_2) and mixing time (X_4) at other three variables constant, (h) mixing speed (X_3) and mixing time (X_4) at other three variables constant, (j) mixing time (X_4) and addition rate (X_5) at the other three variables constant, the response of mixing time was quite arbitrary. When evaluated with mixing speed, the response of mixing time is constant with minimum impact. When evaluated against the addition rate, it was seen that lower mixing time/addition rate or higher mixing time/addition rate is required to achieve a desirable smaller particle size. Regardless of other variables, and the increase in mixing time shows a decrease in particle size. This behavior can be explained as longer mixing time provides adequate hydration time and shows better dispersibility that can further provide smaller and

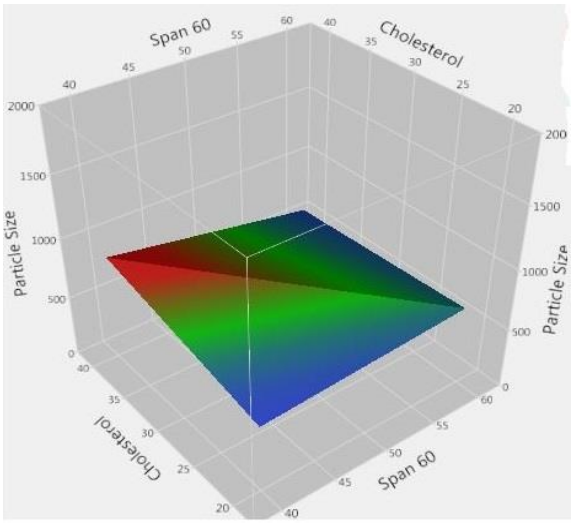
uniform niosomes. Similar findings have been observed in previously published work [40].

3.6.4.2.6. Effect of addition rate on particle size

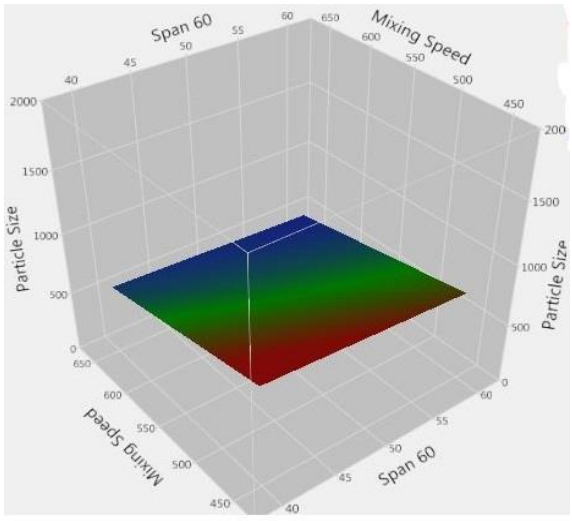
Contour plot analysis showed in the case of (d) Span 60 (X_1) and addition rate (X_5) at other three variables constant, (g) cholesterol (X_2) and addition rate (X_5) at other three variables constant, (i) mixing speed (X_3) and addition rate (X_5) at other three variables constant, (j) mixing time (X_4) and addition rate (X_5) at other three variables constant, the response of addition rate is quite constant. Particle size is comparatively constant when it is evaluated against Span 60, cholesterol, and mixing speed and changes gradually with changes in variables other than the addition rate. This behavior clearly states that other variables have a dominant impact on niosomes particle size as compared to the addition rate. When it is evaluated against mixing time, combinations of lower addition rate/mixing time or higher addition rate/mixing time is seen to be required to achieve desirable smaller particle size.

3.6.4.2.7. Response surface analysis for particle size

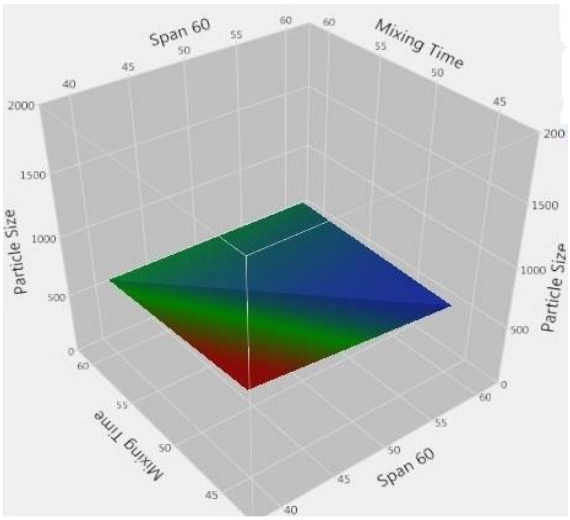
Response surface analysis further explained that in every probable case of interactions, Span 60 (X_1) and cholesterol (X_2) at the other three variables constant was providing a controllable and favorable range of particle size. The surface response curve in Figure 3.5 further justified these conclusions.


Span 60
Cholesterol
Particle Size
Cholesterol
Span 60
Particle Size

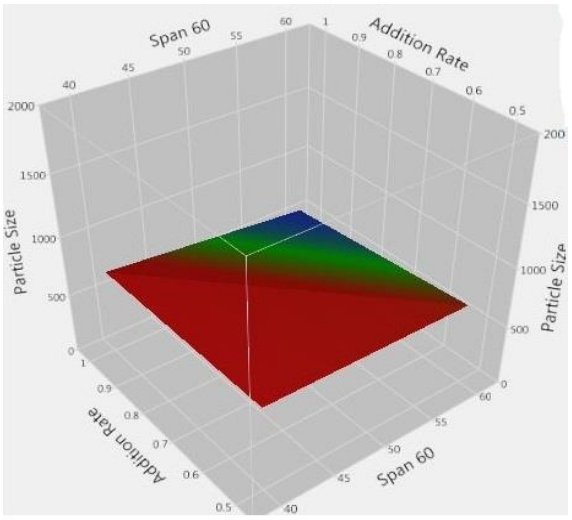
(a)


Span 60
Mixing Speed
Particle Size
Mixing Speed
Span 60
Particle Size

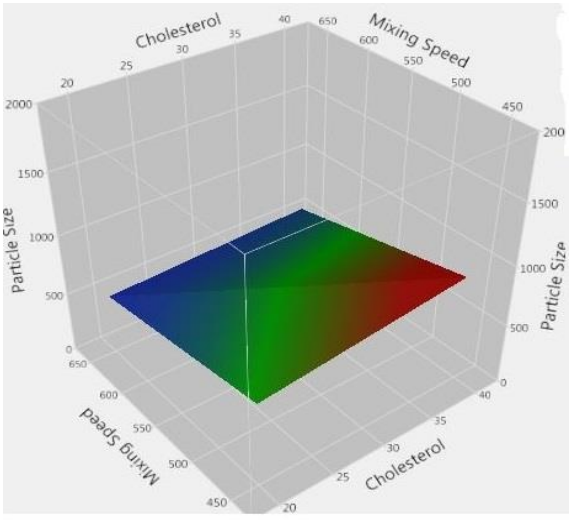
(b)


Span 60
Mixing Time
Particle Size
Mixing Time
Span 60
Particle Size

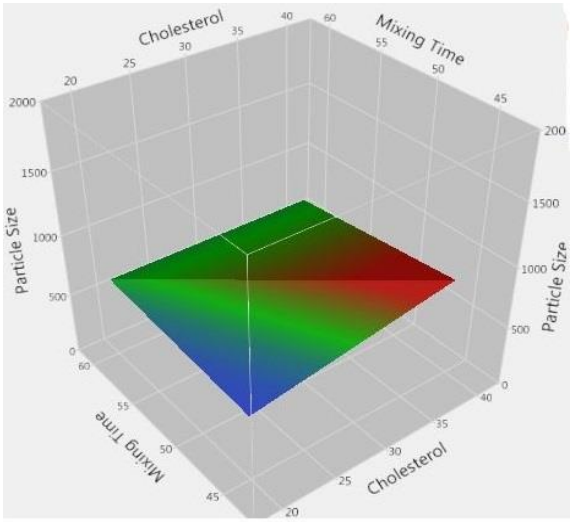
(c)


Span 60
Addition Rate
Particle Size
Addition Rate
Span 60
Particle Size

(d)


Cholesterol
Mixing Speed
Particle Size
Mixing Speed
Cholesterol
Particle Size

(e)


Cholesterol
Mixing Time
Particle Size
Mixing Time
Cholesterol
Particle Size

(f)

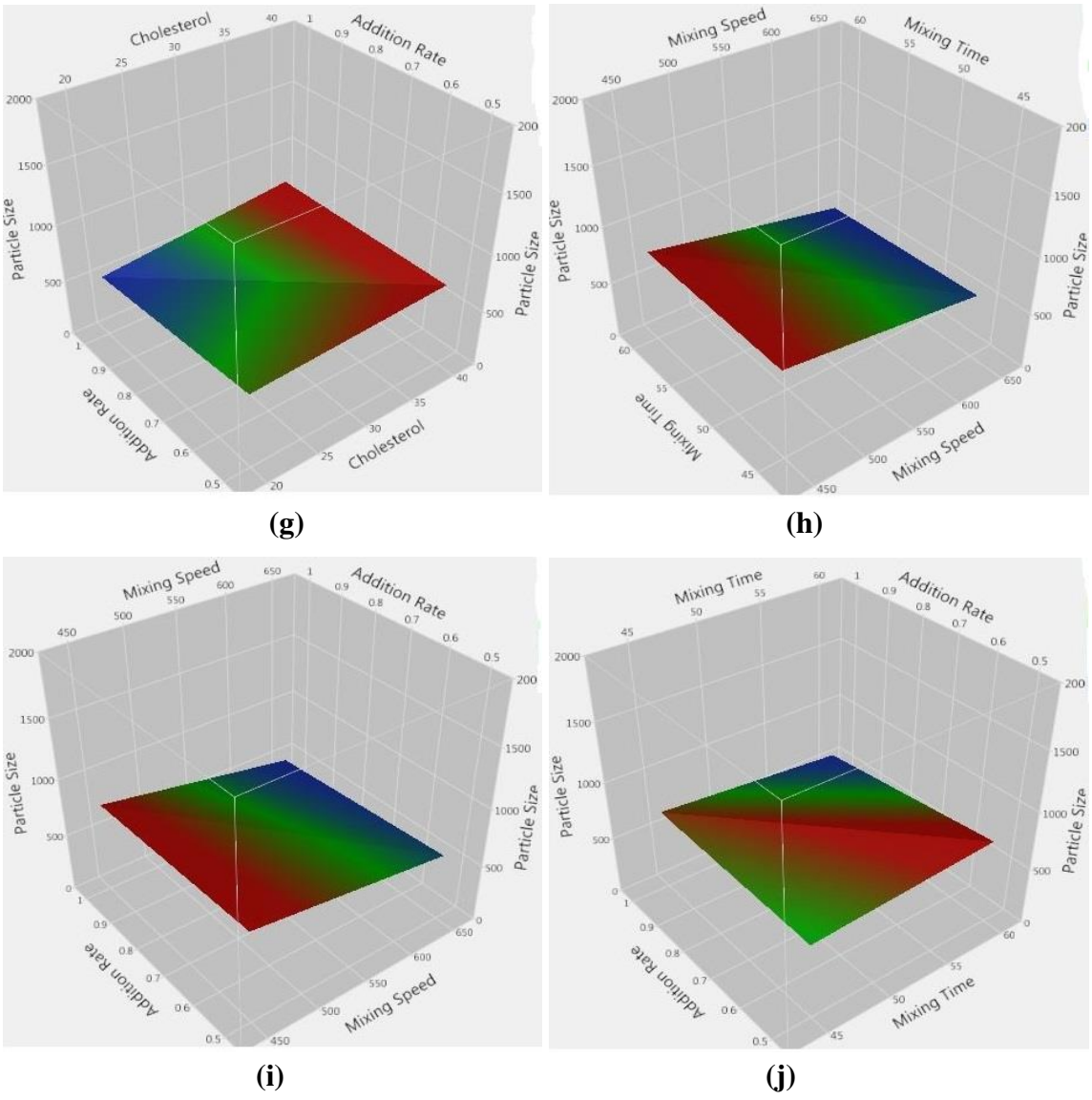


Figure 3.5. 3D surface model graph for particle size (a) Span 60 (X_1) and cholesterol (X_2), (b) Span 60 (X_1) and mixing speed (X_3), (c) Span 60 (X_1) and mixing time (X_4), (d) Span 60 (X_1) and addition rate (X_5), (e) cholesterol (X_2) and mixing speed (X_3), (f) cholesterol (X_2) and mixing time (X_4), (g) cholesterol (X_2) and addition rate (X_5), (h) mixing speed (X_3) and mixing time (X_4), (i) mixing speed (X_3) and addition rate (X_5), and (j) mixing time (X_4) and addition rate (X_5). In the figure, red color indicates the highest particle size, followed by green color and then blue color (lowest particle size) achieved for specific combinations.

Based on contour plots and response surface analysis, specific combinations of Span 60 (X_1) and cholesterol (X_2) keeping the other three variables constant can be

concluded essential to achieve a favorable range of maximum entrapment efficiency and minimum particle size of the niosomes. A further selection is based on the validation checkpoints generated by the software.

3.6.5. Validation of Design of Experiment (profile predictor)

Validating an experimental design model consisted of two checkpoint niosome formulations (DND-62 and DND-63) for accuracy by comparing observed values with predicted entrapment efficiency and particle size values from the profile predictor model provided by JMP® software. Niosome formulations were manufactured for a comparative study of entrapment efficiency and particle size, as provided in Table 3.6 and Table 3.7, respectively.

Table 3.6. Composition of checkpoint formulations, expected and observed value for the response variable of niosomal entrapment efficiency (n=3, mean ± SD).

Batch Detail	Variable parameters					Entrapment efficiency	
	Span 60 (mg)	Cholesterol (mg)	Mixing time (min)	Mixing speed (rpm)	Addition rate (mL/min)	Expected value (%)	Observed value ± S.D (%)
DND-62	40	20	50	650	0.5	91.11	90.19 ± 0.02
DND-63	40	40	60	500	1.0	89.66	87.27 ± 0.01

Table 3.7. Composition of checkpoint formulations, expected and observed value for response variable of niosomal particle size (n=3, mean ± SD).

Batch Detail	Variable parameters					Particle size	
	Span 60 (mg)	Cholesterol (mg)	Mixing time (min)	Mixing speed (rpm)	Addition rate (mL/min)	Expected value (nm)	Observed value ± S.D (nm)
DND-62	40	20	50	650	0.5	475.20	449.40 ± 29.2
DND-63	40	40	60	500	1.0	840.85	813.43 ± 173.8

No significant differences between the obtained and the expected values were observed using this model, which concluded that the model accurately predicted the entrapment efficiency and particle size using the experimental design mentioned. Formulation DND-62 resulted in significantly higher entrapment efficiency and smaller particle size compared with the formulation DND-63.

DND-62 was selected for use in a further study to optimize desoximetasone-loaded niosomes based on the desired criteria of particle size and maximum entrapment efficiency. Formulation composition containing – drug: surfactant: cholesterol (1:2:1), diethyl ether: methanol (75:25), external phase temperature (65°C), external phase volume: internal phase volume (2:1), mixing speed (650 rpm), mixing time (50 min), addition rate (0.5 mL/min) successfully developed a niosomal formulation with optimal particle size and drug entrapment efficiency.

3.6.6. A non-ionic surfactant verification study

Non-ionic surfactants contain a polar head and a non-polar tail group with different alkyl chain lengths. The stability and entrapment efficiency of the vesicle formed by various non-ionic surfactants were directly affected by the surfactant's intrinsic properties like HLB value, chemical arrangement, and phase transition temperature [41]. Niosomes directly impact the entrapment efficiency and particle size of the final product; therefore, it is highly recommended to choose correct non-ionic surfactant to develop robust niosome with better entrapment efficiency and optimum particle size.

The non-ionic surfactant selected for this study is Span 60 due to high transition temperature (53°C) and the longer alkyl chain, which is required for the higher entrapment efficiency. As mentioned earlier, entrapment efficiency and particle size

depending on the type of the surfactant; therefore, the new formulation was manufactured with a span-20 surfactant. This was executed by keeping all other CMAs and CPPs constant as formulation DND-62. Detail about the manufacturing parameters and observed results for the surfactant evaluation study are presented in Table 3.8 and Table 3.9, respectively.

Table 3.8. Niosome surfactant evaluation batches parameters.

Batch Detail	Critical Material Attributes					Critical Processing Parameters					
	Drug (mg)	Organic Phase Composition	Surfactant (mg)	Cholesterol (mg)	Stearic Acid (mg)	External Phase Temperature (°C)	External Phase Volume (mL)	Internal Phase Volume (mL)	Mixing speed (RPM)	Mixing time (Minutes)	Addition Rate (mL/Min)
DND-62	20	diethyl ether : methanol	40 (Span 60)	20	5	65	20	10	650	50	0.50
DND-64	20	diethyl ether : methanol	40 (Span 20)	20	5	65	20	10	650	50	0.50

Table 3.9. Surfactant evaluation batches result comparison.

Batch Detail	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
DND-62 (Span 60)	90.19 $\pm$ 0.02	449.40 $\pm$ 29.2	0.272 $\pm$ 0.03	-73.50 $\pm$ 0.87
DND-64 (Span 20)	81.02 $\pm$ 0.01	468.30 $\pm$ 76.8	0.305 $\pm$ 0.05	-51.90 $\pm$ 2.47

Data from the surfactant evaluation study (DND-62 and DND-64) demonstrates that formulation with Span 20 has lower entrapment efficiency and larger particle size while comparing with batch manufactured with Span 60. This behavior can be explained as surfactant intrinsic properties such as HLB value, chemical arrangement, and phase transition temperature play a significant role in the formation of niosomes. Span 60 is a hydrophobic surfactant with low HLB value 4.7 with longer saturated alkyl chain length (C_{18}) that accommodates a higher amount of drug into its hydrophobic region and shows better entrapment efficiency. Additionally, Span 60 has a high transition temperature

(53°C) more likely in the ordered form, forming less leaky bilayers, further increasing the entrapment efficiency of the drug. On the other side, Span 20 has a high HLB value of 8.6, a small alkyl chain (C12), and low transition temperature; thus, it has less capacity to incorporate hydrophobic drugs [42-44].

3.6.7. Characterization of optimized niosomal formulation

3.6.7.1. Entrapment efficiency of niosomes

The drug entrapment efficiency of the optimized desoximetasone loaded niosomes was determined using the validated HPLC method, and entrapment efficiency was found to be 90.19 $\pm$ 0.02 %, which describes an appreciable drug loading in niosomes vesicles.

3.6.7.2. Niosome size and distribution

The size of the optimized desoximetasone loaded niosomes was 449.40 $\pm$ 29.2 nm, with a polydispersity index (PDI) of 0.272 $\pm$ 0.03.

3.6.7.3. Zeta potential

Zeta potential of the optimized niosome formulation DND-62 was found to be -73.50 $\pm$ 0.87 mV. Favorable stability of niosomes associated with higher absolute values $\pm$ 30 mV mitigate stability risk while using surfactants [45]. Higher zeta potential values typically increase due to repulsion charges, which prevent particle aggregation.

3.7. Conclusion

Optimization of niosomes for use in topical applications requires a comprehensive understanding of numerous variables that may be interdependent. This work demonstrated the effectiveness of a systematic full factorial design methodology to successfully predict an ideal niosome vesicles containing desoximetasone for topical applications. Formulations obtained for relevant performance efficacy characteristics using *in vitro*, *ex vivo,* and *in vivo* testing techniques. The results show how niosomes were modified to achieve an effective drug delivery profile selecting appropriate parameters for Span 60, cholesterol, mixing time, mixing speed, and addition rate. The optimized niosome formulation (DND-62) was accurately predicted entrapment efficiency and particle size using JMP® software. Further, a surfactant evaluation study also supports that Span 60 formulation (DND-62) shows more favorable results compared to Span 20 formulation. Based on the detailed research about the desoximetasone niosome optimization, a summary of the optimized desoximetasone niosome dispersion is described in Table 3.10.

Table 3.10. Summary of optimized desoximetasone niosomal dispersion formulation (DND).

Ingredients	DND – 62 (% w/w)
Desoximetasone, USP	0.20
Sorbitan Monostearate (Span 60)	0.40
Cholesterol, NF	0.20
Stearic Acid	0.05
Purified Water	Q.S. to 100.00

The methods described to create desoximetasone-loaded niosomes may potentially optimize drug-vehicles in other formulations as an effective way to develop various drug delivery applications.

3.8. References

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4. CHAPTER – 4. DEVELOPMENT, FORMULATION AND EVALUATION OF NON-IONIC SURFACTANT VESICLES (NIOSOMES) BASED TOPICAL GEL

4.1. Introduction

Skin is the largest organ in the body that occupies almost 16% of that total body mass of an adult with approximately 2 m^2 surface area [1]. Due to its complex arrangement of structure, it provides a physical barrier to the environment by acting as a protective barrier by limiting the loss of water, electrolytes, heat and prevents microbial infection [2].

Psoriasis is an autoimmune chronic inflammatory skin disease. Psoriasis starts from a small, localized area to coverage of the full body. The word psoriasis derived from the Greek word “psora” stands for “itch.” Psoriasis categorized in different levels as plaque psoriasis, nail psoriasis, invasive psoriasis, scalp psoriasis, psoriatic arthritis, and many more. According to the International Federation of Psoriasis Association, approx. 3% of the total world population has partial to complete symptoms of psoriasis. Psoriasis patient numbers are increasing by time. Therefore it is imperative to find a better and improved cure for psoriasis treatment [3, 4]. In 2018, the estimated annual cost of 32.5 billion USD for psoriasis treatment in the United States [5].

In recent times, topical drug delivery for pharmaceutical delivery is more acceptable and accessible. Administration of the drugs to the skin by two major pathways: topical and transdermal absorption. Topical formulations are specially designed to deliver the drug into a deeper region of the skin. Transdermal formulations

aim to deliver the drug into the systemic circulation. Topical dosage forms are widely used for localized therapy and accepted for their effect at the site of administration onto the skin [6, 7].

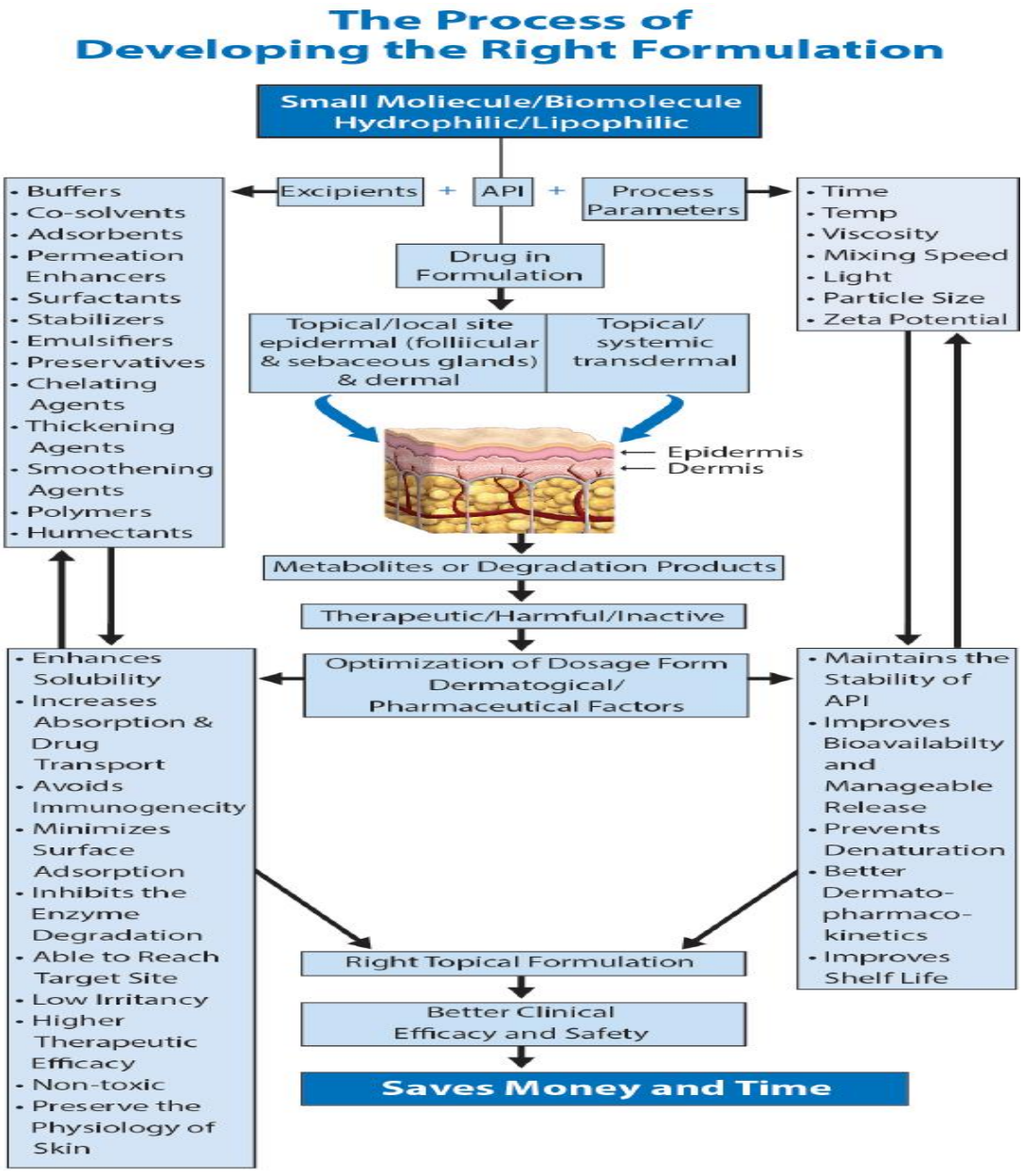

Figure 4.1. The process for correct formulation development for semisolids [8].

Formulations for skin delivery can be efficiently developed using frequently used vehicles across the pharmaceutical industry to treat patients with various skin conditions

[9]. It is widely accepted in the treatment of cutaneous disorders such as acne or the universal disease like psoriasis. The main target of this drug delivery method is confining the pharmacological or other effects of the drug to the surface of the skin or within the skin [10].

Topical medications are preferred to treat inflammation and relieve itching caused by several skin conditions including but not limited to allergic reactions, eczema, and psoriasis. Desoximetasone is used in first-line topical therapy of skin disease. The fundamental mechanism of desoximetasone activates specific intracellular receptors, which bind to distinct DNA sites to modify gene transcription resulting in an induction of the synthesis of anti-inflammatory proteins and suppression of the synthesis of inflammatory mediators. This pathway leads to an overall reduction in chronic inflammation and autoimmune reactions.

Topical formulations for drug delivery are becoming increasingly popular due to many advantages like the ability to deliver drugs more selectively to a specific site; drug level fluctuation can be avoided, improved compliance, and enhanced suitability for self-medication. Desoximetasone topical gel contains several pharmaceutically acceptable thickening agents, like Carbomer, hydroxyl propyl cellulose, hydroxyl propyl methylcellulose was used to prepare viscous formulation for topical application. The gel system not limited that serves as a stabilizer but can also form a uniform drug distribution matrix and increase the contact time of the nanoparticles on the skin resulting in enhanced skin penetration of the payload [11]. The formulation for the topical delivery is a key as the molecule itself because the interaction of the vehicle with the skin can modify the efficacy of the penetrant. The formulation also ensures that the drug substance

is delivered to the targeted site and that it maintains dosage integrity, drug transport, and active duration. Additionally, the change in any physicochemical properties such as pH, viscosity, rheology, stabilizers, droplet size, ionic nature, or the method of preparation can often influence skin absorption and drug efficacy. Ideal formulation development should have preformulation studies and carefully selected excipients [8]. Several studies have been reported in the literature using drug-loaded niosome containing topical gels for the enhanced drug delivery through the skin [12-15].

The main principles of the drug pass through the skin follows laws of passive diffusion and can be described by Fick's first law [16]. According to the law, a permeant will transfer due to the concentration gradient from a high concentration region to a low concentration region. Mathematical models derived by Higuchi explained this passive diffusion process in terms of percutaneous absorption [17, 18]. Moreover, Higuchi used physicochemical principles in describing the importance of the thermodynamic activity of the permeating drug molecule. From the thermodynamic view, the steady-state flux (J) can be described as [18, 19].

$$J = {}^{\alpha D}/_{\Upsilon L}$$

Where α is the thermodynamic activity of the drug in its vehicles, ϒ is the activity coefficient of the drug in the skin, D, and L are the diffusion coefficient of the drug in the skin and skin thickness, respectively.

The objective of this study was to investigate the feasibility of topical delivery of desoximetasone loaded niosomes. This is the first study to investigate the topical flux and skin deposition of desoximetasone to the best of our knowledge. In the beginning, the topical gel was manufactured using various concentrations of Carbomer 980 polymer.

Reverse engineering for the optimization of Carbomer 980 concentration, Rheological evaluation was performed. It was compared with the reference product (Topicort® Gel – marketed product) to identify the correct quantity of the Carbomer 980 polymers. Various topical gel lab batches were manufactured by replacing Carbomer 980 with Carbomer 940, Carbomer 971, Carbomer 98, Carbomer 1342, ethyl cellulose (EC), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC) and xanthan gum to evaluate various gelling agent impact on the final product. Based on the product characterization, ideal topical gel formulation was selected and further evaluated for skin permeation and skin deposition studies compared with the Topicort® Gel – reference marketed gel product.

4.2. Materials

Desoximetasone was gifted by Flavine, New Jersey, USA. Diethyl ether, Stearyl amine, HPLC water, chloroform, calcium chloride dihydrate, docusate sodium, and hydroxypropyl methylcellulose (HPMC)were purchased from Sigma-Aldrich, Saint Louis, MO, USA. Ethanol was procured from Decon Labs, Inc., King of Prussia, PA, USA. Acetone, methanol, and acetonitrile were purchased from BDH VWR Analytical, Radnor, PA, USA. Cholesterol and sorbitan monostearate (Span 60) were gifted from Croda Inc., Mill Hall, PA, USA. Stearic acid was received from BASF Corporation, Edison, NJ, USA. Glacial acetic acid was purchased from Fisher Scientific, Fair Lawn, NJ, USA. Edetate disodium, trolamine, and xanthan gum were purchased from Spectrum Chemical, New Brunswick, NJ, USA. Ethyl cellulose (EC) and hydroxypropyl cellulose (HPC) were gifted by Ashland Specialty Ingredients, Parlin, NJ, USA. Transcutol was

gifted from Gattefosse Corporation, Paramus, NJ, USA. Carbomer 940, Carbomer 974P, Carbomer 980, Carbomer 981, and Carbomer 1342 were gifted from Lubrizol Advanced Materials Inc., Brecksville, OH, USA.

4.3. Methods

4.3.1 Topical gel preparation

Initially, purified water was weighed into a glass beaker. Edetate disodium, docusate sodium, and Transcutol were added into purified water; then, it was mixed using propeller mixer. Next, the thickening agent was carefully weighed and added slowly into the mixture and mixed using a propeller mixer. At the other end, in a separate glass container, purified water and trolamine were added and mixed using a spatula until it thoroughly mix. The mixture of purified water and trolamine was slowly added into a previously mixed thickening agent mixture. Mixing was carried out using the propeller mixer. In the final step of the process, the desoximetasone niosomal dispersion was accurately weighed and added into the previously prepared viscous mixture. Final mixing was carried out using the propeller mixer.

4.3.2. Topical gel chemical characterization

4.3.2.1. High performance liquid chromatography (HPLC) method

The mobile phase was prepared by mixing methanol, HPLC grade water, and glacial acetic acid in ratio 65:35:1 [20]. The diluent was made in a 100 mL volumetric flask by mixing 1.5 g calcium chloride dihydrate into 5 mL of HPLC grade water. The mixture was agitated until calcium chloride dihydrate dissolved completely. The final

volume was made using methanol. The desoximetasone was measured using a Discovery C18 column (Sigma-Aldrich, Saint Louis, MO, USA) with 5 µm particle size, L x I.D. 150 mm x 4.6 mm [20]. The flow rate was 1.5 mL/min, and the injection volume was 20 µL. The sample run time was 10 min at 30°C temperature and retention time for the drug peak was at approximately 4 minutes. Drug content quantification for desoximetasone was performed using HPLC instrument Agilent from 1100 series instrumentation (Agilent Technologies, CA, USA) coupled with UV detection (DAD) at a wavelength λ_{max} 254 nm and HP ChemStation software V. 32.

4.3.2.2. Desoximetasone assay characterization

The desoximetasone niosomal topical gel sample was mixed manually then, a random sample was carefully collected and placed into a diluent, and then using a vortexing mixer it was mixed thoroughly. Then the mixture was sonicated at 60°C for 12 minutes following by cooling down the mixture at room temperature. In the final step, the niosomal topical gel assay sample was further diluted with diluent, and then using a vortexing mixer it was mixed thoroughly. Drug quantification was determined using the pre-determined HPLC method as mentioned in section 4.3.2.1.

4.3.2.3. Content uniformity measurement

The desoximetasone niosomal topical gel sample for the content uniformity measurement was collected to identify drug content at a different location from the sample container. For the content uniformity, a separate sample was collected from top, middle and bottom positions. The sample was carefully collected and placed into a diluent, and then using a vortexing mixer it was mixed thoroughly. Then the mixture was

sonicated at 60°C for 12 minutes following by cooling down the mixture at room temperature. In the final step, the niosomal topical gel assay sample was further diluted with diluent and then using a vortexing mixer, it was mixed thoroughly. Drug quantification was determined using the pre-determined HPLC method, as mentioned in section 4.3.2.1.

4.3.3. Topical gel physical characterization

4.3.3.1. pH measurement

The pH of various desoximetasone niosomal topical gel formulations was determined using the pH meter (VWR pH meter symphony B10P, Radnor, PA, USA). 1 gm of niosomal gel was mixed in 10 gm of DI water. Then, pH was determined at room temperature.

4.3.3.2. Spreadability measurement

Spreadability was measured in millimeters (mm). A 100 mg sample was carefully placed in the center of a microscopic glass slide and covered with another slide, a total of 50 gm of standardized weight was kept on the slide for 1 minute, and at the end of the test, the diameter of the sample was measured in mm.

4.3.3.3. Specific gravity measurement

Specific gravity was measured using the metal pycnometer. Pycnometer was cleaned with purified water, and then it was filled with purified water. The weight of the purified water was measured. Then, the pycnometer was cleaned thoroughly, and the product was filled in the pycnometer. The weight of the product was measured. The

specific gravity of the product is the quotient obtained by dividing the weight of the product contained in the pycnometer by the weight of water at room temperature. Photographs for the specific gravity measurement device stainless steel pycnometer is given in Figure 4.2. The specific gravity of the product was calculated using the following equation.

$$Specific\ Gravity = \frac{Weight\ of\ the\ product\ (g)}{Weight\ of\ water\ (g)}$$

Figure 4.2. Photographs of specific gravity measurement device - stainless steel pycnometer [21].

4.3.3.4. Rheological evaluation

Rheology is the study of how materials deform and flow as an outcome of an external force [22]. The rheological evaluation was performed on TA Rheometer (Model: Discovery HR – 1, Newark, Delaware, USA) equipped with a 40 mm parallel plate. All the tests were completed at 25°C with testing gap 1000.0 µm, loading gap 45,000.0 µm and trim gap offset 50.0 µm. Photograph of the TA rheometer instrument is given in Figure 4.3.

Figure 4.3. Photograph of TA discovery rheometer [23].

4.3.3.4.1. Yield stress measurement

The test temperature was 25°C, and the soak time of the sample was 60.0 seconds. The samples were subjected to an increasing shear rate of 0 to 1000 1/s for 120.0 seconds. The sampling interval was selected 2 s/pt. The graph was plotted for shear rate $\dot{y}$ (1/s) vs stress σ (Pa). This test allows determination of the yield stress value of the sample.

4.3.3.4.2. Flow curve (upward and downward curve) measurement

In flow experiments, the shear stress increased, then decreased and the resultant shear rate measured, producing a flow curve. The test temperature was 25°C and the soak time of the sample was 60.0 seconds. This test was performed in a two-step the initial step for the upward curve and the second step for the downward curve. In the first step, the samples were subjected to increasing shear rate 0 to 1000 1/s for 120.0 seconds. The sampling interval was selected 2 s/pt. In the second step, the samples were subjected to a decreasing shear rate of 1000 to 0 1/s for 120.0 seconds. The graph was plotted for shear rate $\dot{y}$ (1/s) vs stress σ (Pa). This test allows the determination of the flow behavior of the product. The upward curve shows how products behave during an increasing shear rate, and the downward curve shows how products respond while decreasing the shear rate.

4.3.3.4.3. Viscosity (low, medium and high shear rate) measurement

The test temperature was 25°C and the soak time of the sample was 60.0 seconds. The samples were subjected to an increasing shear rate of 0 to 1000 1/s for 120.0 seconds. The sampling interval was selected 2 s/pt. The graph was plotted for shear rate $\dot{y}$ (1/s) vs viscosity η (Pa.s). This test allows determination of the viscosity at a low, medium, and high shear rate.

4.3.3.5. Physicochemical properties evaluation

4.3.3.5.1. Color

Desoximetasone niosomal topical gel was examined visually for their color property evaluation.

4.3.3.5.2. Texture

Desoximetasone niosomal topical gel was examined visually for their texture property evaluation.

4.3.3.5.3. Homogeneity

Physically niosomal gel was examined by placing the gel between the thumb and the index finger and the homogeneity or any aggregates were observed.

4.3.3.5.4. Phase separation

Desoximetasone niosomal topical gel was examined visually to evaluate their physical stability consistency.

4.3.3.5.5. Description

The description is a visual state for a general statement describing how the product looked when examined.

4.4. Selection of the thickening agent

Thickening or viscosity build-up agent is the crucial ingredient in the gel formulation. It is essential to optimize the thickening agent to achieve the targeted viscosity in the final product. Topicort® (Desoximetasone) gel USP, 0.05%, is currently listed as “RLD - reference listed product” by the FDA in the Orange book database [24]. Therefore, our target is to compare topical niosomal gel with the Topicort® gel reference marketed product. As listed in “Dailymed” an official source for FDA approved marketed products, Carbomer 940 was used as a thickening agent in the Topicort® gel USP, 0.05%

[25] formulation manufactured by Taro Pharmaceuticals. Carbomer 940 material manufactured by Lubrizol is not recommended to use for formulation activity by FDA due to higher benzene content. Lubrizol replaces Carbomer 940 with Carbomer 980 with the same physical and chemical properties and safe to use due to controlled benzene level. Therefore, Carbomer 980 is selected for the formulation development activity.

4.5. Optimization of thickening agent concentration study

It is important to optimize the thickening agent to achieve the targeted viscosity in the final product. We are considering the Topicort® gel, 0.05% as reference product; therefore, it was necessary to optimize thickening agent concentration to compare the viscosity and other physicochemical parameters of the test product with the reference product. Rheological evaluation (yield stress and viscosity) was performed to optimize the concentration of Carbomer 980. Yield stress measurement and viscosity measurement at low, medium, and high shear rate techniques were used to compare test and reference products. Formulations with 0.62%, 0.70%, 1.00%, 1.50%, and 2.00% Carbomer 980 were manufactured with identical method and rheological evaluation was performed.

4.5.1. Rheological evaluation parameters

The rheological evaluation was performed on TA Rheometer (Model: Discovery HR – 1, Newark, Delaware, USA) equipped with a 40 mm parallel plate. All the tests were completed at 25°C with testing gap 1000.0 µm, loading gap 45,000.0 µm and trim gap offset 50.0 µm. The test temperature was 25°C and the soak time of the sample was 60.0 seconds. The samples were subjected to an increasing shear rate of 0 to 500 1/s for

120.0 seconds. The sampling interval was selected 5 s/pt. The same flow-ramp run was used to measure yield stress and viscosity (at the low, medium, and high shear rate) of the sample. For yield stress and viscosity evaluation, the graph was plotted for shear rate ẏ (1/s) vs stress σ (Pa) and shear rate ẏ (1/s) vs viscosity η (Pa.s) respectively.

4.6. Selection of the formulation ingredients

Niosomal topical gel formulation is developed based on the reference listed drug Topicort® desoximetasone gel USP, 0.05% [25]. Formulations composition for the niosomal topical gel was selected based on the reference listed product.

Table 4.1. Composition of desoximetasone niosomal topical gel formulation (% w/w).

Ingredients	DNTG-1	DNTG-2	DNTG-3	DNTG-4	DNTG-5	DNTG-6	DNTG-7	DNTG-8	DNTG-9	DNTG-10	DNTG-11	DNTG-12	DNTG-13
Desoximetasone	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Carbomer 980	1.00	1.50	2.00	0.62	0.70	-	-	-	-	-	-	-	-
Carbomer 940	-	-	-	-	-	0.70	-	-	-	-	-	-	-
Carbomer 974P	-	-	-	-	-	-	0.70	-	-	-	-	-	-
Carbomer 981	-	-	-	-	-	-	-	0.70	-	-	-	-	-
Carbomer 1342	-	-	-	-	-	-	-	-	0.70	-	-	-	-
Ethyl cellulose	-	-	-	-	-	-	-	-	-	0.70	-	-	-
Hydroxy propyl cellulose	-	-	-	-	-	-	-	-	-	-	0.70	-	-
Hydroxy propyl methyl cellulose	-	-	-	-	-	-	-	-	-	-	-	0.70	-
Xanthan gum	-	-	-	-	-	-	-	-	-	-	-	-	0.70
Edetate disodium	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
Docusate sodium	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014
Transcutol	8.20	8.20	8.20	8.20	8.20	8.20	8.20	8.20	8.20	8.20	8.20	8.20	8.20
Trolamine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DI Water to QS	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

4.7. Selection of ideal desoximetasone niosomal gel formulation

All desoximetasone niosomal topical gel and reference marketed gel formulations are evaluated for their physicochemical properties. Based on the results, the ideal desoximetasone niosomal gel was selected. Data comparison between selected desoximetasone niosomal gel and reference marketed gel product are further discussed in section 4.10.9.

4.7.1. Statistical analysis

Yield stress (Rheology) study data are reported as mean $\pm$ SD (n=3). The statistical analysis of the data was performed by using one-way ANOVA, the Tukey posthoc tests, and Student's t-test and p-values > 0.05 were considered significantly similar.

4.8. In-vitro permeation study using human cadaver skin

4.8.1. Study design

In vitro skin permeation studies were executed using a Franz diffusion cell (FDC) with a donor area of 0.64 cm^2 and a receptor volume of 5.0 mL (Permegear Inc., Hellertown, PA). The samples of dermatomed human cadaver skin (Source: The New York Firefighters Skin Bank, Donor: White Male, 47 years old, Section location: Posterior Torso) were slowly thawed at room temperature, cut into appropriate pieces, and soaked in filtered phosphate buffer saline with pH 7.4 for 15 minutes. After that, the skin was mounted on FDC with the epidermal side in contact with the reference and test

formulations or donor compartment. The receptor compartment of each cell was filled with receptor media ethanol: water (40:60) and was maintained at 37°C under synchronous continuous stirring using a magnetic stirrer at 600 rpm. The diffusional membranes were left to equilibrate at 37°C for 15 minutes. Once equilibrium was achieved, at time zero formulated gel and reference product (Topicort® gel) were placed over the skin to the donor compartment of each Franz diffusion cell in a zig-zag manner. At each time point, 300 µL of receptor samples were withdrawn from the sampling port. Same time 300 µL of receptor media was added into the receptor compartment of each cell to maintain the sink condition. Samples were collected at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 22, and 24 hours. At the end of experimental hours, receptor aliquots of 300 µL were then analyzed using a valid HPLC method described in section 4.8.2. Photograph of Franz cell is provided in Figure 4.4.

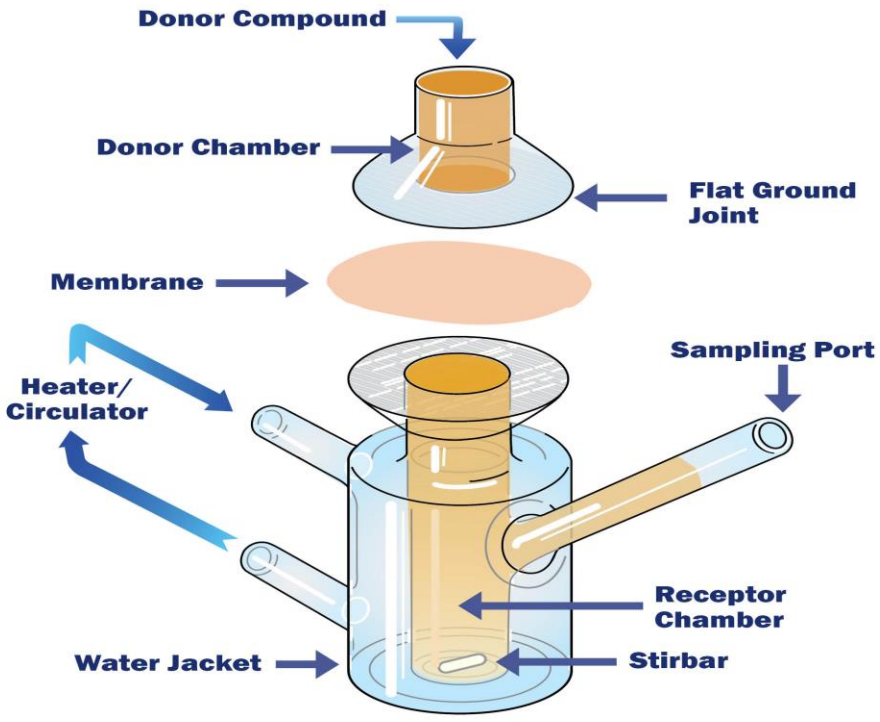

Figure 4.4. Diagram of a typical Franz diffusion cell [26].

4.8.2. Analytical testing parameters

The HPLC instrument used was Agilent 1100 series instrumentation (Agilent Technologies, CA, USA) coupled with UV detection (DAD) and HP ChemStation software V. 32. For the analysis of desoximetasone, a mobile phase of 60% methanol and 40% water was pumped through a Discovery C18 column (Sigma-Aldrich, Saint Louis, MO, USA) with 5 µm particle size, L x I.D. 150 mm x 4.6 mm column. Injection volumes of 20 µL with a flow rate of 1.0 mL/min was set to 30°C with UV detection of 254 nm were used with the retention time of 4 minutes.

4.8.3. Data analysis

Penetration parameters were obtained from the cumulative amount of desoximetasone permeated per unit skin surface area (µg/cm^2) versus time (hours) plot.

4.8.4. Statistical analysis

Results are reported as mean $\pm$ SD (n=6). The statistical analysis of the data was performed by using one-way ANOVA, the Tukey posthoc tests and Student's t-test, and p-values < 0.05 were considered significant.

4.9. Skin deposition study

At the end of the permeation study, the skin was removed from the diffusion cell, cut around the diffusional area, air dried, and weighed accurately. The skin samples were then placed into bead bug tubes, and they were cut in tiny pieces using a scissor. An aliquot of 1 mL ethanol was added to each tube, and they were homogenized for 9

minutes by using BeadBug™ Microtube homogenizer, D1030 (Benchmark Scientific, Sayreville, NJ). All the skin samples were then placed in a Julabo SW22 shaker (Julabo USA Inc., Allentown, PA) and were agitated at 37°C for 24 hours. Next, all the skin samples were centrifuged at 1200 rpm for 5 minutes and were filtered through a 0.45 um polypropylene filter media with polypropylene housing. Desoximetasone concentrations were expressed as ng of desoximetasone per skin weight in mg.

4.9.1. Data analysis

Drug deposition in the skin was obtained from the cumulative amount of desoximetasone deposited in the skin (ng/mg) versus formulation. Results are reported as mean $\pm$ SD (n=6).

4.10. Results and Discussion

4.10.1. Thickening agent concentration determination

Rheological evaluation is the most suitable method for concentration identification of thickening agent in the gel formulation. The thickening agent is a critical ingredient in building the viscosity of the product. It is essential to optimize the thickening agent concentration of the gel formulation to achieve the targeted viscosity to match with the reference product. As we have mentioned in section 4.4, the Carbomer 980 thickening agent was selected for gel formulation for the sameness with Topicort® gel USP, 0.05% reference marketed gel product. Yield stress (Figure 4.5) and viscosity (Figure 4.6) for the various Carbomer 980 concentration contain desoximetasone

niosomal gel formulations, and reference gel product is provided in Table 4.2 and Table 4.3, respectively.

Table 4.2. Yield stress data for the various desoximetasone niosomal gel formulations contain a different concentration of Carbomer 980 and reference gel product.

Batch Detail	Yield stress (Pa)
H882532755 (Reference gel)	78.97
DNTG-1 (1.00% Carbomer 980)	128.95
DNTG-2 (1.50% Carbomer 980)	137.36
DNTG-3 (2.00% Carbomer 980)	194.60
DNTG-4 (0.62% Carbomer 980)	-20.05
DNTG-5 (0.70% Carbomer 980)	93.44

Table 4.3. Viscosity data at low, medium and high shear rates for the various concentration of Carbomer 980 contains desoximetasone niosomal gel and reference gel product.

Batch Detail	Viscosity (Pa.s)		
	~ 10 Shear rate	~ 240 Shear rate	~ 490 Shear rate
H882532755 (Reference gel)	18.61	2.03	1.27
DNTG-1 (1.00% Carbomer 980)	23.05	2.29	1.43
DNTG-2 (1.50% Carbomer 980)	27.28	2.69	1.66
DNTG-3 (2.00% Carbomer 980)	30.82	2.99	1.87
DNTG-4 (0.62% Carbomer 980)	15.36	1.82	1.11
DNTG-5 (0.70% Carbomer 980)	19.96	2.02	1.24

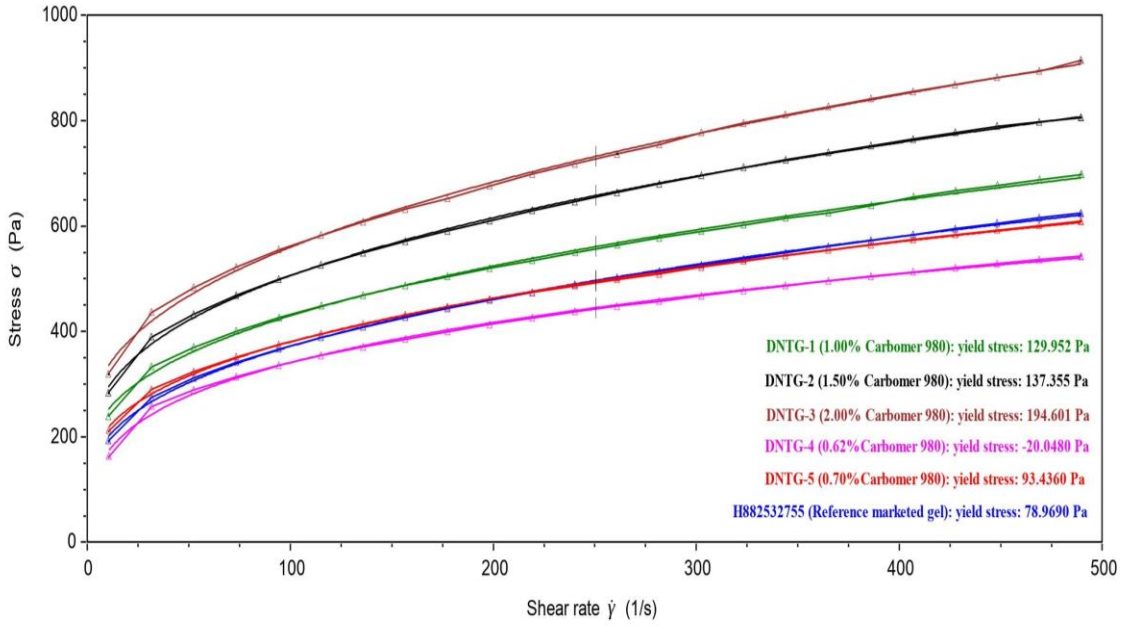

Figure 4.5. Yield stress profile overlay for formulations contains Carbomer 980 concentration 0.62% (DNTG-4), 0.70% (DNTG-5), 1.00% (DNTG-1), 1.50% (DNTG-2) and 2.00% (DNTG-3), and Topicort® gel reference product (H882532755).

For yield stress evaluation, the graph was plotted for shear rate γ̇ (1/s) vs stress σ (Pa) and yield stress value for all the samples were determined by applying the Herschel-Bulkley model. Yield stress values from Table 4.2 shows that reference marketed gel yield stress value was 78.97 Pa. Yield stress value for the desoximetasone niosomal gels were varying based on Carbomer 980 concentration. An increasing trend was observed for the yield stress of the gel formulation was observed with increasing concentration of Carbomer 980. Yield stress value for the 0.70% Carbomer 980 (Lot# DNTG-5) formulation is 93.44 Pa, which is the closest value in comparison with reference gel yield stress.

For viscosity evaluation, the graph was plotted for shear rate γ̇ (1/s) vs viscosity η (Pa.s).Viscosity values from Table 4.3 shows that reference gel viscosity was 18.61 Pa.s (~ 10 shear rate), 2.03 Pa.s (~ 240 shear rate) and 1.43 Pa.s (~ 490 shear rate). Viscosity

data follows the identical trend as yield stress measurement and viscosity for the various desoximetasone niosomal gels were varying based on the Carbomer 980 concentration. Viscosity value for the 0.70% Carbomer 980 (Lot# DNTG-5) formulation was 19.96 Pa.s (~ 10 shear rate), 2.02 Pa.s (~ 240 shear rate) and 1.24 Pa.s (~ 490 shear rate), which is the closest value in comparison with reference gel.

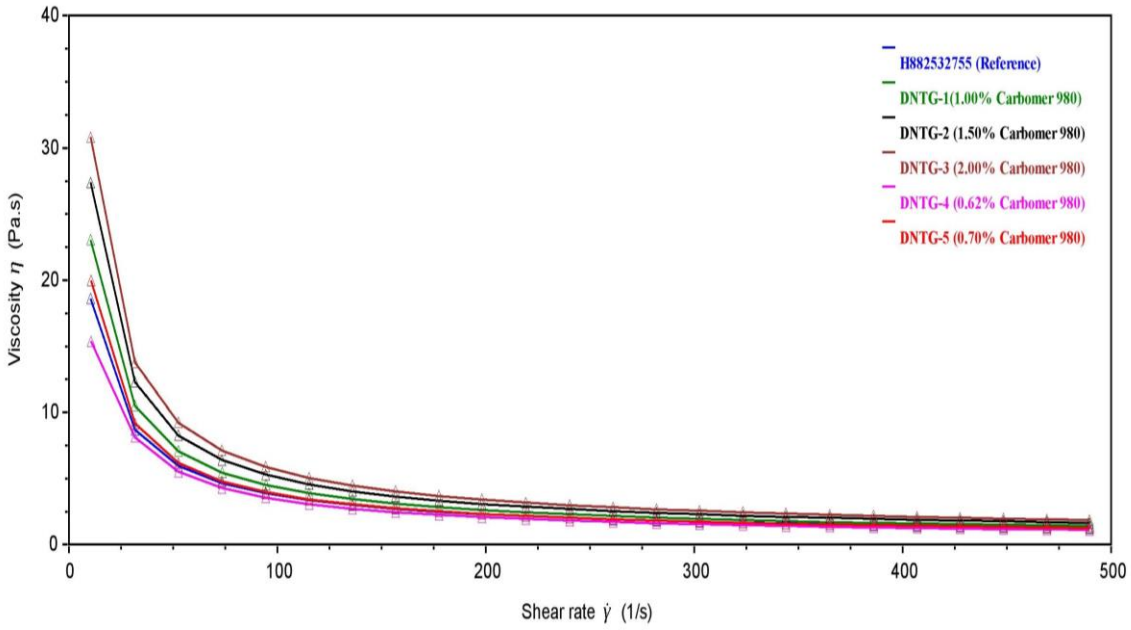

Figure 4.6. Viscosity profile overlay for formulations contains Carbomer 980 concentration 0.62% (DNTG-4), 0.70% (DNTG-5), 1.00% (DNTG-1), 1.50% (DNTG-2) and 2.00% (DNTG-3), and Topicort® gel reference product (H882532755).

Based on the yield stress and viscosity evaluation, 0.70% Carbomer 980 (Lot# DNTG-5) formulation shows the best match with a reference gel product. Therefore, Lot# DNTG-5 will be used for further comparison in detail with reference gel product, and 0.70% concentration will be used to evaluate various thickening agent impact on the final product.

4.10.2. Desoximetasone drug content (assay) determination

Drug content from the various topical gel was analyzed along with the reference product. The drug content assays are performed to ensure that the correct amount of active ingredient has been added to the product during the manufacturing of the formulation. Drug content data from the desoximetasone niosomal topical gel and reference gel products are provided in Table 4.4. The drug contents values of the niosomal topical gel formulations were found in the range of 93.03 to 101.84%. The drug content value of the reference marketed gel product was observed to be 101.80%. All the prepared niosomal gel shows consistent results with a controlled standard deviation. The Data suggests that niosomal gel contains the right amount of the active ingredients in the formulations.

4.10.3. pH measurement study

pH potentially affects the stability of the active ingredient and physicochemical properties of the semisolid product. pH also may impact on the effectiveness of the preservatives and viscosity of the drug product. pH values of the formulations were found in the range of 4.39 - 9.28. pH values. The pH value of the reference marketed product was observed to be 5.64. The data shows the pH of the formulations is well controlled and acceptable. the pH of the desoximetasone niosomal gel formulations depends on the quantity and type of the thickening polymer. pH data from the desoximetasone niosomal topical gel and reference gel products are provided in Table 4.4.

4.10.4. Spreadability study

The spreadability of the formulation is inversely influenced by the viscosity. Good spreadability is a critical criterion for the gel formulation as it shows the product's behavior when it comes out from the container. It is the term that is used to indicate the extent of the area to which gel readily spreads on application. Results justified this statement as the spreadability of the desoximetasone niosomal gel is in the range of 17.00 to 26.33 mm. The spreadability of the reference marketed gel was observed to be 18.67 mm. It was found that the spreadability of desoximetasone niosomal gel decreased by increasing the thickening polymer concentration or spreadability change with change in the type of thickening polymer. Spreadability data from the various desoximetasone topical gel and reference marketed gel products are provided in Table 4.4.

4.10.5. Specific gravity study

The specific gravity of the semisolid product is used to verify the excessive amounts of the product's entrapped air. It is essential to observe the specific gravity of the semisolid product because excess air in the product can lead to spoiling and degradation of the ingredients, and affect various physical measurement such as viscosity. Specific gravity data for the desoximetasone niosomal topical gel was observed in the range of 0.952 to 1.009. The specific gravity value for the reference marketed gel was found to be 0.947. Specific gravity data from the desoximetasone niosomal topical gel and reference gel products are provided in Table 4.4.

Table 4.4. Desoximetasone niosomal gel drug content, pH, spreadability and specific gravity data (n=3, mean ± SD).

Batch Detail	Drug content (%)	pH	Spreadability (mm)	Specific gravity
DNTG-1	98.07 ± 0.05	5.19 ± 0.01	18.00 ± 0.00	0.957 ± 0.00
DNTG-2	95.05 ± 0.08	4.77 ± 0.00	17.33 ± 0.58	0.964 ± 0.00
DNTG-3	94.43 ± 0.95	4.39 ± 0.01	17.00 ± 0.00	0.972 ± 0.00
DNTG-4	93.03 ± 0.23	5.97 ± 0.01	19.33 ± 0.58	0.952 ± 0.00
DNTG-5	95.92 ± 0.55	5.67 ± 0.01	19.00 ± 0.00	0.954 ± 0.00
DNTG-6	98.18 ± 0.88	5.64 ± 0.01	19.00 ± 0.00	0.979 ± 0.00
DNTG-7	93.08 ± 0.96	5.80 ± 0.00	23.67 ± 2.08	0.971 ± 0.00
DNTG-8	94.69 ± 0.38	5.43 ± 0.02	26.33 ± 0.58	0.972 ± 0.00
DNTG-9	95.15 ± 0.72	5.46 ± 0.01	23.00 ± 0.00	0.972 ± 0.00
DNTG-10	94.35 ± 0.05	9.12 ± 0.01	Not available	1.007 ± 0.00
DNTG-11	96.84 ± 1,25	9.22 ± 0.00	Not available	1.009 ± 0.00
DNTG-12	101.84 ± 0.11	9.28 ± 0.02	Not available	1.008 ± 0.00
DNTG-13	97.61 ± 0.49	9.06 ± 0.01	Not available	1.006 ± 0.00
H882532755 - Topicort® Gel, 0.05%	101.80 ± 0.38	5.64 ± 0.01	18.67 ± 0.58	0.947 ± 0.00

4.10.6. Topical gel content uniformity study

The content uniformity evaluation is essential in the case of semisolid products contains the drug in the dispersed phase. The content uniformity test is essential to identify the drug content level at a different location in the finished product. To analyze the content uniformity samples were collected from the undisturbed finished product from the top, middle, and bottom position. The content uniformity study data from the desoximetasone niosomal topical gel and reference gel products are provided in Table 4.5.

Table 4.5. Desoximetasone niosomal gel content uniformity data.

Batch Detail	Top sample (%)	% RSD (n=3)	Middle sample (%)	% RSD (n=3)	Bottom sample (%)	% RSD (n=3)
DNTG-1	93.38	0.05	92.60	0.05	94.30	0.04
DNTG-2	93.26	0.18	95.31	0.02	95.24	0.07
DNTG-3	95.15	0.86	94.66	0.91	93.89	0.65
DNTG-4	94.02	0.47	94.33	0.22	95.70	0.18
DNTG-5	93.69	2.01	97.06	0.31	95.28	7.59
DNTG-6	95.90	0.43	98.13	0.94	94.45	0.13
DNTG-7	92.77	0.62	94.41	0.22	92.95	0.25
DNTG-8	93.40	0.97	94.67	1.83	94.95	0.41
DNTG-9	97.05	0.82	96.61	0.04	95.68	1.38
DNTG-10	67.15	0.90	58.21	0.36	112.33	0.17
DNTG-11	59.69	1.32	74.77	0.62	172.61	0.69
DNTG-12	47.44	1.53	48.46	0.96	271.63	0.03
DNTG-13	96.96	1.81	95.5	0.75	94.47	1.03
H882532755 - Topicort® Gel, 0.05%	101.78	1.27	100.74	0.34	100.88	1.39

The content uniformity data are well within the trend. Formulation DNTG – 1 to DNTG – 9, DNTG – 13 and Topicort® gel finished products are in semisolid form; therefore, drug content between top, middle and bottom samples are identical; where formulation DNTG – 10 to DNTG – 12 finished products are in liquid form, thus we observed that dispersed drug niosomes are settled down, and that results in higher drug content in the bottom sample compared to middle and top samples.

The content uniformity study data reveals that in the semisolid gel product drug loaded niosomes are distributed uniformly, and drug content in the top, middle, and bottom samples are identical. This behavior may prove that the gel manufacturing process is efficient in uniform distributing desoximetasone loaded niosomes throughout the batch and the gel matrix is able to hold the desoximetasone loaded niosomes.

4.10.7. Rheological study

The rheological studies of gels help to understand the microstructure of the gel product, which in turn governs the viscoelastic properties of the gel. The rheology of semisolid formulations provides important information for product and process performance including product stability. The rheological study can help to predict which formulations might exhibit flocculation, coagulation, or coalescence, resulting in undesired effects, such as settling, creaming or separation. Various gel formulations were manufactured by either changing Carbomer 980 concentration or replace Carbomer 980 with a various thickening agent such as Carbomer 940, Carbomer 974P, Carbomer 981, Carbomer 1342, xanthan gum, hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), and ethyl cellulose (EC). Rheological yield stress and viscosity data of the various niosomal gel and reference marketed gel are provided in Table 4.6 [27].

Table 4.6. Yield stress and viscosity data for niosomal gel and reference marketed gel drug products.

Niosomal gel and reference gel rheology (yield stress and viscosity) data					
Batch Detail	**Formulation Detail**	**Yield stress (Pa)**	**Viscosity (Pa.s)**		
			~ 8	**~ 493**	**~ 991**
DNTG-1	Carbomer 980 - 1.00%	126.26	23.03	1.33	0.84
DNTG-2	Carbomer 980 - 1.50%	163.37	27.53	1.50	0.95
DNTG-3	Carbomer 980 - 2.00%	225.54	37.00	1.87	1.19
DNTG-4	Carbomer 980 - 0.62%	84.56	19.83	1.11	0.70
DNTG-5	Carbomer 980 - 0.70%	91.09	19.79	1.13	0.71
DNTG-6	Carbomer 940 - 0.70%	157.24	26.56	1.39	0.87
DNTG-7	Carbomer 974P - 0.70%	53.95	16.45	1.01	0.64
DNTG-8	Carbomer 981 - 0.70%	31.61	5.99	0.59	0.41
DNTG-9	Carbomer 1342 - 0.70%	117.00	16.98	0.89	0.59

Niosomal gel and reference gel rheology (yield stress and viscosity) data					
Batch Detail	**Formulation Detail**	**Yield stress (Pa)**	**Viscosity (Pa.s)**		
			~ 8	**~ 493**	**~ 991**
DNTG-10 *	Ethyl cellulose – 0.70%	N/Av	N/Av	N/Av	N/Av
DNTG-11 *	Hydroxy propyl cellulose – 0.70%	N/Av	N/Av	N/Av	N/Av
DNTG-12 *	Hydroxy propyl methyl cellulose – 0.70%	N/Av	N/Av	N/Av	N/Av
DNTG-13	Xanthan gum - 0.70%	16.12	1.96	0.06	0.03
H882532755	Reference marketed gel	113.40	20.74	1.23	0.80

* Due to the intrinsic nature of the polymer, the gel was not obtained. Therefore, rheology could not be performed
N/Av = Not available

4.10.7.1. Yield stress measurement study

The yield stress, defined as the minimal stress that must be applied to the sample to disrupt the structure in material and cause it to flow. Yield stress value is required to determine the product flowability point. It is essential to determine yield stress value for the semisolid product to know the product flow behavior at point product start flowing from solid state to liquid state. It was found strong correlate to the thickening agent concentration with a yield stress of the product, and show the change in yield stress of the product by changing either concentration or different type. For yield stress evaluation, the graph was plotted for shear rate $\dot{y}$ (1/s) vs stress σ (Pa) and yield stress value for all the samples were determined by applying the Herschel-Bulkley model. The Herschel-Bulkley equation can be described as [28]:

$$\tau = \tau_0 + k(\gamma)^n$$

Where, τ = shear stress, τ_0 = yield stress, k = consistency factor, γ = shear rate, and n = flow index, a power law exponent.

The yield stress behaviour comparison for all the niosomal gel and reference marketed gel drug products is provided in Figure 4.7 reveals that all the samples exhibit pseudo-plastic flow with a presence of yield stress.

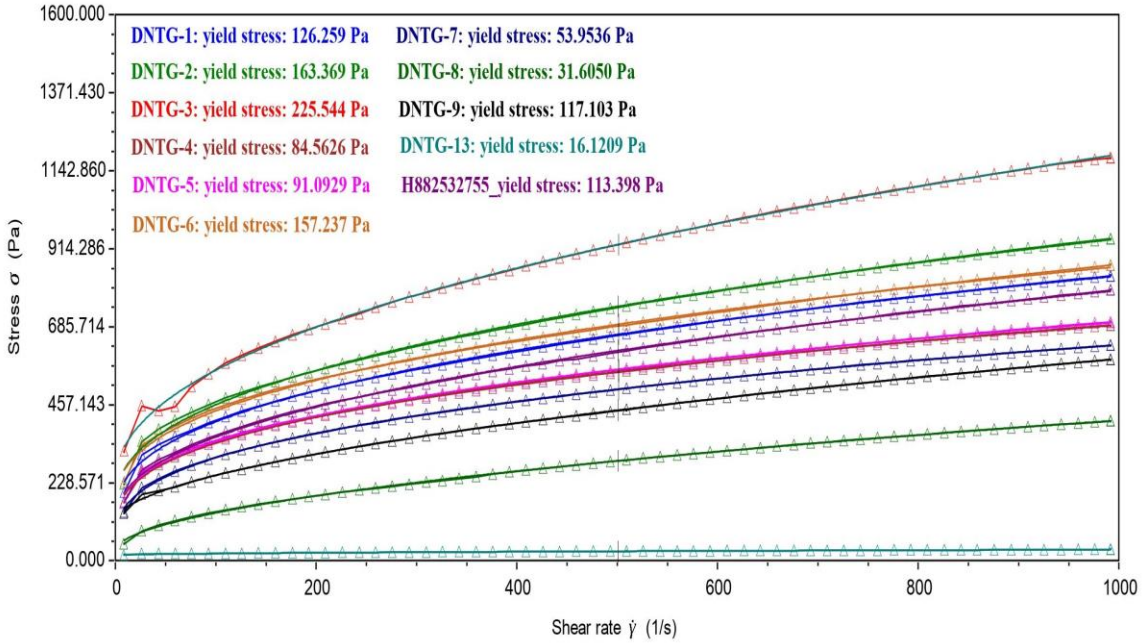

Figure 4.7. Yield stress (Herschel-Bulkley model) rheological data comparison between DNTG-1 to DNTG-9, DNTG-13 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel).

4.10.7.2. Flow (upward and downward) curve behaviour study

Flow curve measurement is important testing as flow predict how semisolid drug product will behave in a real-life situation. The upward and downward curve gives information on sample breakdown and recovery, whether the sample structure will recover quickly when the stress is removed or it will recover over the period of time. In use, the formulation structure should disrupt easily while the tube or packaging component is squeezed, flow readily when being applied to the skin, and then rebuild structure quickly (negligible hysteresis) to prevent runoff from the skin. From the flow

curve profile of all the formulations shown in Figure 4.8, it can be interpreted that all the formulations show non-Newtonian flow behaviour (pseudo-plastic flow) with yield stress. Additionally, the downward curve for all the formulations follows the same path as the upward curve, which suggests that all the formulations are non-thixotropic in nature.

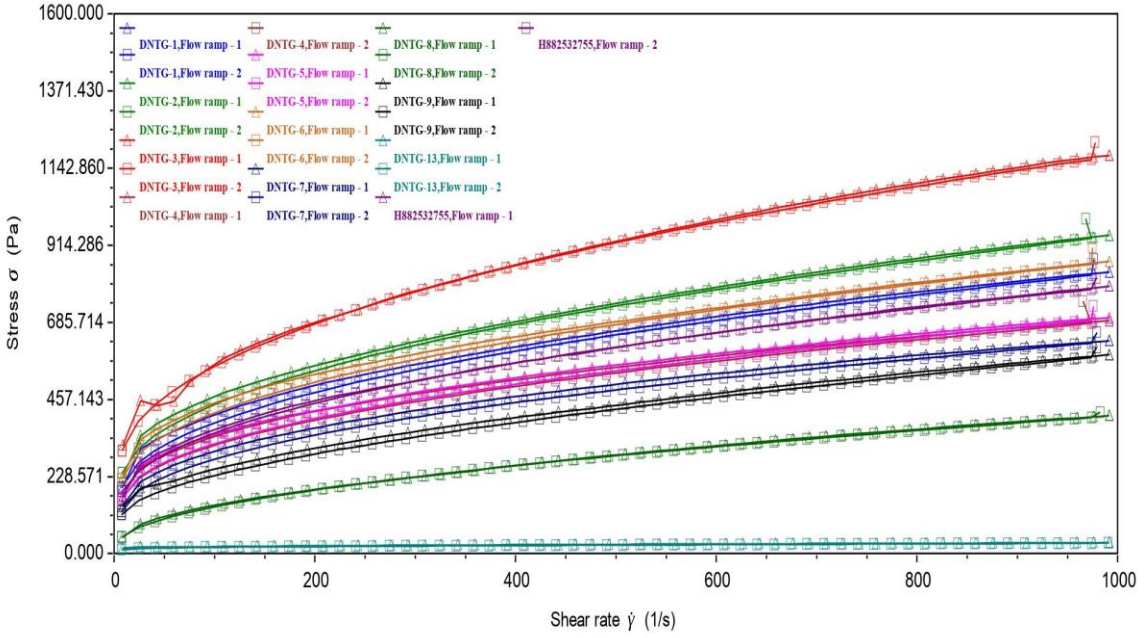

Figure 4.8. Flow curve (upward and downward curve) rheological data comparison between DNTG-1 to DNTG-9, DNTG-13 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel).

4.10.7.3. Viscosity (low, medium and high shear rate) study

Viscosity is a measure of the internal friction of a fluid, the resistance to flow. Viscosity testing requires applying a shear stress to a fluid and measuring the rate of material flow caused by this stress. Table 4.6 lists the viscosity values of all the samples at low shear rate (~ 8 1/s), medium shear rate (~ 493 1/s) and high shear rate (~ 991 1/s). For viscosity evaluation, the graph was plotted for shear rate γ̇ (1/s) vs viscosity η (Pa.s).

From Figure 4.9. it can also be seen that the viscosity for all the samples decreases as the shear rate increases, showing the pseudo-plastic flow behaviour of all the samples.

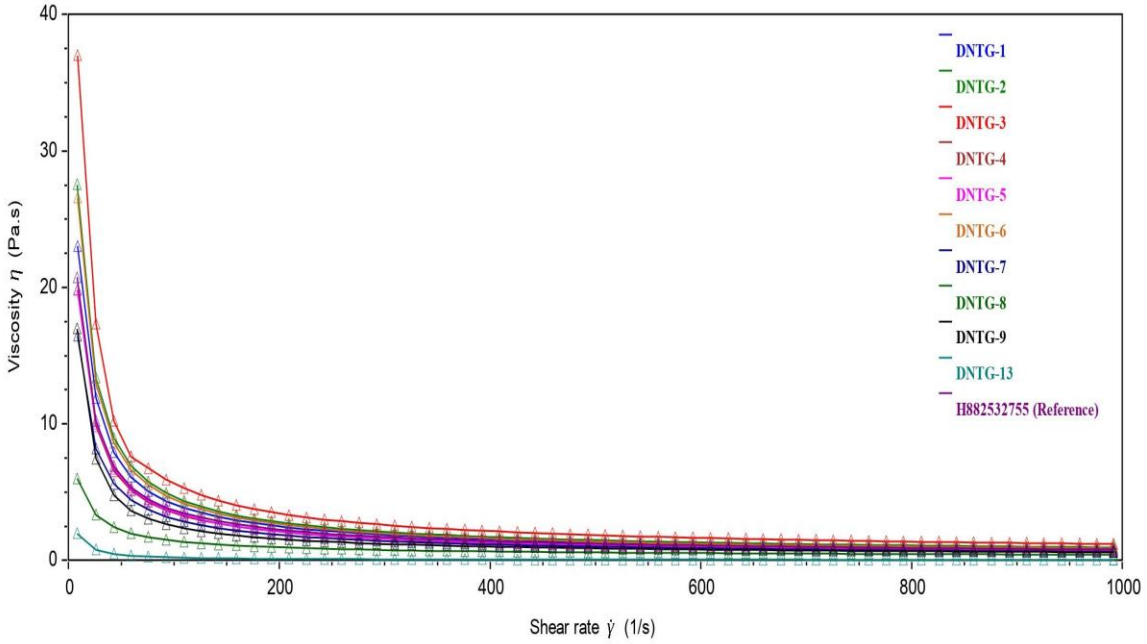

Figure 4.9. Viscosity rheological data comparison between DNTG-1 to DNTG-9, DNTG-13 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel).

4.10.8. Physicochemical properties evaluation

Table 4.7 shows the results of the physicochemical properties of prepared desoximetasone niosomal gel formulations DNTG-1 to DNTG-13 and Topicort® gel marketed product. All the prepared desoximetasone niosomal gel along with marketed product were opaque to white color. Formulation DNTG-1 to DNTG-9, DNTG-13, and Topicort® marketed gel showed good homogeneity with no lumps and smooth homogeneous texture. Formulating DNTG-10 to DNTG-12 showed poor homogeneity and liquidly texture because of the thickening polymer's intended nature in respected formulations. Phase separation was not observed in any gel formulation. Description of

the gel formulations is ‘opaque to light white smooth gel’ for DNTG-1 to DNTG-9, DNTG-13, and Topicort® reference marketed gel, for formulation DNTG-10 to DNTG-12 is ‘opaque to light white solution with dispersed particles.’

Table 4.7. Physicochemical properties of desoximetasone niosomal topical gel.

Batch Detail	Color	Texture	Homogeneity	Phase separation	Description
DNTG-1	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
DNTG-2	opaque to white	Smooth	++	No phase separation	Opaque to white smooth gel
DNTG-3	opaque to white	Smooth	++	No phase separation	Opaque to white smooth gel
DNTG-4	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
DNTG-5	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
DNTG-6	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
DNTG-7	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
DNTG-8	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
DNTG-9	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
DNTG-10	opaque to white	Liquidly	+	No phase separation	Opaque to light white solution with dispersed particles
DNTG-11	opaque to white	Liquidly	+	No phase separation	Opaque to light white solution with dispersed particles
DNTG-12	opaque to white	Liquidly	+	No phase separation	Opaque to light white solution with dispersed particles
DNTG-13	opaque to white	Smooth	++	No phase separation	Opaque to light white smooth gel
H882532755 - Topicort® Gel, 0.05%	opaque to white	Smooth	++	No phase separation	Opaque to white smooth gel

+: not good; ++: good

4.10.9. Selection of ideal desoximetasone niosomal topical gel formulation

From the beginning of the topical gel study, we have targeted ideal desoximetasone niosomal gel formulation, which shows the identical characteristics with Topicort® gel USP, 0.05% reference marketed gel product. The purpose of the developing new formulation is to match physical characteristics with reference product with the only difference is drug entrapped in niosome matrix and in disperse form in niosomal gel where drug in solubilize form in reference marketed gel.

As mentioned in Table 4.1, different 13 formulations were manufactured by changing either concentration of Carbomer 980 or type of the thickening agent to find the most suitable thickening agent for the desoximetasone niosomal gel. To match the similarity of the developed formulations with the reference gel product, we have evaluated various physicochemical characteristics such as drug content, content uniformity, pH, spreadability, specific gravity, color, texture, homogeneity, phase separation, description, and rheological properties. All the 13 desoximetasone niosomal gel formulation and reference marketed gel samples were evaluated for physicochemical properties as described in Table 4.4, Table 4.5, and Table 4.6. The evaluation from available data demonstrates that formulation DNTG-5 with 0.7% Carbomer 980 data matches with Topicort® gel product. Summary of the comparison between DNTG-5 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel) are described in Table 4.8. The schematic representation of rheological overlay graphs is illustrated in Figure 4.10 to Figure 4.12.

Table 4.8. Data comparison of DNTG-5 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel).

Test	H882532755 (reference marketed gel)	DNTG - 5 (Niosomal gel)
Drug content	101.80%	95.92%
pH	5.64	5.67
Spreadability	18.67 mm	19.00 mm
Specific gravity	0.947	0.954
Content uniformity		
Top sample	101.78%	93.69%
Middle sample	100.74%	97.06%
Bottom sample	100.88%	95.28%
Physicochemical properties		
Color	Opaque to white	Opaque to white
Texture	Smooth	Smooth
Homogeneity	Good	Good
Phase separation	No phase separation	No phase separation
Description	Opaque to white smooth gel	Opaque to light white smooth gel
Rheological properties		
Yield stress	118.88 Pa	106.57 Pa
Viscosity @ low shear rate	20.95 Pa.s	20.82 Pa.s
Viscosity @ medium shear rate	1.25 Pa.s	1.21 Pa.s
Viscosity @ high shear rate	0.80 Pa.s	0.78 Pa.s

Figure 4.10 displays the yield stress comparison of DNTG-5 (niosomal gel) and H882532755 (reference marketed gel) (n=3). The graph reveals that all the samples exhibit pseudo-plastic flow with a presence of yield stress. For yield stress evaluation, the graph was plotted for shear rate $\dot{y}$ (1/s) vs stress σ (Pa) and yield stress value for all the samples were determined by applying the Herschel-Bulkley model. The average yield stress values (n=3) obtained for DNTG-5 (niosomal gel) is 106.57 Pa and H882532755 (reference marketed gel) is 118.88 Pa.

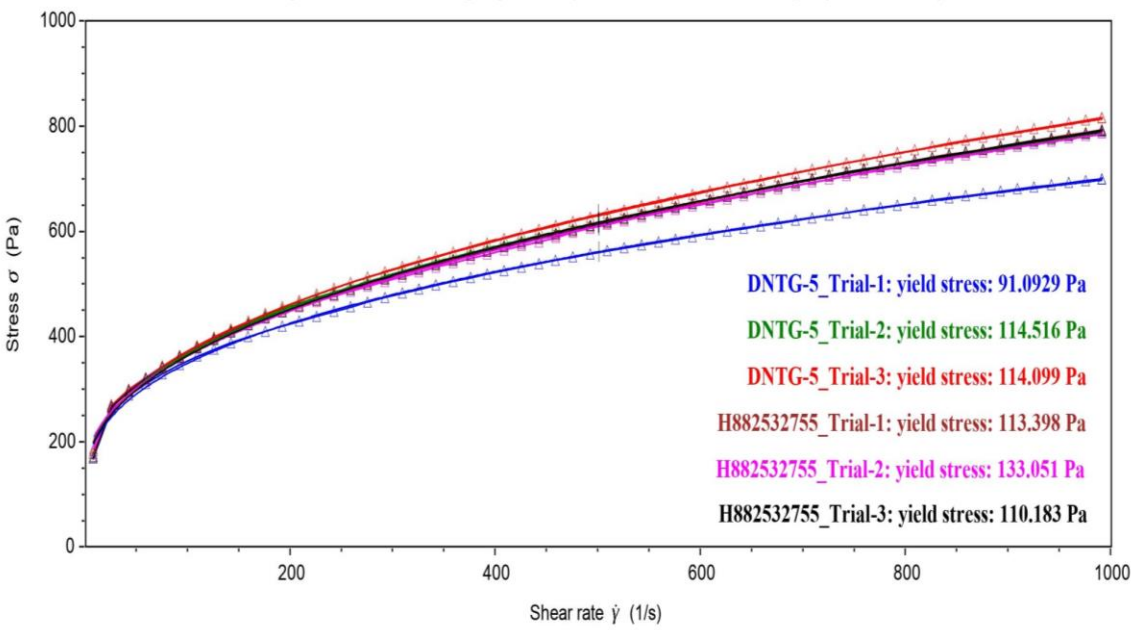

Figure 4.10. Yield stress (Herschel-Bulkley model) rheological data comparison between DNTG-5 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel) (n=3).

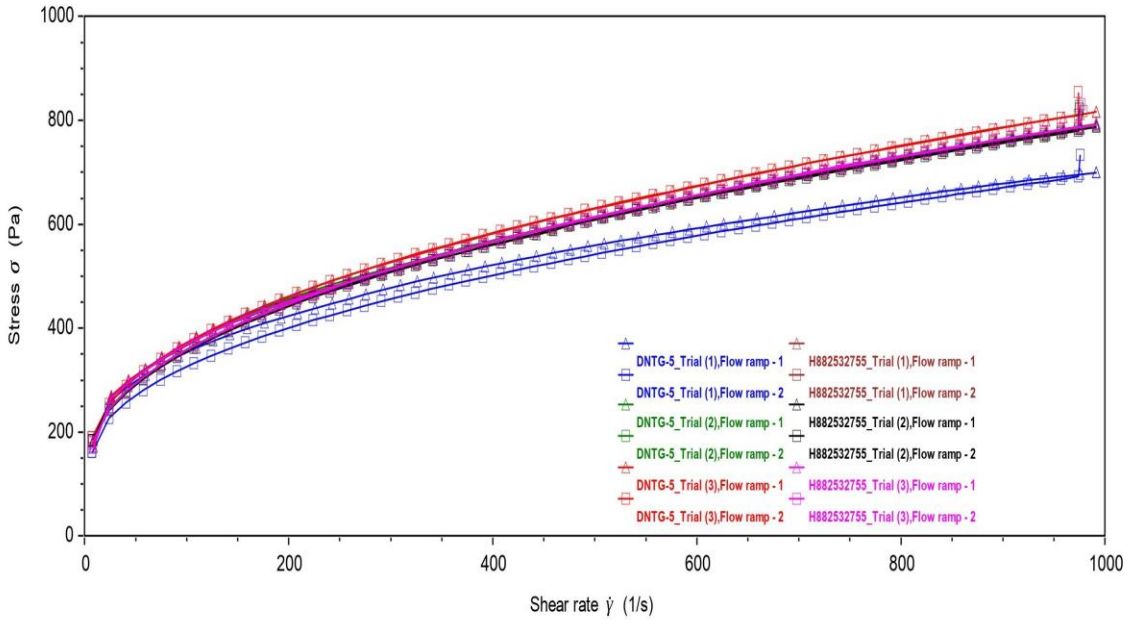

Figure 4.11. Flow curve (upward and downward) rheological data comparison between DNTG-5 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel) (n=3).

Figure 4.11 displays the complete flow curve (upward and downward curve) of shear stress versus shear rate of DNTG-5 (niosomal gel) and H882532755 (reference marketed gel) (n=3). From the flow curve profile of all the samples, it can be interpreted that both niosomal gel and reference marketed gel drug products show non-newtonian flow behaviour (pseudo-plastic flow) with yield stress. Additionally, the downward curve for all the sample runs follows the same path as an upward curve, which suggest that both niosomal gel and reference marketed gel drug product are non-thixotropic in nature.

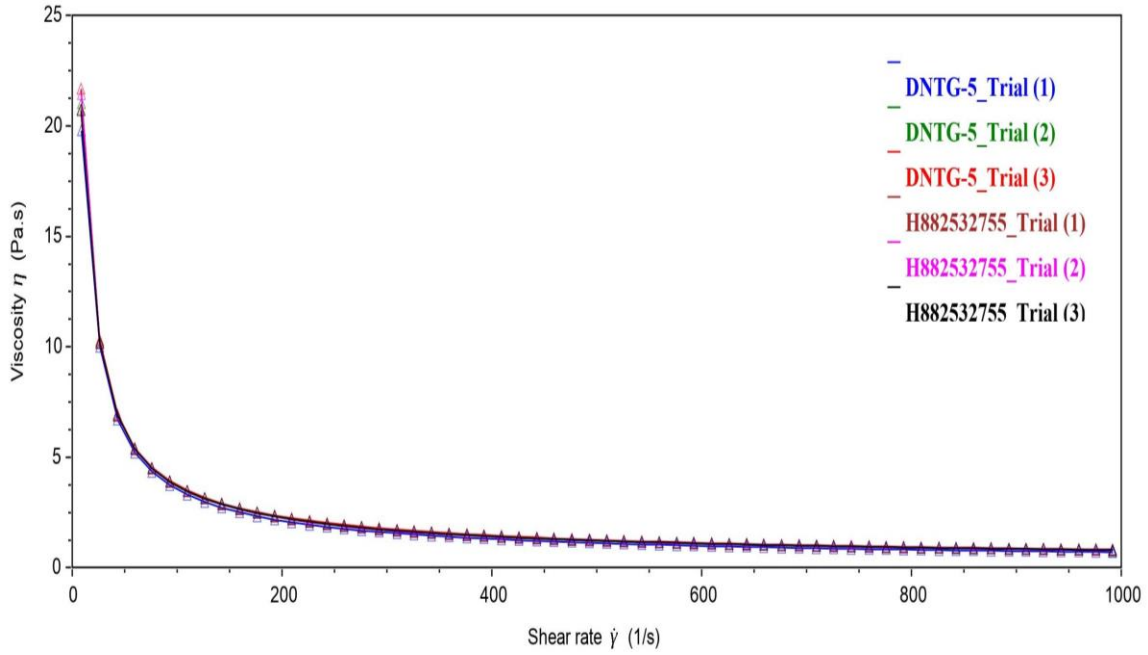

Figure 4.12. Viscosity rheological data comparison between DNTG-5 (desoximetasone niosomal gel) and H882532755 (Topicort® reference marketed gel) (n=3).

Figure 4.12 depicts the viscosity profile comparison of DNTG-5 (niosomal gel) and H882532755 (reference marketed gel) across the range of attainable shear rates. For viscosity evaluation, the graph was plotted for shear rate γ̇ (1/s) vs viscosity η (Pa.s). The average viscosity values (n=3) of niosomal gel and reference marketed gel drug products are identified at a low shear rate (~ 8 1/s), medium shear rate (~ 493 1/s) and high shear

rate (~ 991 1/s). Data suggest that average viscosity for niosomal gel is 20.82 Pa.s, 1.21 Pa.s and 0.78 Pa.s respectively at low shear rate, medium shear rate and high shear rate. Average viscosity for reference marketed gel is 20.95 Pa.s, 1.25 Pa.s and 0.80 Pa.s respectively at low shear rate, medium shear rate and high shear rate. From data in Table 4.8, it can be seen that the average viscosity values obtained for the niosomal gel and reference marketed gel at low, medium and high shear rates are similar. It can also be seen that the viscosity for all the samples decreases as shear rate increases, showing the pseudo-plastic flow behavior of all the samples.

Desoximetasone niosomal gel and reference marketed gel data suggest, DNTG-5 (0.70% Carbomer 980) is the ideal desoximetasone gel formulation. DNTG-5 (desoximetasone niosomal) gel data are identical and comparable with Topicort® gel, 0.05% reference product.

4.10.10. In-vitro permeation study evaluation

The permeation study was performed between the test formulation (DNTG-5) and reference formulation (Lot# H882532755, Taro Pharmaceuticals, Inc.). This study was conducted to evaluate the effect of niosome loaded drugs in a gel formulation for their drug release through human cadaver skin and deposition of the drug in skin layers over time.

4.10.10.1. Drug release through human cadaver skin

Penetration parameters of desoximetasone are summarized, and a comparison between test and reference products are given in Table 4.9. Samples were collected at regular intervals (1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 22, and 24 hours) during the permeation

study. Figure 4.13 shows the desoximetasone permeation profile for the reference and test formulations, and the amount of desoximetasone detected after 24 hours in human cadaver skin.

Table 4.9. Penetration parameters of desoximetasone through human cadaver skin (n=6) up to 24 hours.

Time (hours)	Q up to 24 hours (µg/cm^2)	
	Lot# H882532755 (Reference product)	**Lot# DNTG-5 (Test product)**
1	3.70 ± 0.13	3.73 ± 0.08
2	3.91 ± 0.13	3.94 ± 0.08
3	4.12 ± 0.13	4.15 ± 0.08
4	4.98 ± 0.27	4.75 ± 0.13
6	6.25 ± 0.62	5.51 ± 0.22
8	8.19 ± 1.05	6.08 ± 0.37
10	9.74 ± 1.75	6.56 ± 0.25
12	11.54 ± 2.33	7.11 ± 0.29
16	17.01 ± 3.68	8.19 ± 0.49
20	21.76 ± 4.54	9.02 ± 0.57
22	22.79 ± 4.48	9.24 ± 0.47
24	24.22 ± 4.29	9.75 ± 0.44

Q, the cumulative amount of desoximetasone penetrated per cm^2 up to 24 hours (n=6, mean ± SD)

The results show that the flux from the reference formulation lot# H882532755 is higher in comparison with the test formulation lot# DNTG-5. Niosomes showed biphasic drug release. An initial phase lasting 4 hours was followed by a slower release phase extending from 4 hours to 24 hours. In the initial phase, free unentrapped or loosely bound drug and drug adsorbed at the surface of niosomal bilayer diffuses into the release medium [29]. In contrast, whereas in the slow release phase, the entrapped drug leaks out gradually from the niosome vesicles into the medium. The addition of stearic acid into niosomes formulation reduced both release rate and extent the drug release from the niosome formulation through the study period, the effect being concentration-dependent.

The sustained release drug from niosomes was due to the release retarding effect of bilayer stabilized by the cholesterol [30].

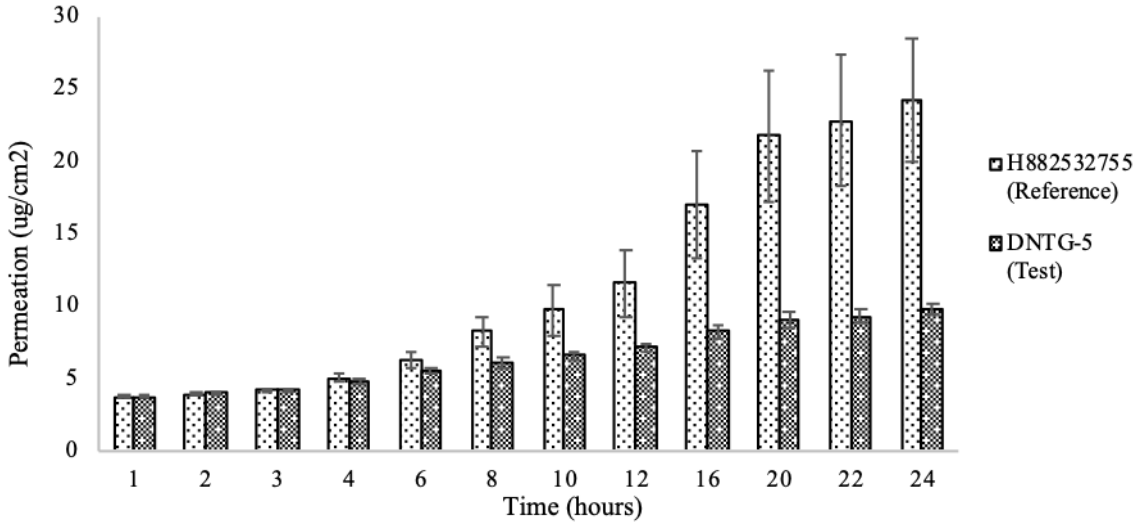

Figure 4.13. Desoximetasone permeation profile for H882532755 (reference – marketed gel) and DNTG-5 (test – niosomal gel) formulations. Time points were measured at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 22, and 24 hours. Each point represents the mean ± SD; n=6).

The additional possibility of the sustained release drug from the negatively charged niosome vesicles could be attributed to retaining the positive drug by the negative bilayers. A similar trend was observed and published in various research articles [31, 32].

The drug release study indicates that the release pattern between the reference and test product is identical; however, drug release from the desoximetasone niosomal gel is steady and slower compared to the referenced gel marketed product.

4.10.10.2. Drug deposition in human cadaver skin

Drug deposited in the skin during the permeation study was evaluated at the end of the permeation study. It is essential to identify drug deposited in the skin during the permeation study, as skin deposition show direct relation with drug release duration.

Table 4.10. Amount of desoximetasone detected after 24 hours in human cadaver skin (n=6, mean ± SD).

Skin deposition of desoximetasone (ng/mg)	
H882532755 (Reference)	**DNTG-5 (Test)**
26.01	30.88

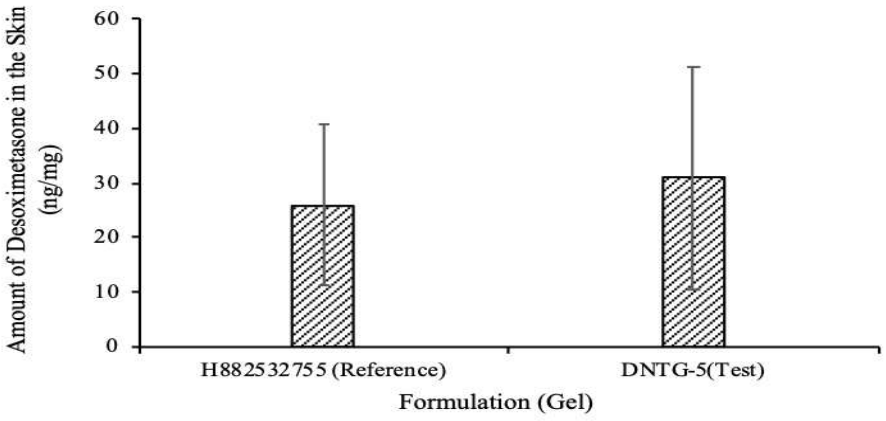

Figure 4.14. Amount of desoximetasone detected after 24 hours in human cadaver skin (n=6, mean ± SD) using reference (marketed gel) and test (niosomal gel) desoximetasone formulations.

The skin deposition of desoximetasone is summarized, and a comparison between test and reference products is given in Table 4.10. Figure 4.14 shows the desoximetasone permeation profile for the reference and test formulations and the amount of desoximetasone detected after 24 hours in human cadaver skin. It was observed that test product (lot# DNTG-5) was able to retain more drugs in human cadaver skin compared to the reference product (lot# H882532755) that might be useful in the treatment and management of skin disease to reduce the frequency of drug product application at the site of action.

4.10.10.3. Kinetic analysis of the drug release data

The first-order and Higuchi square root law model has been used to evaluate the drug release kinetics from the polymer matrix systems. The first-order and Higuchi models were applied to the release data. The R^2 value of the first-order was less fit to test product (lot# DNTG-5) niosomal gel formulation, and the Higuchi model was less fit to reference product (lot# H882532755) marketed gel product. Whereas, the drug release kinetic data fitted the Higuchi model for test product (lot# DNTG-5) niosomal gel and the first-order model for reference product (lot# H882532755) marketed gel product. The in vitro release results showed that the release of the drug from the test formulation DNTG-5 niosomal gel is most fitted to the diffusion-controlled mechanism (Higuchi model) according to the higher correlation coefficient. (Figure 4.15). Therefore, it can be stated that the drug molecules are released from the niosomal gel matrix by the following mechanisms: (a) in the initial phase, free unentrapped or loosely bound drug and drug adsorbed at the surface of niosomal bilayer diffuses into the release medium. This study in agreement with the research conducted by Akbari, V., et al., Release studies on ciprofloxacin loaded non-ionic surfactant vesicles [29]. (b) Whereas in the slow release phase, the entrapped drug release slowly from the niosome vesicles. The addition of charge molecule into niosomes reduced both release rate and extent the drug release through the study period, the effect being concentration dependent. The sustained release drug from niosomes was due to the release retarding effect of bilayer stabilized by the cholesterol. This study in agreement with the published research by Gurrapu, A., et al., Improved oral delivery of valsartan from maltodextrin based proniosome powders [30]. The diffusion exponent of the Korsmeyer Peppas equation was less than 0.5 (DNTG-5

exponent n value 0.3). This indicates a Fickian mechanism is dominant and controls the drug release from the DNTG-5 desoximetasone niosomal gel formulation. Similar results were observed with the published study by Feith, G., Fluconazole loaded niosomal gels as a topical ocular drug delivery system for corneal fungal infections [33]. The kinetic release parameters and correlation coefficients (r^2) calculated for the lot# H882532755 (reference) and lot# DNTG-5 (test) gel formulations are summarized in Table 4.11.

Table 4.11. Kinetic models of desoximetasone release from marketed gel and niosomal gel products (n= 6, mean ± SD).

Formulation	First order (r^2)	Higuchi model (r^2)	Exponent of Korsemeyer Peppas equation (n)
H882532755 (Reference - gel)	0.975	0.928	0.673
DNTG-5 (test - niosomal gel)	0.945	0.991	0.332

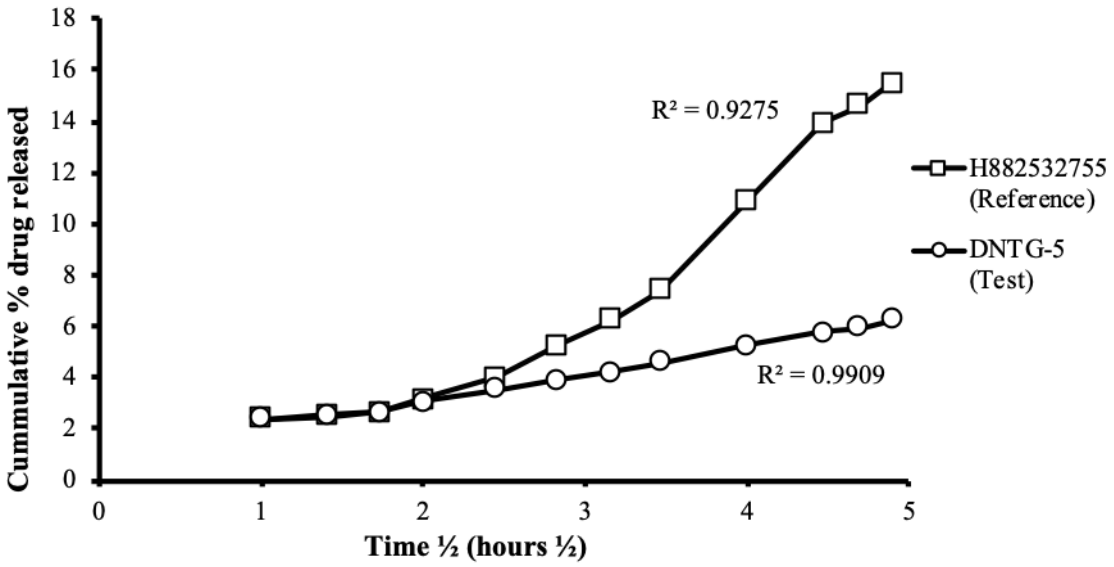

Figure 4.15. Higuchi release kinetics marketed reference gel and niosomal gel products (n=6, mean ± SD).

4.11. Conclusions

The semisolid dosage form is the ideal method to deliver the drug locally. Skin diseases such as inflammation, itching, redness, psoriasis are very common and desoximetasone is a widely accepted corticosteroid drug for the common skin disease. Desoximetasone is available in gel, cream, spray or ointment form in the market. The information listed in FDA's Orange Book database Topicort® (Desoximetasone) gel, 0.05%, is currently listed as "RLD - reference listed product." Therefore, we have developed desoximetasone niosomal gel with similar physicochemical characteristics with Topicort® gel USP, 0.05%. The significant difference between the desoximetasone niosomal gel and Topicort® gel is a drug is in the niosomal matrix and niosomes are dispersed state in the test gel product (lot# DNTG-5) in comparison with the drug is solubilized form in Topicort® gel reference product (lot# H882532755).

In this study, novel desoximetasone niosomal topical gel was developed. In the first step of the gel formation, different concentration of Carbomer 980 was utilized to optimize thickening agent concentration through comparing yield stress and viscosity of the reference gel. Additionally, desoximetasone niosomal gel batches were manufactured using different types of thickening agents to evaluate their impact. Desoximetasone niosomal gels were evaluated for drug content, pH, specific gravity, spreadability, yield stress, viscosity, color, texture, homogeneity, phase separation, and description. Evaluated data were compared with the results obtained from the reference gel product. Based on the preliminary results, it was concluded that desoximetasone niosomal gel's 0.70% Carbomer 980 (DNTG-5) was the formulation that shows identical physicochemical properties compared to the reference product. After finalizing the

desoximetasone niosomal gel formulation, in vitro drug release study was conducted in comparison with reference product using human cadaver skin. This study results suggested that the desoximetasone niosomal gel's drug release is slower compared to the Topicort® gel marketed product. In vitro permeation study data indicates that drug release from desoximetasone niosomal gel DNTG-5 was fitted to the diffusion-controlled mechanism (Higuchi model) and diffusion exponent of Korsmeyer Peppas equation was less than 0.5 which indicates a Fickian mechanism is dominant and controls the drug release from the DNTG-5 desoximetasone niosomal gel. Skin deposition study was also conducted between desoximetasone niosomal gel and reference gel products. Data suggests that DNTG-5 formulations retain a higher amount of the drug in the skin, which could be the prime reason for drug release for a more extended time, which can reduce dosing frequency. Detail about the optimized desoximetasone niosomal topical gel formulation is described in Table 4.12.

Table 4.12. Summary of optimized desoximetasone niosomal topical gel formulation (DNTG).

Ingredients	**DNTG – 5 (% w/w)**
Desoximetasone, USP (niosomal dispersion)	0.05
Carbomer 980, NF	0.70
Edetate disodium, USP	0.018
Docusate sodium	0.014
Transcutol	8.20
Trolamine, NF	0.30
Purified Water	QS to 100.00

This study would provide the additional evidence of DNTG-5 desoximetasone niosomal gel's significant potential for controlled release of the drug and higher drug retained in the skin, which can help to reduce the drug concentration and reduce dosing frequency. However, further research is required to confirm this novel finding.

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5. CHAPTER - 5. STABILITY EVALUATION OF FINAL DESOXIMETASONE NIOSOMAL DISPERSION AND DESOXIMETASONE NIOSOMAL TOPICAL GEL

5.1. Introduction

Nanoparticles drug delivery systems have recently been extensively researched for various health and medical applications due to their multiple advantages compared to conventional formulations. The significant benefits as discussed in the previous section like increased solubility of hydrophobic drugs increased stability of labile drugs, increased permeability through biological membranes, controlled release, and increased drug availability. Despite all these benefits, the nanoparticle drug delivery system has not been scaled up for commercial development due to challenges involved with their physical and chemical stability. Physical instability includes agglomeration of nanoparticles, which leads to a reduction in size related advantages like increased permeability, large surface area, etc. In contrast, chemical stability such as hydrolysis of the carrier, drug leakage, and drug instability leads to non-viability to the formulations.

Commonly nanoparticle systems are suspension/dispersion in an aqueous system and non-enzymatic hydrolysis reactions (because of the presence of water) are primarily responsible for the nanoparticle drug delivery system's instability. Therefore, it is essential to monitor the stability of the nanoparticle formulation over time at various temperatures to evaluate time and temperature effects on the product.

It is essential to monitor the gel product's stability containing drugs incorporated into the niosome vesicles. Semisolid products may show various kinds of physical

instability such as creaming, flocculation, Ostwald ripening, change in viscosity, or rheological behavior either by structure build-up or structure breakdown, change in particle size either by increasing or decreasing particle size. Sedimentation, color change, pH change, or conductivity may also be a problem during stability. The typical examples of the chemical instability are reduction in drug assay or increase degradation products, due to the degradation of the drug substance following chemical reaction with other ingredients in the formulation, or environmental instability like thermal, oxidative, or light-induced degradation.

Formal stability testing is essential to support the shelf-life of the product along with support the regulatory submission. The ICH stability testing guidelines [1] provide details on the storage temperatures and testing frequency for different product types, different packaging container-closure type, and intended markets. Suggestions from the FDA regarding the stability guidelines that testing covers physical and chemical attributes apply to the products.

In this study, stability for the desoximetasone niosomal dispersion and desoximetasone niosomal topical gel was performed at 15 days, one month, two months, and three months time intervals. For desoximetasone niosomal dispersion, 2 mL samples were aliquot into Eppendorf tubes, and desoximetasone niosomal topical gel, 4 gm of samples were aliquot into glass scintillation vials. Samples were kept at 15 days, one month, two months, and the three months time point at room temperature and 40°C temperature. Stability sample of desoximetasone niosomal dispersion was evaluated for drug content, entrapment efficiency, particle size, polydispersity index and zeta potential,

and desoximetasone niosomal topical gel samples were evaluated for assay, pH, spreadability, color, phase separation, texture, homogeneity, and description.

5.2. Stability evaluation of desoximetasone niosomal dispersion

Lab batch was manufactured based on the final selected desoximetasone niosomal dispersion (DND). The batch was evaluated for entrapment efficiency, particle size, polydispersity index, and zeta potential for time 0 (initial) sample. At the end of the batch manufacturing process, 2 mL samples were aliquots in Eppendorf tube.

5.2.1. Materials

Desoximetasone was gifted by Flavine, New Jersey, USA. Diethyl ether and Stearyl amine were purchased from Sigma-Aldrich, Saint Louis, MO, USA. Ethanol was procured from Decon Labs, Inc., King of Prussia, PA, USA. Acetone, methanol, and acetonitrile were purchased from BDH VWR Analytical, Radnor, PA, USA. Cholesterol and Span 60 (sorbitan monostearate) were gifted from Croda Inc., Mill Hall, PA, USA. Stearic acid was received from BASF Corporation, Edison, NJ, USA. HPLC water and chloroform were procured from Sigma-Aldrich, Saint Louis, MO, USA. Glacial acetic acid was purchased from Fisher Scientific, Fair Lawn, NJ, USA.

5.2.2. Methods

5.2.2.1. Niosome vesicle preparation

Initially, the drug was dissolved into an organic phase; then it was mixed thoroughly until it completely dissolved. Next, surfactant, cholesterol, and lipid were

added into the solution and mixed using a suitable magnetic spin bar in a 20 mL glass scintillation vial. Purified water was heated in a separate 50 mL glass beaker at 65°C temperatures using a hot plate with magnetic stirring. The organic phase was then filled into a 10 mL syringe with a 26 G needle. The organic phase mixture was injected into the preheated water phase at 0.5 mL/min addition rate. Mixing was carried out at 650 rpm for 50 minutes. In the final step of the process, the batch was cooled down to room temperature, and the formulation was stored in a suitable glass storage container.

5.2.2.2. Desoximetasone analysis

HPLC method is required to perform desoximetasone analysis to identify drug content (assay value) and entrapment efficiency of the formulation.

5.2.2.3. High performance liquid chromatography (HPLC) method

The mobile phase was prepared by mixing methanol, HPLC grade water, and glacial acetic acid in ratio 65:35:1 [2]. The diluent was prepared by mixing methanol and acetonitrile in ratio 50:50. The desoximetasone was measured using a Discovery C18 column with 5 µm particle size, L x I.D. 150 mm x 4.6 mm [2]. The flow rate was 1.0 mL/min, and the injection volume was 10 µL. The sample run time was 10 min at room temperature and retention time for the drug peak was at approximately 5 minutes. Drug content quantification for desoximetasone was performed using HPLC (Agilent 1100-Chemstation software) coupled with UV analysis at a wavelength of λ_{max} 254 nm.

5.2.2.4. Niosome vesicle characterization and data analysis

5.2.2.4.1. Organoleptic properties

Niosomal dispersions were evaluated for visual appearance, color, and odor to confirm the presence of any residual solvent standard quality check.

5.2.2.4.2. Assay determination (Drug content)

The desoximetasone niosomal dispersion was carefully collected and placed into an intermediated solvent containing a mixture of chloroform: methanol (40:60) and then using a vortexing mixer was mixed until it completely dissolved at room temperature. In the final step, niosomal dispersion samples were further diluted with equal ratios of diluent. Drug quantification was determined using the pre-determined HPLC method as mentioned in section 5.2.2.3.

5.2.2.4.3. Drug entrapment efficiency of niosome vesicles

The desoximetasone free drug was separated and determined from the entrapped drug in niosomal formulation. To separate the free drug from the formulation, ultracentrifugation at 14,000 rpm for 30 minutes using an ultracentrifuge (Branson Ultrasonics Corporation, CT, USA) at room temperature was performed. The supernatant containing the free drug was carefully collected and separated from the sediment of the sample without disturbing the formulation. The supernatant was further dissolved into chloroform: methanol (40:60) mixture using a vortex mixer. After mixing, the sample was further diluted with an equal amount of the diluent. Drug quantification was determined using the pre-determined HPLC method, as mentioned in section 5.2.2.3.

Drug % entrapment efficiency was calculated in triplicate by using the following formula [3].

$$\% \text{ Entrapment Efficiency} = \frac{Total\ amount\ of\ Drug - Free\ amount\ of\ Drug}{Total\ amount\ of\ Drug} \times 100 \qquad [4]$$

5.2.2.4.4. Niosomes vesicle size and polydispersity index (PDI)

The mean vesicle size and its distribution were evaluated at room temperature using a Delsa Nano S Particle Sizer (Beckman Coulter, CA, USA) in triplicate based on the light scattering spectroscopy principles.

5.2.2.4.5. Niosomes zeta potential

The zeta potential of the niosomal dispersion was measure in triplicate using a Malvern Particle Sizer 2000 (Malvern Technologies, Worcestershire, UK).

5.2.3. Desoximetasone niosomal dispersion stability formulation

DND-62 is the final selected desoximetasone niosomal formulation. The replicate batch of the DND-62 was manufactured, and the number for the new batch was given to DND-69. Summary of the desoximetasone niosomal dispersion (DND-69) formulation concentrations is provided in Table 5.1.

Table 5.1. Desoximetasone niosomal dispersion stability study batch detail (DND-69).

Ingredients	DND – 69 (% w/w)
Desoximetasone, USP	0.20
Sorbitan Monostearate (Span 60)	0.40
Cholesterol, NF	0.20
Stearic Acid	0.05
Purified Water	Q.S. to 100.00

5.2.4. Stability samples, time points and stability stations detail

2 mL samples were aliquots into Eppendorf. These samples were kept at different stability times and temperatures, as described in Table 5.2.

Table 5.2. Desoximetasone niosomal dispersion DND-69 detail on sample quantity and sample storage plan for the stability study.

Batch detail	Aliquot quantity	Packaging component	Stability time	Storage condition
DND-69	2 mL	Eppendorf	Initial	N/A
	2 mL	Eppendorf	15 days	Room temperature
	2 mL	Eppendorf	1 month	Room temperature
	2 mL	Eppendorf	2 months	Room temperature
	2 mL	Eppendorf	3 months	Room temperature
	2 mL	Eppendorf	15 days	40°C temperature
	2 mL	Eppendorf	1 month	40°C temperature
	2 mL	Eppendorf	2 months	40°C temperature
	2 mL	Eppendorf	3 months	40°C temperature

5.3. Stability evaluation of desoximetasone niosomal topical gel (DNTG)

The stability batch of the desoximetasone niosomal topical gel was manufactured based on the final selected gel formulation. The batch was evaluated immediately for drug content, pH, spreadability, homogeneity, color, texture, phase separation, and description for time 0 (initial) sample. At the end of the batch manufacturing process, 4 gm samples were aliquots in 20 mL glass scintillation vial.

5.3.1. Materials

Desoximetasone was gifted by Flavine, New Jersey, USA. Diethyl ether and Stearyl amine were purchased from Sigma-Aldrich, Saint Louis, MO, USA. Ethanol was procured from Decon Labs, Inc., King of Prussia, PA, USA. Acetone, methanol, and acetonitrile were purchased from BDH VWR Analytical, Radnor, PA, USA. Cholesterol and sorbitan monostearate (Span 60) were gifted from Croda Inc., Mill Hall, PA, USA. Stearic acid was received from BASF Corporation, Edison, NJ, USA. HPLC water, chloroform, calcium chloride dihydrate, docusate sodium, and hydroxypropyl methylcellulose (HPMC) were procured from Sigma-Aldrich, Saint Louis, MO, USA. Glacial acetic acid was purchased from Fisher Scientific, Fair Lawn, NJ, USA. Edetate disodium, trolamine, and xanthan gum were purchased from Spectrum Chemical, New Brunswick, NJ, USA. Ethyl cellulose (EC) and hydroxypropyl cellulose (HPC) were gifted by Ashland Specialty Ingredients, Parlin, NJ, USA. Tarscutol was gifted from Gattefosse Corporation, Paramus, NJ, USA. Carbomer 940, Carbomer 974P, Carbomer 980, Carbomer 981, and Carbomer 1342 were gifted from Lubrizol Advanced Materials Inc., Brecksville, OH, USA.

5.3.2. Methods

5.3.2.1. Topical gel preparation

Initially, purified water was weighed into a glass beaker. Edetate disodium, docusate sodium, and Transcutol were added into purified water; then, it was mixed using propeller mixer. Next, the thickening agent was carefully weighed and add slowly into the mixture and mixed using propeller mixer. At the other end, in a separate glass

container, purified water and trolamine were added and mixed using a spatula until it thoroughly mixes. The mixture of purified water and trolamine was slowly added into a previously mixed thickening agent mixture. Mixing was carried out using the propeller mixer. In the final step of the process, the desoximetasone niosomal dispersion was accurately weighed and added into the previously prepared viscous mixture. Final mixing was carried out using the propeller mixer.

5.3.2.2. Topical gel chemical characterization

5.3.2.2.1. High performance liquid chromatography (HPLC) method

The mobile phase was prepared by mixing methanol, HPLC grade water, and glacial acetic acid in ratio 65:35:01 [2]. The diluent was prepared in a 100 mL volumetric flask by mixing 1.5 g calcium chloride dihydrate into 5 mL of HPLC grade water. The mixture was agitated until calcium chloride dihydrate dissolved completely. The final volume was made using methanol. The desoximetasone was measured using a Discovery C18 column (Sigma-Aldrich, Saint Louis, MO, USA) with 5 µm particle size, L x I.D. 150 mm x 4.6 mm [2]. The flow rate was 1.5 mL/min, and the injection volume was 20 µL. The sample run time was 10 min 30°C temperature and retention time for the drug peak was at approximately 4 minutes. Drug content quantification for desoximetasone was performed using HPLC instrument Agilent from 1100 series instrumentation (Agilent Technologies, CA, USA) coupled with UV detection (DAD) at a wavelength λ_{max} 254 nm and HP ChemStation software V. 32.

5.3.2.2.2. Desoximetasone assay characterization

The desoximetasone niosomal topical gel sample was mixed manually. Then, a random sample was carefully collected and placed into a diluent, and then using a vortexing mixer it was mixed thoroughly. Then the mixture was sonicated at 60°C for 12 minutes following by cooling down the mixture at room temperature. In the final step, the niosomal topical gel assay sample was further diluted with diluent and then using a vortexing mixer, it was mixed thoroughly. Drug quantification was determined using the pre-determined HPLC method as mentioned in section 5.3.2.2.1.

5.3.2.3. Topical gel physical characterization

5.3.2.3.1. pH measurement

The pH of various desoximetasone niosomal topical gel formulations was determined using the pH meter (VWR pH meter symphony B10P, Radnor, PA, USA). 1 gm of niosomal gel was mixed in 10 gm of DI water. Then, pH was determined at room temperature.

5.3.2.3.2. Spreadability measurement

Spreadability was measured in mm. A 100 mg sample was placed carefully in the center of a microscopic glass slide and covered with another slide, total 50 gm of standardized weight was kept on it for 1 minute and at the end of the test, the diameter of the sample was measured in mm.

5.3.2.3.3. Physicochemical properties evaluation

Color

Desoximetasone niosomal topical gel was examined visually for their color property evaluation.

Texture

Desoximetasone niosomal topical gel was examined visually for their texture property evaluation.

Homogeneity

Physically niosomal gel was examined by placing the gel between the thumb and the index finger and the homogeneity or any aggregates were observed.

Phase separation

Desoximetasone niosomal topical gel was examined visually for their physical stability phase consistency property evaluation.

Description

The description is a visual state for a general statement describing how the product looked when examined.

5.3.3. Desoximetasone niosomal topical gel stability formulation

DNTG-5 is selected as the final optimized desoximetasone niosomal topical gel formulation. The replicate batch of the DNTG-5 was manufactured, and the number for the new batch was given to DNTG-14. Summary of the desoximetasone niosomal topical gel (DNTG-14) formulation concentrations are provided in Table 5.3.

Table 5.3. Desoximetasone niosomal topical gel stability study batch detail (DNTG-14).

Ingredients	DNTG-14 (% w/w)
Desoximetasone, USP (niosomal dispersion)	0.05
Carbomer 980, NF	0.70
Edetate disodium, USP	0.018
Docusate sodium	0.014
Transcutol	8.20
Trolamine, NF	0.30
Purified Water	QS to 100.00

5.3.4. Stability samples, time points and stability stations detail

4 gm samples were aliquots into 20 mL glass scintillation vial. These samples were kept at different stability times and temperatures, as described in Table 5.4.

Table 5.4. Desoximetasone niosomal topical gel (DNTG) DNTG-14 detail on sample quantity and sample storage plan for the stability study.

Batch detail	Aliquot quantity	Packaging component	Stability time	Storage condition
DNTG-14	4 gm	glass vial	Initial	N/A
	4 gm	glass vial	15 days	Room temperature
	4 gm	glass vial	1 month	Room temperature
	4 gm	glass vial	2 months	Room temperature
	4 gm	glass vial	3 months	Room temperature
	4 gm	glass vial	15 days	40°C temperature
	4 gm	glass vial	1 month	40°C temperature
	4 gm	glass vial	2 months	40°C temperature
	4 gm	glass vial	3 months	40°C temperature

5.4. Results and Discussion

Stability study was conducted for desoximetasone niosomal dispersion (DND) and desoximetasone niosomal topical gel (DNTG) at room temperature and 40°C temperature for up to 3 months.

5.4.1. Desoximetasone niosomal dispersion (DND)

Niosomes are composed of non-ionic surfactant and cholesterol, manufactured in the lab have numerous applications in the medical field. Similar to other nanoparticle delivery systems, niosomes can control the drug release, increase labile drug stability, demonstrate sustained release, and have shown efficacy in many cosmetic products. However, to be a viable, commercially available treatment option, niosomes and the drug(s) they encapsulated must be stable for extended periods. Typically, the shelf life of two years is the advised amount of time for which a formulation should remain stable. Additionally, storage at room temperature is preferable in comparison to store the product at reduced temperatures because of additional logistics and distribution expenses associated with lower temperature freezer storage. Desoximetasone niosomal dispersion DND-69 batch was used for the stability study, and study data are presented in Table 5.5.

Table 5.5. Observed results for entrapment efficiency, particle size, polydispersity index and zeta potential for samples were stored at room temperature and 40°C temperature (n=3, mean ± SD).

Stability time	Temperature conditions	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
Initial	N/A	90.05 ± 0.65	411.83 ± 8.22	0.428 ± 0.05	-30.70 ± 0.36
15 days	Room temperature	93.01 ± 0.31	404.67 ± 16.38	0.407 ± 0.02	-38.47 ± 0.75
1 month	Room temperature	91.23 ± 0.31	375.03 ± 4.89	0.434 ± 0.02	-42.73 ± 0.95

Stability time	Temperature conditions	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
2 months	Room temperature	90.70 ± 0.44	393.80 ± 15.61	0.483 ± 0.05	-59.77 ± 1.01
3 months	Room temperature	91.72 ± 0.45	451.47 ± 15.01	0.468 ± 0.01	-32.90 ± 0.72
15 days	40°C temperature	91.21 ± 0.41	417.93 ± 8.16	0.468 ± 0.02	-41.63 ± 0.35
1 month	40°C temperature	90.01 ± 0.59	336.10 ± 1.61	0.376 ± 0.01	-27.33 ± 0.96
2 months	40°C temperature	87.38 ± 0.13	380.57 ± 1.31	0.456 ± 0.01	-24.77 ± 1.15
3 months	40°C temperature	89.56 ± 0.06	487.77 ± 9.11	0.471 ± 0.02	-43.23 ± 1.00

5.4.1.1. Effect of stability conditions on entrapment efficiency of the niosomal dispersion

The niosomal formulation DND-69 was subjected to various stability conditions to evaluate their impact on the formulation's entrapment efficiency. The experimental data demonstrate that the entrapment efficiency of the multiple stability samples is constant. No significant change was observed in drug entrapment between the various stability samples. Data for these experiments are given in Table 5.5. Entrapment efficiency comparisons of room temperature and 40°C temperature samples are illustrated in Figure 5.1.

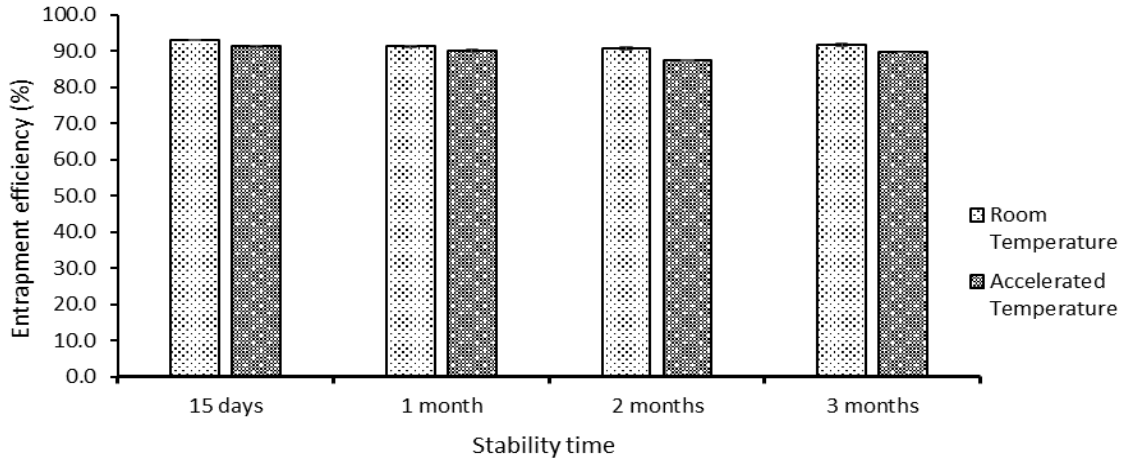

Figure 5.1. Effect of stability conditions on entrapment efficiency (%) of niosomes (n=3, mean ± SD).

Entrapment efficiency results are consistent and no change at an even higher temperature (40°C) or longer time samples. Data suggests that niosomes are stable at higher temperatures or for a longer time and do not shows any breakdown of niosome vesicles. This behavior may explain as the niosomes formulation is optimized for cholesterol concentration, which plays an essential role in manufacturing niosomes. Ideal formulation combination helps to stabilize niosome vesicles for a longer time.

5.4.1.2. Effect of stability conditions on particle size and polydispersity index of the niosomal dispersion

The niosomal formulation DND-69 were subjected to various stability conditions, and their impact on the particle size and polydispersity index of the niosomes were evaluated. The experimental data shows that particle size and polydispersity index of the various stability samples are similar. No significant change was observed in the drug niosome particle size and PDI index between the different stability samples. Data for these experiments are given in Table 5.5. Particle size and PDI of room temperature and 40°C temperature samples are illustrated in Figure 5.2 and Figure 5.3, respectively.

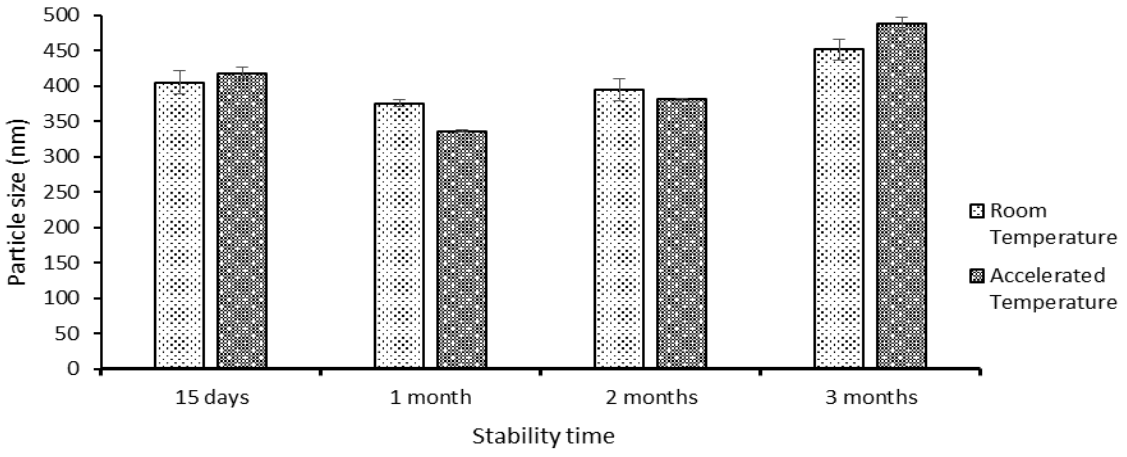

Figure 5.2. Effect of stability conditions on particle size (nm) of niosomes (n=3, mean ± SD).

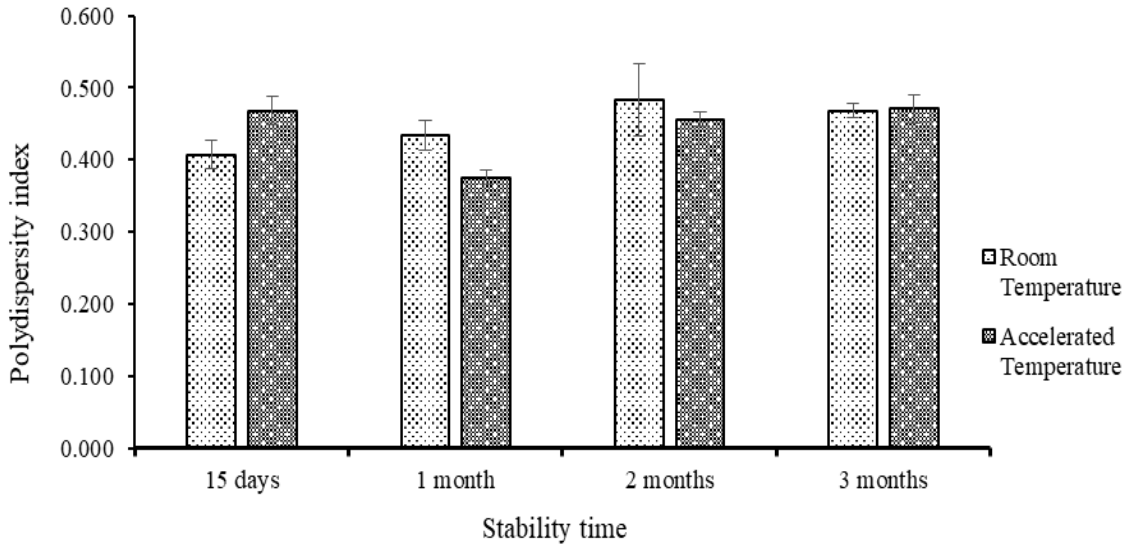

Figure 5.3. Effect of stability conditions on polydispersity index of niosomes (n=3, mean ± SD).

Results show no significant change in particle size and polydispersity index at a higher temperature (40°C) and a longer time. Data suggests that niosomes are stable and do not shows any agglomeration or clumps. This behavior may explain the presence of a charge-inducing agent in the formulation, which prevents the agglomeration and shows the repulsion effect between the niosome particles.

5.4.1.3. Effect of stability conditions on zeta potential of the niosomal dispersion

The lab formulation DND-69 was subjected to various stability conditions and their impact on the zeta potential of the niosomes. The experimental data indicates the similarity of zeta potential. Data for these experiments are given in Table 5.5. Zeta potential of room temperature and 40°C temperature samples are illustrated in Figure 5.4.

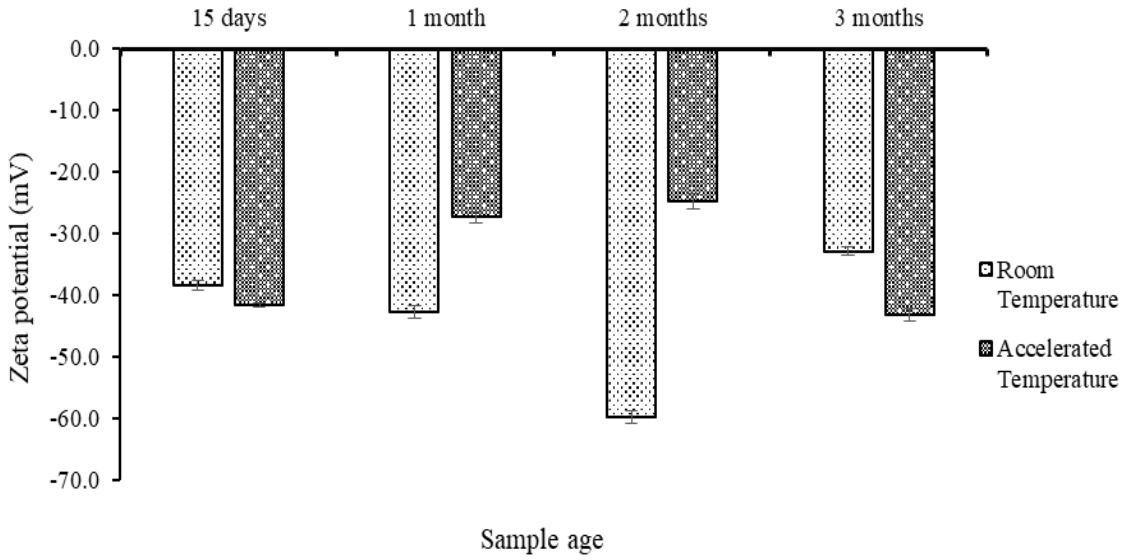

Figure 5.4. Effect of stability conditions on zeta potential of niosomes (n=3, mean ± SD).

The stability study result concludes that temperature and time have no impact on the zeta potential of the various stability batches. Data suggests that niosomes are stable and do not show less than 30 mV zeta potential in any stability batches, which is sufficient to prevent aggregation between the niosomal vesicles [4].

5.4.2. Desoximetasone niosomal topical gel (DNTG)

Desoximetasone niosomal gel is composed of edetate disodium, docusate sodium, Transcutol, Carbomer 980, and trolamine, manufactured in the lab have numerous applications in the medical field. Similar to other semisolid products such as cream, ointment, lotion, the topical gel can deliver the drug at the target site and drug release can be modified by changing viscosity build-up polymer. However, to be a viable, commercially available treatment option, topical gel, and the drug(s) must be stable for extended periods. Typically, a shelf-life of two years is the advised amount of time for which a formulation should remain stables. Additionally, storage at room temperature is

preferable in comparison to store the product at reduced temperatures because of additional logistics and distribution expenses associated with lower temperature freezer storage. Desoximetasone niosomal topical gel DNTG-14 batch was used for the stability study, and study data are presented in Table 5.6.

Table 5.6. Observed results for drug content, pH, spreadability for samples were stored at room temperature and 40°C temperature (n=3, mean ± S.D).

Stability time	Temperature conditions	Drug content (%)	pH	Spreadability (mm)
Initial	N/A	93.10 ± 0.80	5.65 ± 0.01	18.67 ± 0.58
15 days	Room temperature	94.72 ± 0.62	5.72 ± 0.01	18.67 ± 0.05
1 month	Room temperature	93.57 ± 2.19	5.68 ± 0.02	19.00 ± 1.00
2 months	Room temperature	91.33 ± 0.94	5.72 ± 0.02	19.00 ± 1.00
3 months	Room temperature	93.70 ± 0.69	5.57 ± 0.01	19.33 ± 0.58
15 days	40°C temperature	96.58 ± 2.48	5.70 ± 0.01	19.33 ± 0.58
1 month	40°C temperature	95.15 ± 0.15	5.70 ± 0.01	19.33 ± 0.58
2 months	40°C temperature	98.71 ± 23.96	5.74 ± 0.03	18.67 ± 0.58
3 months	40°C temperature	94.65 ± 1.14	5.55 ± 0.03	19.67± 0.58

5.4.2.1. Effect of stability conditions on the drug content of the niosomal topical gel

The niosomal topical gel DNTG-14 were subjected to various stability conditions to evaluate their impact on the drug content of the formulation. The experimental data demonstrate that the drug content of the different stability samples is constant. The various stability samples have no considerable impact on the drug content. Data for these experiments are given in Table 5.6. Drug content comparisons of room temperature and 40°C temperature samples are illustrated in Figure 5.5.

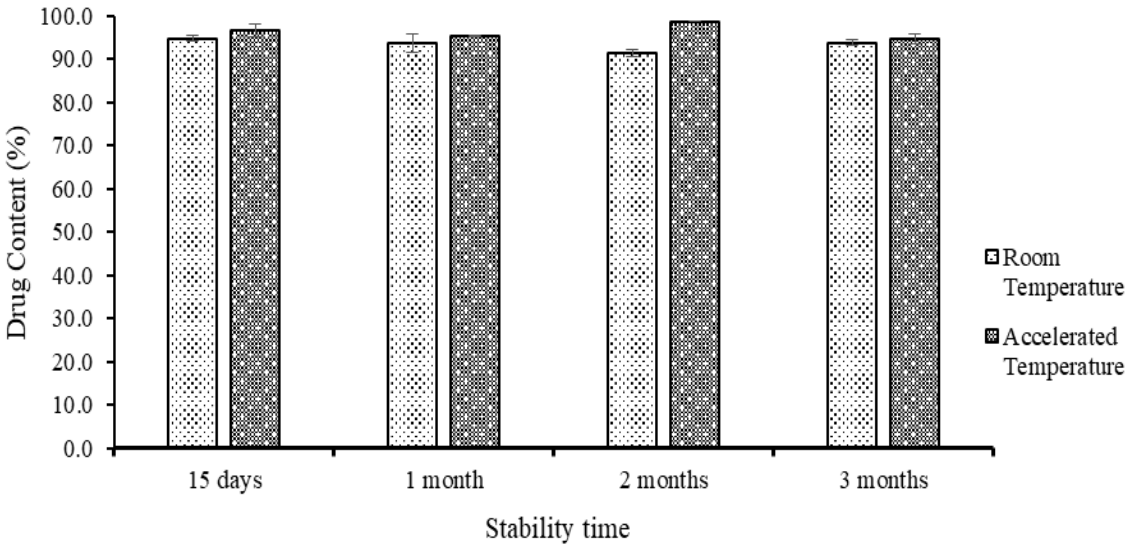

Figure 5.5. Effect of stability conditions on drug content (%) of niosomal topical gel (n=3, mean ± SD).

Drug content results are consistent and no change at the even higher temperatures (40°C) or longer time samples. Data suggests that desoximetasone niosomal gel is stable and does not show any degradation of the drug substance during the stability study.

5.4.2.2. Effect of stability conditions on the pH of the niosomal topical gel

pH potentially affects the stability of the active ingredient and physicochemical properties of the semisolid product. pH also may affect the effectiveness of the preservatives and viscosity of the drug product. pH values of the formulations were found in the range of 5.57 – 5.74 pH values. the pH of the desoximetasone niosomal gel formulations depends on the quantity and type of the thickening polymer. pH data from the desoximetasone niosomal topical gel and reference gel products are provided in Table 5.6. pH data comparison between room temperature and 40°C temperature samples are illustrated in Figure 5.6.

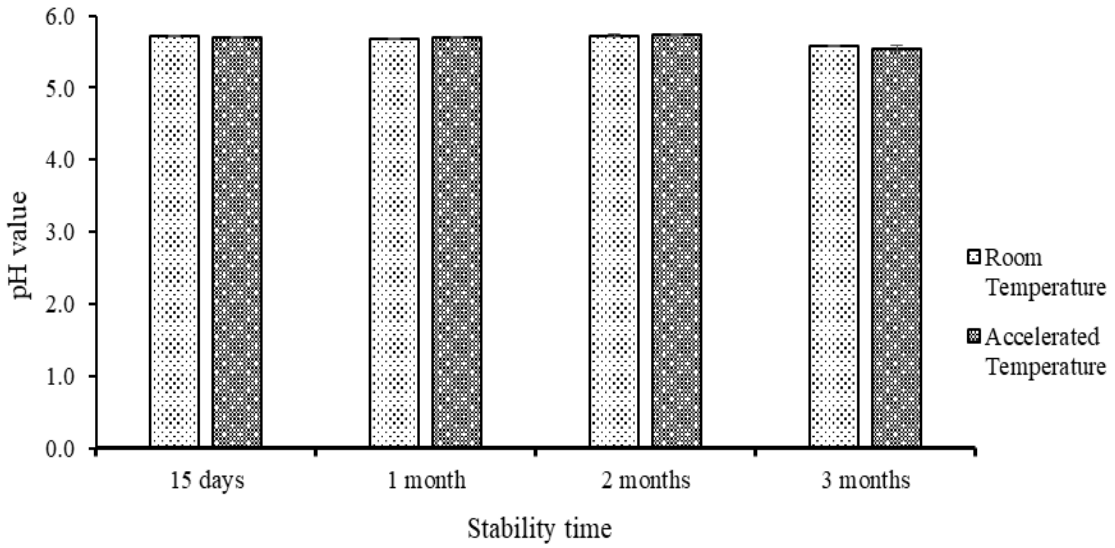

Figure 5.6. Effect of stability conditions on the pH of niosomal topical gel (n=3, mean ± SD).

The data shows the pH of the formulations is well controlled and acceptable. pH results are consistent and no change at an even higher temperature (40°C) or longer time samples. Data suggests that desoximetasone topical gel is stable and does not show any pH fluctuation during the stability study. Formulation pH lied in the normal pH range of skin to avoid any risk of irritation upon the application to the skin.

5.4.2.3. Effect of stability conditions on the spreadability of the niosomal topical gel

Good spreadability is a critical criterion for the gel formulation as it shows the product's behavior when it comes out from the container. It is the term that is used to indicate the extent of the area to which gel readily spreads on application. Results justify this statement as the spreadability of the topical niosomal gel is in the range of 18.67 to 19.67 mm. It was observed that the spreadability of desoximetasone niosomal gel decreased by increasing the thickening polymer concentration or spreadability change

with change in the type of thickening polymer. Spreadability data from the various stability samples of desoximetasone topical gel products are provided in Table 5.6. A comparison of the room temperature and accelerated temperature samples are illustrated in Figure 5.7.

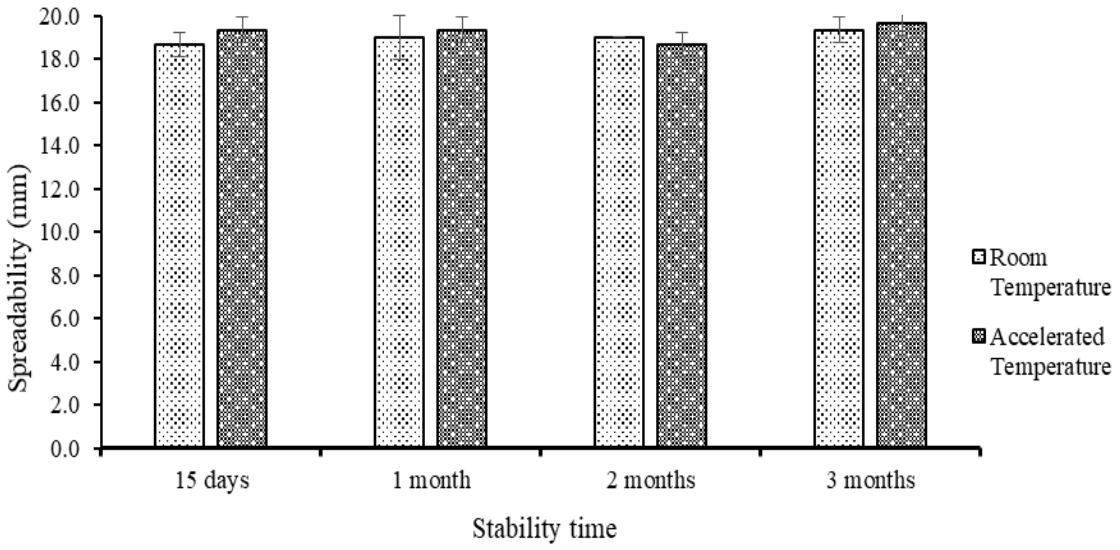

Figure 5.7. Effect of stability conditions on the spreadability of niosomal topical gel (n=3, mean ± SD).

5.4.2.4. Stability conditions effect on the physicochemical properties of topical niosomal gel.

Table 5.7 shows the results of the physicochemical properties of various stability samples of desoximetasone niosomal gel formulation DNTG-14. All the samples are opaque to white color. All stability samples show good homogeneity without lumps and smooth homogeneous texture. Additionally, all the samples show an identical description ‘Opaque to white smooth gel’.

Table 5.7. Physicochemical properties of desoximetasone niosomal topical gel stability batches.

Stability time	Temperature conditions	Color	Phase Separation	Texture	Homogeneity	Description
Initial	N/A	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
15 days	Room temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
1 month	Room temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
2 months	Room temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
3 months	Room temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
15 days	40°C temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
1 month	40°C temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
2 months	40°C temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel
3 months	40°C temperature	Opaque to white	No phase separation observed visually	Smooth	++	Opaque to white smooth gel

+: not good; ++: good

The physicochemical properties of the stability samples are identical and comparable with the initial sample data. There is no observable change in the physical properties of any stability samples regardless of time and temperature. Data suggests that desoximetasone niosomal gel DNTG-14 is stable for a longer time at a various temperature range.

5.5. Conclusions

Stability analysis of the desoximetasone niosomal dispersion was performed using entrapment efficiency, particle size, polydispersity index, and zeta potential at room temperature and 40°C temperature storage condition up to 3 months. Data revealed that

niosomal dispersion stored at room temperature (91.72%, 451.47 nm, 0.468 PDI, and -32.90 mV of entrapment efficiency, particle size, polydispersity index, and zeta potential respectively) and 40°C temperature (89.56%, 487.77 nm, 0.471 PDI, and -43.23 mV of entrapment efficiency, particle size, polydispersity index, and zeta potential respectively) is stable for three months. Although three months may be short-term stability, long term stability up to 2 years or longer suggested by Food and Drug Administration in ICH guideline Q1A(R2) for stability testing of new drug substances and products [5] necessary to further increase shelf-life.

Desoximetasone niosomal dispersion (DND-69) formulation is showing excellent entrapment efficiency with ideal particle size and zeta potential value and during the stability evaluation of this formulation further indicates that no significant changes in properties exist after three months of storage at accelerated temperature condition indicating that progress towards long term stability of the niosomal dispersion formulation has been completed.

Stability analysis of the desoximetasone niosomal topical gel was executed using drug content, pH, spreadability, and physicochemical properties at two different conditions room temperature and 40°C temperature for storage for up to 3 months. Data demonstrates that niosomal topical gel stored at room temperature (93.70%, 5.57 and 19.33 mm of drug content, pH and spreadability respectively) and 40°C temperature (94.65%, 5.55, and 19.67 mm of drug content, pH and spreadability respectively) is stable for three months. Although three months may be short-term stability, long term stability up to 2 years or longer suggested by Food and Drug Administration in ICH

guideline Q1A(R2) for stability testing of new drug substances and products [5] necessary to further increase shelf-life.

Desoximetasone niosomal topical gel (DNTG-14) formulation is showing good drug content assay with ideal pH (favorable to skin condition) and spreadability value. Stability samples show similar physicochemical properties as the initial (time 0) sample with no observable change in formulation. During the stability evaluation of this formulation, further indicates that no significant differences in properties exist after three months of storage at accelerated temperature conditions indicating that progress towards long term stability of the niosomal topical gel formulation has been accomplished.

5.6. References

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THESIS SUMMARY AND FUTURE PERSPECTIVES

The scope of this thesis was the development of a niosome loaded topically applied gel system to deliver the corticosteroids such as desoximetasone to the targeted dermal site with control release to avoid multiple applications or reduce the drug concentration in the formulation. Firstly, this research aimed to identify critical material attributes (CMAs) and critical process parameters (CPPs) for manufacturing ideal niosome system by considering the solvent system, drug concentration, surfactant concentration, cholesterol concentration, and types of lipid as CMAs and external phase temperature, external phase volume, internal phase volume, mixing speed, mixing time, and addition rate as CPPs. These studies suggest that surfactant concentration, cholesterol concentration, mixing speed, mixing time, and additional rate are the only impacting variables on the final product of the desoximetasone loaded niosomal system. Secondly, by considering, impacting CMAs and CPPs derived from the previous study and utilizing JMP® statistical software, we have designed 2^5 full factorial design of experiment study. Based on the input variables and selected full factorial study, the design of the experiment (DOE) suggested 32 formulation combinations optimize the ideal combination for the desoximetasone niosome system. Based on the DOE, 32 batches were manufactured and evaluated for entrapment efficiency, particle size, polydispersity index, and zeta potential. Experimental results were added into the JMP® software full factorial design, based on the results input and by selecting fit model software created profile predictor for the desoximetasone niosome system. Using the profile predictor model, we have chosen an ideal formulation combination for the desoximetasone niosome system. Additionally, for profile prediction model validation, we have generated

two formulation combinations and predicted data for these formulations were verified with experimental data. Thirdly, we have used niosomal dispersion to develop and manufacture a novel niosomal topical gel system. In this study, we have developed topical gel and compared with marketed product Topicort® (desoximetasone) gel USP, 0.05%. To develop topical gel, we have optimized thickening agent (Carbomer 980) concentration by manufacturing various concentration batches and comparing rheological properties of the experimental batches with the reference product. Desoximetasone niosomal topical gel was selected by comparing drug content, pH, spreadability, and physicochemical properties with the reference product. Studies reveal that 0.7% Carbomer 980 formulation data is comparable and identical with the reference product. Additionally, in vitro permeation study with human cadaver skin was performed, and data revealed that drug entrapped in the niosomal system was also able to generate desoximetasone reservoirs in the skin may be useful to exert sustained release of desoximetasone from the stratum corneum over a more extended period. This fact provided information to drug entrapped in the niosomal system can be the permeation modified of choice to develop a novel topical formulation of desoximetasone further. Fourthly, developed and optimized niosomal dispersion and niosomal topical gel were evaluated for stability at room temperature and 40°C temperature for up to 3 months. Niosomal dispersion and niosomal topical gel both formulations were periodically assessed at 15 days, one month, two months, and three months period. This data suggests that there is no observable chemical or physical change in niosomal dispersion and niosomal topical gel formulations during the stability study. It also indicates that formulations are stable with the hope of longer shelf life.

This research indicates that the desoximetasone niosomal system has the potential to become an activity of interest for use in the pharmaceutical industry. Desoximetasone's anti-inflammatory properties can be applied to treat various skin conditions like itching, allergic reactions, eczema, and psoriasis. Since the various topical product of desoximetasone is approved by Food and Drug Administration and available in the United States market, desoximetasone niosomal system is not available in any drug delivery system for topical application, it has the potential to offer a new strategy to controlled release drug to avoid multiple application or reduce the drug concentration in the formulation. In the future work, investigating commercially viable scale-up of the desoximetasone niosome system and more extensive stability study to establish a longer shelf-life might expand the scope of this research. It will be important that future research investigates the combination therapy of desoximetasone with other corticosteroid agents to evaluate the potential benefit of combination therapy in different disease conditions such as rosacea, psoriasis, skin and soft tissue infection, etc. Additionally, studies could investigate the various strategies to improve desoximetasone delivery systems (topical cream, topical ointment, topical foam, topical spray, etc.) for the successful delivery of desoximetasone.