Gelled and Foamed Microemulsion-based Systems for Cutaneous Drug Delivery of Diclofenac Sodium

By

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Abstract

Topical formulations of diclofenac have become popular for treating various painful inflammatory conditions. Yet, not all formulations are suitable candidates for dermal delivery as the skin acts as a natural barrier that limits drug penetration. This barrier challenges the efficacy of topically administered medications. This dilemma drove the attention to the development of new topical drug delivery systems that enhance drug penetration without compromising its efficacy. Microemulsions (MEs) have gained interest from the pharmaceutical industry due to their ability to provide enhanced topical penetration of wide-ranging hydrophilic and lipophilic compounds. Accordingly, the first study aimed to develop ME based systems loaded with Diclofenac sodium (DS) and investigated their *in vitro* release performance in comparison to different marketed formulations. The second study was designed to develop, evaluate and *in vitro* compare the potential of microemulsion-based foam for improving the topical delivery of DS.

In the first study, an ME was prepared using caprylocaproyl polyoxyl-8 glycerides, diethylene glycol monoethyl ether, and propylene glycol monolaurate. An ME-based gel was developed to enhance the viscosity of the ME by using carbopol polymer as a gelling agent. The prepared formulations were subjected to different physiochemical stability testing and *in vitro* drug release using Franz diffusion cells. The drug-loaded ME and its gelled form were physio-chemically stable and had a cumulative release after 6 hr of 76.67 ± 8.63% for the former and 69.28 ± 7.14 % for the latter. This was statistically significant ($p < 0.0001$)

compared to different formulations. However, the high viscosity of the ME-based gel might put it in superiority for topical administration without dripping.

In the second study, foam-based MEs were prepared using caprylocaproyl polyoxyl-8 glycerides, polyglyceryl-3 dioleate, caprylic capric triglycerides, and water. The prepared formulations were studied for physiochemical stability, in-vitro drug release, foamability and foam stability. The formulated foamable systems exhibited good stability profile. The cumulative release profile of DS from the foam-based ME was the highest among the tested formulations 75.586 ± 9.074 % after 6 hr. According to the foamability and foam stability assessments, the foam generated from the DS-loaded ME had higher stability profile at room temperature and 32°C in comparison to the drug-free foam. Findings from the current research work suggested that the developed DS-loaded ME- based systems might be potential vehicles for enhancing the topical penetration of DS.

Preface

This thesis is an original work by Braa Mohammad Hajjar completed under the supervision of Prof. Dr. Raimar Löbenberg at the University of Alberta. Most of this thesis work was carried out at Dr. Löbenberg lab's facilities and Drug Development Innovation Center (DDIC). Some of the experiments were performed in different lab facilities at the University of Alberta.

Chapter 2 of this thesis has been published as Braa Hajjar, Kira-Isabel Zier, Nayab Khalid, Shirzad Azarmi & Raimar Löbenberg with the title of "Evaluation of a microemulsion-based gel formulation for topical drug delivery of diclofenac sodium" in **Journal of Pharmaceutical Investigation** *April 2017,10*

Dedication

This thesis is dedicated in memory of my father, Mohammed Hajjar, who had always loved me unconditionally and whose good examples have encouraged me to chase my dreams no matter where they take me.

To my mother, Najyah Hamdan, who made me believe that anything is possible. And to my beloved husband, Ammar Redwan, for making everything possible. Both of you were the light that kept me motivated throughout my life, in general, and on this journey, in particular.

To the one and only angle, my son Mohammed Redwan, you have decorated my life and made it full of hope, happiness, and joy.

To my brothers and sisters, your prayers, energy, passion, support, and, most of all, your love; to these I owe where I stand today.

To all the amazing students I have met and those I have not, who battle with dark days.

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List of Abbreviations

API	Active pharmaceutical ingredients
Cox	Cyclooxygenase
CVS	Cardiovascular
Da	Dalton
DETP	Diclofenac epolamine topical patch
DS	Diclofenac Sodium
DLS	Dynamic Light Scattering
DMSO	Dimethyl sulfoxide
f2	Similarity factor
FD	Foam density
FDA	Food and drug administration
FE	Foam expansion
Fig.	Figure
FIB/AAPS	International Pharmaceutical Federation/American Association of Pharmaceutical Sciences
FLS	Foam liquid stability
FVS	Foam volume stability
GI	Gastrointestinal
HPLC	High-performance liquid chromatography
HT	Heated temperature (32 °C)
IVRT	*In vitro* release testing
Log D	Distribution coefficient
Log P	Partition coefficient

ME	Microemulsion
NSAIDs	Non-Steroidal Anti-Inflammatory drugs
o/w	Oil/water
PBS	Phosphate-buffered saline
PDI	PDI Polydispersity Index
PLO	Pluronic Lecithin Organogel
PVDF	Polyvinylidene Fluoride
RCF	Relative centrifugal force
rpm	Round per minute
RT	Room Temperature
SC	Stratum corneum
SD	Standard deviation
Smix	Surfactant/cosurfactant
SUPAC-SS	Scale-Up and Postapproval Changes
%T	Percentage transmittance
TEM	Transmission electron microscopy
UV	Ultraviolet-visible
w/o	Water/oil
%w/w	Percentage Wight/Wight

Chapter 1

1. Literature review

1.Introduction

Centuries ago, people have applied substances on the skin for therapeutic purposes, currently, a variety of topical preparations have been developed to provide not only local therapeutic effect on diseased skin (topical delivery) but also for systemic delivery effect (transdermal delivery) (1,2). The use of topical drug delivery is advantageous compared to the other routes of administration, as it generally bypasses the first-pass metabolism of the liver and avoids the gastrointestinal tract (GI) for poorly bioavailable drugs (3). In addition to that, it's non-invasive, can be self-administered and provides greater patient compliance (4). On the other hand, as nonsteroidal anti-inflammatory drugs (NSAIDs) have a popular and widespread use for reliving chronic and acute musculoskeletal conditions, this gave rise to a number of topical (NSAIDs) including diclofenac, which has become popular for treating various acute and chronic painful inflammatory conditions (3,5). Topical diclofenac formulations have significantly lowered the systemic exposure associated with oral diclofenac. Although topical diclofenac preparations have generally been shown to be relatively safe for years, more recently, some significant safety risks have been observed such as hepatic, cardiac, renal and gastrointestinal toxicity (6). Since then, the overall safety and efficacy of topical diclofenac formulations remain controversial (7). The main reason behind such conflict is probably due to the fact that topical formulations are complex systems and have different release mechanisms. Even with their invention several centuries ago, the dynamics of the drug release from these preparations is still the focus of pharmaceutical investigation (8).

Generally, when an active substance is applied topically, it must be released from its vehicle first before contacting the skin surface and then to be available to penetrate the stratum corneum (SC) and later lower layers of the skin (9). However, in order to acquire more information regarding the way a compound will interact with and permeate through the skin,

it was of great importance to obtain the most relevant data needs from *in vivo* in humans such as the drug absorbance, the apparent steady-state levels, and the drug elimination based on an SC concentration-time curve (10). Yet the process of obtaining *in vivo* data is expensive, time-consuming, has a wide biological variability and require "meeting with ethical approval" (11). Therefore, there has been an increased interest in developing *in vitro* testing techniques which are simple, reproducible and generally applicable to all topical dosage forms and mimic *in vivo* conditions as closely as possible so that the yielded results can then be extrapolated (8).

Diffusion cells are a widely-used technique for release-rate testing of topically administered products. This test method is designed to produce meaningful and relevant data for these dosage forms and produce a release profile for the pharmacologically active drug substance from the formulation matrix as a function of time. Which then allow assessing the general performance of products and in our case topically applied diclofenac formulations (12). Therefore, this thesis was aimed at summarizing the performance of topical diclofenac preparations and some advances in topical diclofenac drug delivery with an emphasis on the *in vitro* release testing.

1.1. Diclofenac

Diclofenac is one of the most potent and commercially successful worldwide NSAIDs that has been extensively used for many years for treating mild to moderate pain and inflammation (13,14,15). Orally, rectally and intramuscularly administered diclofenac around 0.25 to 0.5 mg/kg in children and approximately 50 to 150 mg/day in adults ensure good analgesic and anti-phlogistic effect (16).This relates to the drug concentration in the plasma, or tissue to be treated, of about 0.5 µg/g tissue (17). The absorption of diclofenac in the

gastrointestinal tract is usually offset by the first-pass hepatic metabolism of ~ 50% besides possessing short biological half-life (~ 2h) (18). Even though, oral preparations of diclofenac are the most frequently used, and extensive clinical application for many years has demonstrated their effectiveness in relieving pain (13). Nevertheless, the main drawback of utilizing oral diclofenac is not the insufficient bioavailability nor the short biological half-life but rather the severity of adverse reactions, affecting the gastrointestinal tract (gastrointestinal bleeding, small bowel injury, upper and lower gastrointestinal harm), acute renal complications and cardiotoxicity (19). Such side effects can be troublesome and might trigger great discomfort for the long-term use, and that may necessitate discontinuation of the drug usage and may worse, lead to hospitalization and mortality.

Therefore, orally administered diclofenac is considered to be poorly tolerated. For this reason, advances in pharmaceutics have extensively been focused on the development of options to address these serious adverse effects. The concentrated have been on modifying the pharmacological properties of diclofenac and, developing novel modes of delivery (15). The goal was to reach the therapeutic drug concentration in the targeted tissue while simultaneously maintaining the systemic concentrations as low as possible. For that, topical applications of special diclofenac preparations were introduced (17). However, understanding the structure and function of the human skin is fundamental for designing optimal topical diclofenac dosage forms (20).

1.2. The Skin Barrier

The human skin is the largest organ of the body with an area of nearly 2 m^2 (21). The primary function of the skin is to protect the body from water loss and the entrance of potentially toxic compounds, allergens, and microbes. It comprises the epidermis (about 100 μm thick nonvascular layer), the dermis (about 500 to 3,000 μm thick highly vascularized layer) and the underlying subcutaneous tissue, that contains sebaceous and sweat glands. The outermost layer of the epidermis is known as the stratum corneum (SC) and is 10-40 μm thick. This layer act as the main barrier to skin penetration and permeation by topically applied administered formulations. It consists of dead, partially desiccated, and keratinized epidermal cells. The SC employs its greatest barrier function against hydrophilic compounds, and this is due to its exceptional lipid composition that involves long chain ceramides, free fatty acids and cholesterol (22,23). On the other hand, the viable epidermis is mostly resistant to highly hydrophobic compounds (24). As the different layers consist of cells with varying compositions and functions, accordingly, the different layers have different water content, which may be as low as 15% in the SC and as high as 70% at the dermis. Therefore, hydrophilic molecules tend to remain on the surface of the skin while the lipophilic ones tend to accumulate at the SC and therefore will not provide the desired pharmacological action (25).

The structure of the SC is believed to be an analog to a brick wall, where the corneocytes creating the bricks, and the extracellular lipid arranged into lamellar lipid bilayers for forming continuous lipid phases are the mortar (26,27). Nevertheless, the trans-appendageal route might be vital for ions and large polar molecules that usually permeate slowly through the SC. The epidermal route, on the other hand, offers two potential micro-pathways through the SC: transcellular route and intercellular route (Figure 1-1) (28). Polar or hydrophilic

compounds usually travel through the transcellular route, while hydrophobic molecules move along the intercellular route. The latter route might be the primary pathway of entry for most drugs; however, it is also the major barrier to drug permeation(29). Assuming that the intercellular pathway is predominant, factors influence the movement of a drug into and within this environment are of utmost importance.

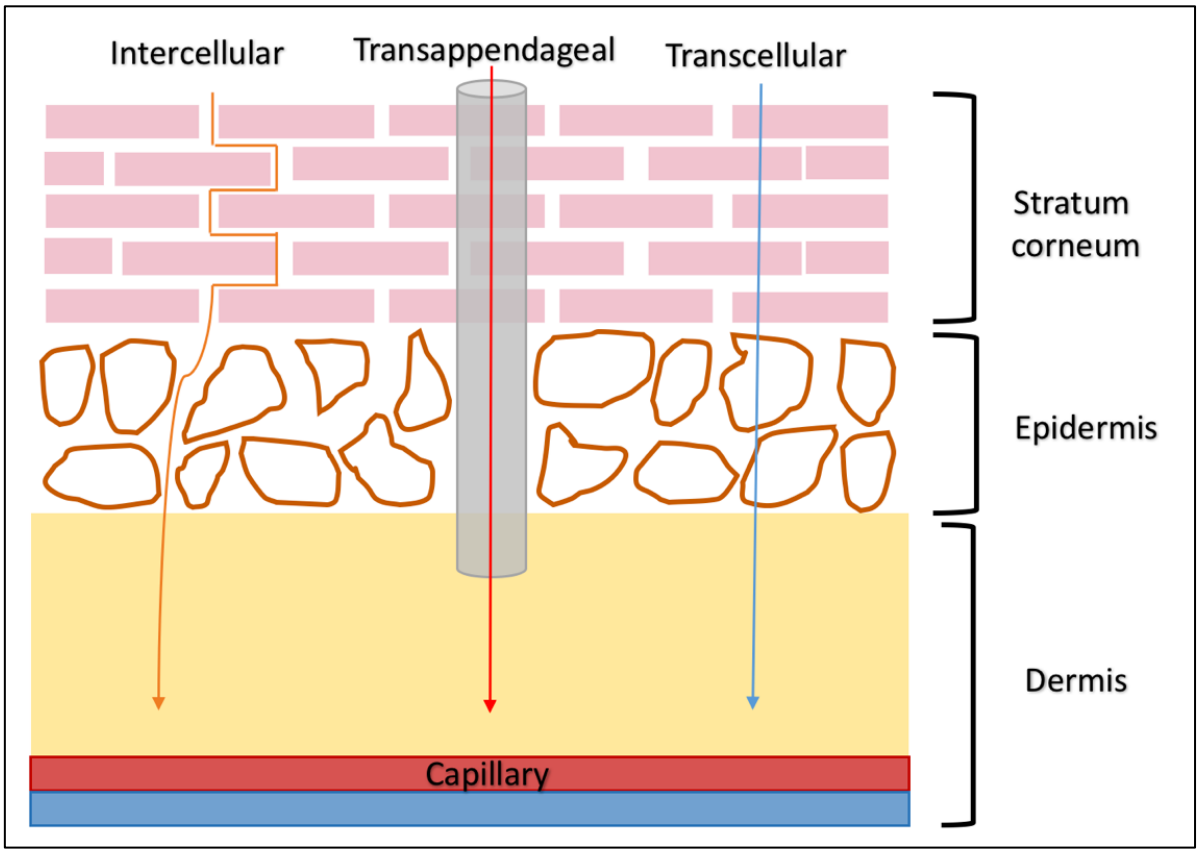


Figure 1-1 Potential micro-pathways of topical drug through the brick and mortar model of the SC. This non-living layer locates on the outer surface of the skin and consists of keratinized cells surrounded by a lipid-rich extracellular medium that acts as the primary barrier on the skin. The underneath epidermis is a viable tissue devoid of blood vessels. The dermis, which is located beneath the dermal-epidermal junction, contains capillary loops that have the ability to uptake drugs into the systemic circulation. Reproduce from ref (30).

1.3. Principles of Topical Drug Use

The topical application of medications is one of the oldest drug delivery routes. Recently, applying medications topically has become increasingly popular due to their various advantages. One of which is that they can achieve efficacy similar to oral preparations, due to their capability to accumulate locally, and maintain therapeutic concentrations with significantly lower systemic drug exposure, which reduces the risks of developing systemic adverse effects (31). Other advantages include localization by direct access to the site of action, the option of prolonged use since it involves avoiding extensive first-pass metabolism, ease of use which might enhance patient compliance and adherence to the prescribed regimen, especially for those who cannot use oral medications (32). To be effective, topically applied drugs, such as diclofenac, should penetrate the skin and pass from one tissue layer to the next. Usually, most topical agents are unable to achieve this if administered alone. Nevertheless, if the drug was part of a formulation, then, the drug permeation process involves a series of steps that start with the release of the pharmaceutically active ingredient from the dosage form. Followed by the diffusion into and through the layer of SC, then partitioning to the more aqueous epidermal layers and diffusion to deeper tissues or uptake into the blood vessels within the skin to the systemic circulation (Figure 1-2) (20). These processes are extremely reliant on the solubility and diffusivity of the drug in each environment. The release of the permeant from the topical vehicle and the uptake into the SC is determined by the relative solubility in each environment, and hence the stratum corneum – vehicle partition coefficient.

The process of drug partitioning depends on many factors related to the environment anddrug properties. Many studies have shown that increasing drug lipophilicity increases the skin

permeation. Very lipophilic agents will have high solubility in the intercellular lipid domains, which will influence the diffusion coefficient within the stratum corneum and thus increases the flux (33,34). Nevertheless, the high lipophilicity might hinder drug partitioning to the more aqueous epidermis, limiting skin permeation rate. Thus, increasing the skin hydration is a necessity to increase the compound permeability. Indeed, water is considered as a natural skin penetration enhancer in topically applied formulations (35-37). The skin permeation rate is also determined by the concentration of soluble drug within the vehicle, as the solubility of the drug in a vehicle might influence both the drug concentration gradient and the partition coefficient between the vehicle and the skin. Hence, if a lipophilic permeant has limited solubility in a topical vehicle, the permeant might partition into the SC, resulting in depletion in the vehicle and so that might reduce the drug flux across the skin. Therefore, the ideal compound requires lipid solubility with reasonable aqueous solubility to maximize flux (20).

The size of the permeant will also affect the diffusivity within the SC where It has been proven that there is an inverse relationship between the size of the permeant and its skin permeation profile. Generally, drugs selected for topical delivery tend to be less than 500 Da (38,39). Thus, it is of great importance to note that large molecules might not be good candidates for topical drug delivery (20). The degree of drug ionization is also another factor that might influence a drug permeation. Unionized agents, predominantly, diffuse through the lipophilic intercellular domains in the SC. Whereas, the ionized drug molecules might permeate, but in a slow and low mode (40).

Therefore, for developing an ideal topical drug delivery, drugs are required to be of a low molecular weight, possess hydrophobic characteristics for traversing the SC, have sufficient hydrophilicity to traverse the aqueous epidermis, and have low ionization degree.

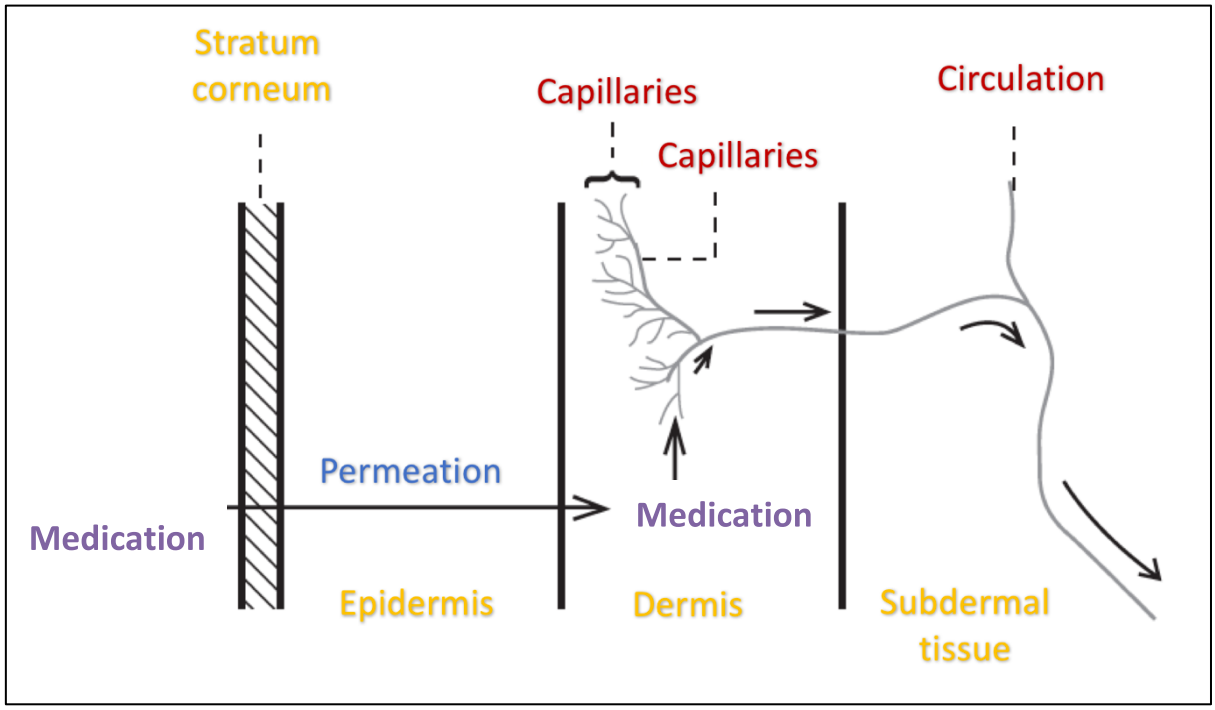


Figure 1-2 The transport process of topically applied drugs through the skin into the surrounding tissues and/or circulation. Reproduced from ref (30).

1.4. Diclofenac physicochemical properties

The physicochemical properties of diclofenac are the primary factors that influence its absorption after topical application (125). Normally, the lipophilic SC has a pH of 4.2-5.6, while that of the hydrophilic epidermis is ranged between 7.3–7.4 (126). Moreover, as previously mentioned, very hydrophilic drugs are unable to penetrate the skin, while very lipophilic drugs have the propensity to remain in the SC. Therefore, drugs with biphasic (water and lipid) solubility have better skin permeation ability than those with high monophasic (water or lipid) solubility (125). Diclofenac is hydrophobic, while its salts are water soluble at neutral pH, which imply its partially soluble in aqueous and hydrophobic environments (figure 1-3). The lipophilicity of diclofenac at lower pH would enhance its penetration through the SC, on the other hand, its higher hydrophilicity at higher pH would increase its ability to diffuse through deeper skin layers.

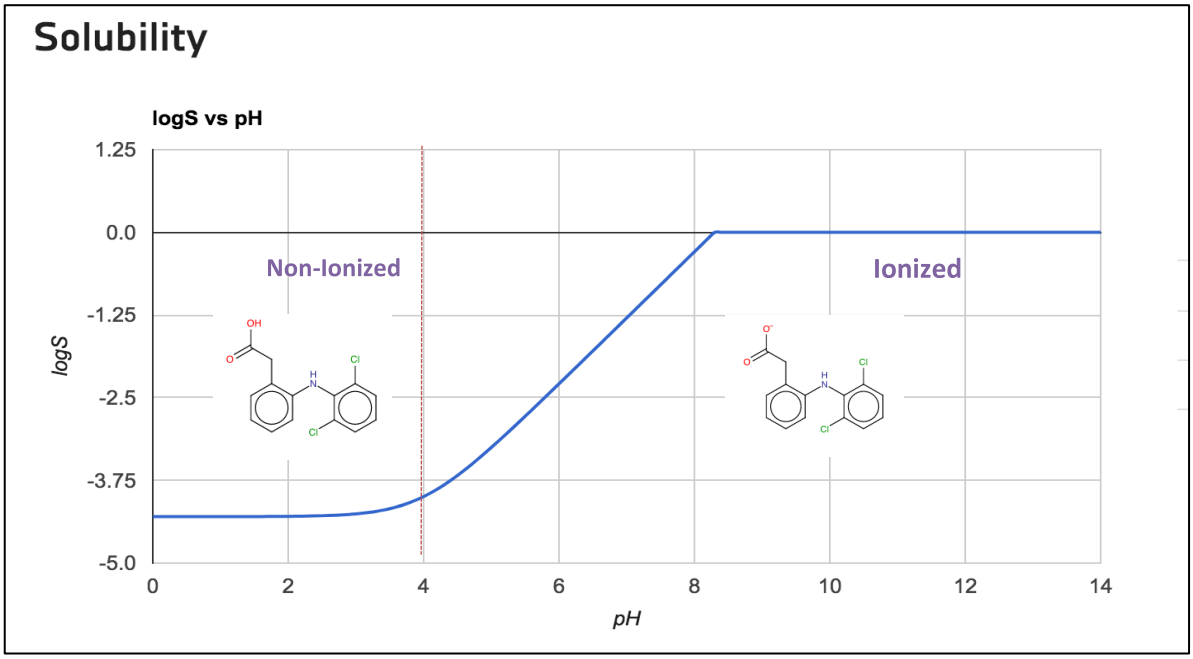


Figure 1-3 The representative Diclofenac Solubility as a function of pH. As the pH increases the ionized diclofenac increase its solubility. The drug solubility is shown in logarithmic form where logS is the solubility 10-based logarithm measured in mol/l.

As an organic acid, diclofenac is a phenylacetic acid with a pKa value of 4, and it can exist in three different protonation states (Figure1-4), the predominant existence of the neutral form is usually at pH ≤ 4, while the anionic form is at pH ≥ 4. The marginal existence of the cationic form usually found to be at pH < 0.5. Such distribution indicates that diclofenac is ionized at skin pH. The lipophilicity of the SC had led to the idea that ionized compounds would perform poorly for cutaneous delivery; thus, the ionization of diclofenac might hinder its penetration through the SC (125). Generally, penetration of the SC requires that a drug partitions into the membrane. Such partitioning is a crucial step in the penetration of the skin. The distribution coefficient (log D) value, which is the partition coefficient (log P) value at a specific pH of a drug is usually a good indication as to whether a compound lipophilicity would be favorable for skin permeation or not. Normally, the higher the log P value, the more

lipophilic is the compound. Drugs with a log P less than −1 would, thus, have difficulty in passing from the vehicle into the SC. Subsequently, only drugs with log P higher than −1 should be considered for cutaneous delivery (125). However, it has been reported that molecules with a log P ranged between 1–3, would exhibit both aqueous and lipophilic properties that are sufficient enough to achieve proper cutaneous permeation, as they can pass the lipophilic (SC) and hydrophilic layers (epidermis) of the skin (127).

Diclofenac was found to have a log P of 4.26-4.51 (128,129). However, the log D was found to be 3.7-1.1 at the pH ranged between 4.2 and 7.4 (Figure 1-5). The high lipophilicity of diclofenac at lower pH indicate that this compound would have partition coefficient good enough to be efficiently delivered across the lipophilic SC layer. In contrast, the low hydrophobicity at higher pH then would help the drug to pass through the deeper hydrophilic skin layers.

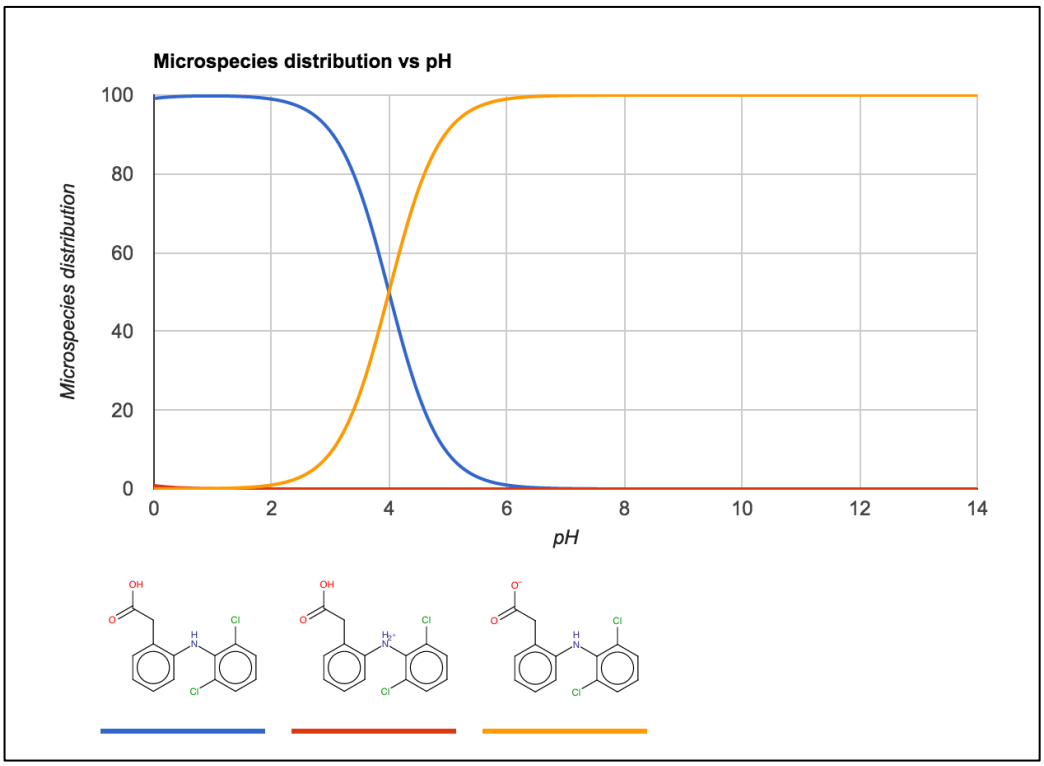


Figure 1-4 The representative pH-dependent distribution of diclofenac micro-species.

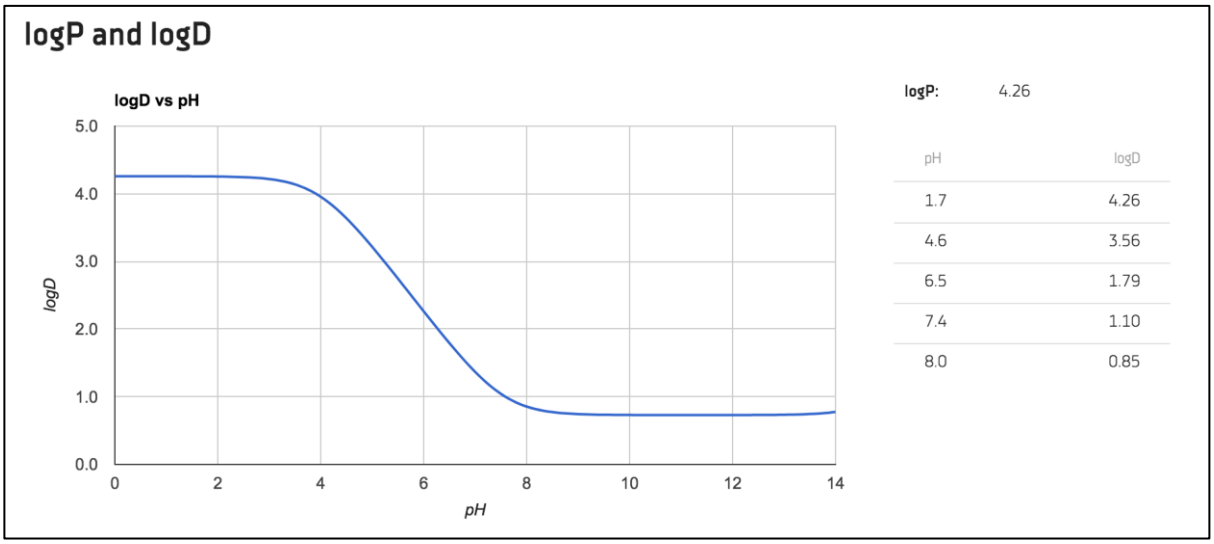


Figure 1-5 The representative lipophilicity of diclofenac as a function of pH

1.5. Topical Administration of Diclofenac Formulations

Topical diclofenac formulations were developed with the aim of treating local pain and inflammation by reducing the systemic exposure of diclofenac, which potentially minimizes the risk of adverse reactions. As an organic acid with pKa value of 4, a Log P of 4.26 and a molecular size of 296 Da, the combination of these properties renders diclofenac to penetrate through the synovial lining of joints and the skin (15). Pharmacokinetic studies revealed that diclofenac has a high rate of transdermal penetration and it is absorbed continuously across the skin to a depth of at least 3–4 mm throughout the underlying dermis and subcutaneous tissue. At that level, the uptake of diclofenac from the dermal microcirculation into the systemic circulation does occur (13). Yet, many pharmacokinetic studies confirm that diclofenac preferentially distributes in the local tissues, which leads to higher concentrations in these areas. After topical application, approximately 119–3320 ng/mL of diclofenac was

found in synovial fluid while around 131–1740 ng/g was found in synovial tissue. These figures are up to 20 times higher than the plasma 6–52 ng/mL, and also higher than those seen for other NSAIDs; suggesting that topically applied diclofenac reaches the target tissues (soft tissue/joint) at seemingly sufficient concentrations to exert a therapeutic response (41). Furthermore, diclofenac is found in significantly higher concentrations in inflamed tissues in comparison to non-acidic NSAIDs such as aminopyrine and mepirizole, which distribute almost equally throughout the body (42).

Evidence suggests that the different formulation of a topical diclofenac dosage form might notably affect its pharmacokinetic properties. Nevertheless, building on its good permeation properties along with the strong anti-inflammatory effect when applied topically, several diclofenac preparations in different salts (sodium, potassium, epolamine, and diethylamine) have been developed for topical delivery, including gels, solutions, creams, sprays, lotions and patches (43,44).

1.6. Diclofenac Formulations in The Market

Lately, topical diclofenac formulations have been widely employed as a therapy for many musculoskeletal disorders (44). Several topical diclofenac preparations are available as prescriptions and over the counter (45). Some of the formulations that have been extensively used are topical sodium gel 1%, which was approved by the FDA in 2007 for relieving the pain of osteoarthritis amenable for topical treatment such as the knees and hands, topical sodium emulgel 1% (45). Topical diclofenac diethylamine gel 1.16% and the extra strength 2.32% are also available for relief pain related to (acute), localized muscle or joint injuries. Similarly, topical diclofenac sodium solution 1.5% and 2%, containing dimethyl sulfoxide (DMSO) as solvent, are also available in the market and are indicated to treat signs and

symptoms of osteoarthritis of the knee. The diclofenac epolamine topical patch (DETP) 1.3% was FDA approved in 2007 for the topical treatment of acute pain caused by minor sprains, strains, and contusions (30,46). A formulation of diclofenac sodium gel 3% is also available but is indicated only for short-term treatment of actinic keratosis (47). Additionally, some countries, such as the United Kingdom, Ireland, and Hungary, have approved the use of cutaneous diclofenac sodium spray gel 4%. Italy, however, is the only country which has produced diclofenac 3% cutaneous foam (48-50).

As the pharmaceutical industries keep developing and enhancing topical diclofenac preparations, the safety and the efficacy of these formulations are skeptically evaluated. Nevertheless, few studies have proven the superiority of some of the topical diclofenac dosage forms in terms of safety and efficacy in comparison to placebo, oral diclofenac or other topical NSAIDs (51).

1.7. Topical Diclofenac Efficacy

1.7.1. Topical diclofenac vs. oral NSAIDs

Regarding efficacy, a comparative effectiveness review of analgesics has shown that topically applied NSAIDs (with diclofenac being the most studied) seem to have a similar efficacy profile to oral NSAIDs in pain relief, mostly in trials with patients with knee osteoarthritis (52). In the five-arm study that was conducted by Simon *et al.* with 775 patients for a 12-week period, patients were randomly assigned to either diclofenac in DMSO solution, DMSO vehicle, oral diclofenac, a combination of topical and oral diclofenac, or a control group. It was found that the topical diclofenac solution was superior over the placebo solution and the DMSO vehicle in terms of efficacy outcomes (physical function, pain, and

patient global assessment), and there was no significant difference in the efficacy between the topical and the oral diclofenac. Additionally, the combination of topical and oral diclofenac did not show any improvement in the outcome evaluations (71).

1.7.2. Topical diclofenac vs. placebo

Some studies have confirmed that the tested topical diclofenac preparations are superior to placebo for pain relief. Niethard *et al.* compared 1.16% diclofenac diethylamine gel to placebo gel and found some improvements in efficacy endpoints that peaked at week 2, and maintained up to the 3rd week of the treatment (54). Bookman *et al*. also showed a significant improvement in knee osteoarthritis treated with 1.15% diclofenac sodium in a carrier containing DMSO, compared to a carrier containing DMSO alone, as well as to a placebo solution containing a token amount of DMSO for blinding reasons. The topical diclofenac arm exhibited an improvement of about 43 % for pain 40 % for stiffness, 39 % for physical function, and almost 45% for pain on walking (55). Additionally, Roth *et al. did* show that the improvements yielded with topical diclofenac solution sustained for up to 12 weeks in comparison to the vehicle control arm (53).

1.7.3. Topical diclofenac vs. other topical NSAIDs

When compared with other topical NSAIDs, data from single-blind and non-randomized studies suggested moderate evidence that diclofenac diethylamine gel is just as effective as indomethacin gel for pain relieving from mixed rheumatic disorders. It was also indicated by some single-blinded trials that the effectiveness of diclofenac diethylamine gel in relieving pain associated with knee osteoarthritis is similar to that of ketoprofen gel, piroxicam gel and diclofenac spray (one trial each) (13).

1.8. Topical Diclofenac Controversy

Although some studies have confirmed that topical diclofenac formulations are more effective in relieving acute and chronic pain than the placebo, oral diclofenac, and other NSAIDs, many other studies did contradict these findings. In a randomized, double-blind, placebo-controlled crossover study, Burian *et al.* found that the overall pain relief over in a well characterized experimental model of cutaneous inflammation in humans was 1.7-fold greater with oral diclofenac than with topical diclofenac (56). In a meta-analysis of randomized controlled trials, Lin et al. revealed that the reduction in pain, the improvement in function and stiffness showed the superiority of the topical NSAIDs including diclofenac over placebo in the first two weeks, however, not in the following two weeks. Topical diclofenac was inferior to oral NSAIDs in the first week of treatment, and no difference was detected in the clinical response rate ratio between topical diclofenac and oral NSAIDs (57). Radermacher *et al.* have compared a diclofenac gel with placebo gel through a double-blind, randomized, placebo-controlled trial for patients with an inflammatory arthropathy of both knees, using a different agent on each knee and it was found that there is no significant difference in the improvement between knees in clinical parameters (58). The different outcomes of the trials could be due to the inter-individual variability of skin properties that might influence the percutaneous absorption and distribution of a topically applied drug.

The therapeutic effects of topically applied medications are known for their dependence on the rate, amount, and depth of the drug penetration into the skin(43).These factors may play an important role in the variability of both analgesic response and systemic drug exposure (59).

1.9. Compounded Diclofenac Preparations

Despite the availability of many marketed topical diclofenac formulations, compounding pharmacies began to provide non-commercially available strengths in different semisolid bases as physicians began to request more amounts of drug, and prescribed 3%, 5% and in some cases 10%, or more of topical diclofenac preparations (60-62). The anecdotal motive behind the increments was the belief that higher doses would improve pain relif, or that the prescribed dose did not effectively reduce pain and thus more drug was needed to reach the desired compliance. The higher amount of diclofenac in compounded transdermal medications was believed to relive pain. Additionally, it was assumed that compounded topical formulations might avoid some of the challenges associated with commercially available preparations, such as being customizable to a specific patient needs, which might improve patient compliance, and permit for less frequent dosing (63). However, it has been demonstrated in literature reports that the best-known marketed topical diclofenac formulation (Voltaren Emulgel), has only 6.6 % bioavailability when applied to the skin, which might necessitate combination with diclofenac tablets to reach a therapeutic effect. Based on that, compounding pharmacies have developed various recipes for extemporaneous preparation of topical diclofenac dosage forms that consist of different strengths in different pharmaceutical bases such as Pluronic Lecithin Organogel base (PLO), Versapro gel base, Lipoderm base or Pentravan cream base (64-68). In some cases, diclofenac might be included with multiple analgesics in a single compounded formulation as an extra strength effect (68).

Even though these formulations have been available for prescription for a while, however, such preparations are rarely evaluated for bioavailability and objective clinical endpoints (59). Lately, a review of published literature revealed the lack of data offering guidance on

the stability of extemporaneously prepared NSAIDS for topical application. Plus, to our knowledge, no published study so far has inspected the efficacy *in vivo*, and the extended stability of any of the available compounded topical diclofenac formulations, except of PLO gel, and there have not been any studies correlating the chemical and physical stability of diclofenac to its topical penetration ability (64,70). Limited *in vivo* studies suggested that PLO might be beneficial as a delivery vehicle for local action. In these studies, diclofenac-loaded in PLO gel was applied 3 times/day to the affected areas for two weeks to treat osteoarthritis knees and one week to treat lateral epicondylitis. Following application, patients experienced less pain. However, drug levels in blood were not measured, and drug absorption into patients' systemic circulation could not be assumed. Generally, research on compounded topically applied diclofenac dosage forms does not prove the presence or absence of their efficacy. Some evidence has revealed the significant variation between the stated potency of a compounded product and the actual ingredients (68). Thus, in all cases, as long as there are no regulatory requirements, or enough research addressing the safety and efficacy of topically compounded diclofenac, patients must be aware that these products may carry some risks (69).

1.10. Safety of Topical Diclofenac Formulations

Most patients using topical diclofenac preparations as a treatment for musculoskeletal disorders are likely to use them over the course of many weeks and, with the rise in the number of topically applied diclofenac preparations, it was of great importance to gain an understanding of their long-term safety profile (51). In most clinical trials using topical diclofenac, cutaneous, gastrointestinal (GI), cardiovascular (CVS), renal and laboratory parameters have been scrutinized for rates of side effects. Placebo controlled studies of topically applied diclofenac in several musculoskeletal disorders found that a considerable

number of patients did develop local skin adverse reactions (dryness, rash, and pruritus), but not GI, CVS, or renal reactions (51). The most common adverse event reported in various studies regrading topical diclofenac was dry skin at the application site. Simon et al. evaluated topical diclofenac with DMSO in comparison to topical placebo, a topical vehicle with DMSO, and oral diclofenac in a knee osteoarthritis population and reported 45.1 % cutaneous adverse reactions involving dry skin, contact dermatitis and contact dermatitis with vesicles. This is probably caused by the higher amount of DMSO incorporated into the topical formulations (71).

Nevertheless, several studies have reported systemic side effects associated with the use of topical diclofenac preparations. Tugwell *et al.* compared topical diclofenac solution with oral diclofenac of the treatment of knee osteoarthritis. It was found that topical preparation users developed GI adverse events, which included abdominal pain (12%), diarrhea (9%), dyspepsia (15%) and nausea (8%). Additionally, some of the patients in the topical treatment group had developed irregularities in the hepatic transaminases, hemoglobin, and renal function (72). Niethard et al. assessed the efficacy and safety of 1.16% diclofenac diethylamine gel compared to placebo gel. He reported two GI adverse reactions in the topical diclofenac group compared to zero in the placebo group (54). Shainhouse et al. conducted an open label study on topical diclofenac in DMSO vehicle as a treatment of knee osteoarthritis and reported GI events in 12% of the patients; these side effects included gastroesophageal reflux, nausea, dyspepsia, diarrhea, abdominal pain, and liver function test abnormalities. GI bleeding was developed in 8 patients (1%) and some CVS events were reported in 9.1% of patients (angina, palpitations, myocardial infarction, arrhythmia, venous thrombosis, and hypertension) (73). In patients with hand osteoarthritis in a trial, Zacher et al. compared the safety of topical diclofenac to oral ibuprofen and found a comparable rates of overall side effects (22% *vs.* 27%) for topical diclofenac and oral ibuprofen, respectively. The

topical diclofenac group reported GI adverse events in 9% of the patients versus 14% in the oral group (13). Zimmerman et al. also reported a case series of four patients with severe GI bleeding after initiating topical diclofenac emulgel. These cases were among 110 patients admitted for upper gastroesophageal hemorrhage to a single hospital in one year. Two of the patients used the topical diclofenac incorrectly for back pain. The remaining two had preexisting history of GI ulcer disease before applying the topical diclofenac gel (74). Another serious CVS side effect, which was also considered to be possibly treatment related was reported by Baraf et al. This trial was conducted assessing topical diclofenac sodium 1% gel. In this study, one patient had experienced deep vein thrombosis and pulmonary embolism. (75).

Therefore, since systemic absorption of diclofenac from topically applied formulations has been documented in many studies, caution should be exercised when employing these formulations, especially in patients with a history of GI and CVS diseases.

1.11. Obstacles with Topical Diclofenac

Owing to the shortage in studies evaluating the safety and efficacy of marketed and compounded topical diclofenac formulations, and based on the currently available data from the existing literature, it can be said that the reported efficacy profiles of topically applied diclofenac preparations varied among different topical dosage forms. However, the safety profile of some of the tested formulations, mainly diclofenac in solution vehicles, involve a wide range of adverse reactions that varied from minor cutaneous side effects to few but severe GI and CVS reactions.

The recommended dose of topical diclofenac, according to the commonly prescribed strength 1% diclofenac sodium emulgel, is 2- 4 g of gel for a normal application at one location. The total dose should not exceed 32 g per day over all affected joints. For lower extremities, 4 g gel 4 times daily and not more than 16 g daily to any one affected joint of the lower extremities is recommended. For the upper extremities 2 g 4 times a day and not more than 8 g daily is recommended. Furthermore, it should only be used at the lowest dose possible and for the shortest time needed. Even with the pre-existing knowledge regarding the toxicity of diclofenac, higher concentrations of this drug, up to 10 %, are being requested, formulated, and dispensed in different topical formulations to different age groups (Table 1-1), and thesest might be at risk to develop, or already suffering from, GI and/or CVS (60-62).

Table 1-1 Comparison of diclofenac sodium concentrations in mg using the recommended doses of the manufacturer in Bold and of compounded preparations. Doses in red exceed the recommended daily dose of 32 g

Diclofenac formulation	Diclofenac in mg after single application using 2g	Diclofenac in mg after single application 4g	Upper recommended concentration using 32g gel	lower extremities 4g @ 4 times	upper extremities 2 g @ 4 times
1%	**2**	**4**	**32**	**16**	**8**
5%	10	20	160	80	40
10%	20	40	320	160	80

This situation raises the question of why higher amounts of diclofenac are requested and formulated in topical formulations? Also, why side effects are not seen after administering higher concentrations?

One possible justification is the prepared diclofenac formulations did not efficiently deliver the drug to the site of action. Otherwise, by increasing the drug doses, the aimed efficacy should be met and/or an increase in the side effects should be detected. It is known that the ability of a topically applied drug in a formulation to penetrate the skin and exert its effect depends on the diffusion of the drug out of the vehicle to the skin surface, and then penetrating the skin's natural barrier to reach the site of action, or becoming systemically available (76). Also, it has been reported by many authors that a topical vehicle greatly influences drug percutaneous absorption (77). Accordingly, it has been documented in many studies that differences in diclofenac formulations may greatly impact its absorption and penetration through the skin (59). This scenario probably explains the poor performance of some of the currently available topical diclofenac preparations. Thus, the problem that needs to be addressed is how to enhance the skin penetration of topical diclofenac formulations?

1.12. Advances in Topical Diclofenac Formulations

In an attempts to overcome the problem related to low skin penetration ability of some diclofenac cutaneous formulations, advances in pharmaceutics have been focused on the development of novel modes of drug delivery, with the main goal of enhancing its indications and improving its tolerability (15). Various permeation enhancement technologies have been studied to improve skin penetration of diclofenac after topical application for years. One of which is applying some chemical modifications to increase the therapeutic efficacy of topical diclofenac. Employing suitable salt form(s), epolamine salt of diclofenac is one example of such modification. This salt has an improved solubility in both water and organic solvents, facilitating epidermal penetration (15).

Another popular technique is using penetration enhancers that reduce the permeability barrier of the SC, such as dimethyl sulfoxide, and ethanol, that have been suggested to enhance the permeation of diclofenac through the skin, yet their significant cutaneous side effects were problematic and limits their use (78). However, the excellent barrier function of the SC restricts the rate and extent of topical drug delivery, for that, the focus has moved to the development of new technologies that reversibly impair barrier function. Several approaches to ablate the layer of SC have been studied; including microneedles by way of mechanical ablation, or by employing laser-assisted microporation to form transport channels in the skin and enable controlled enhancement of diclofenac delivery. There are also more sophisticated energy-based strategies to selectively remove or enter the SC, such as using radiofrequency high-voltage currents based on an ablation of outer layers of skin to facilitate diclofenac penetration (79,80).

Despite the potential for topical delivery of diclofenac and the advancements in fabrication technologies, these techniques often pose problems in the delivery of accurate dose administration and patient compliance. Besides, the concerns regarding the possible damage to skin and instrumentation costs are challenging factors to prove the clinical benefits of these systems (81). On the other hand, efforts have been made in another approach related to the modification of the formulation (vehicle) that acts as a key in the percutaneous absorption. Recently, vesicle and particulate systems, such as liposomes and nanoparticles, have been investigated as vehicles for the dermal absorption of diclofenac, with a surge in the development of microemulsions (82).

1.12.1. Microemulsions

The concept of a microemulsion was first introduced in the 1940s for describing a transparent single-phased system, that was generated through titrating a milky emulsion with hexanol (83). Since then, microemulsions have been extensively studied as a potential topical delivery system for their multiple advantages over conventional formulations. These systems have been gaining attention due to their thermodynamic stability, excellent biocompatibility, and capability to incorporate hydrophobic and hydrophilic drugs of varying solubilities (84). MEs seem to be a promising carrier system for topical diclofenac delivery, as they offer additional advantages compared to other vehicles, for example, spontaneous formation, droplet range of 0.1-1 μm, long shelf life, high solubilization capacity, and suitability for large-scale production (85). In principle, these systems are known for their clarity, optical isotropicity, and the ability to be sterilized by filtration (86).

1.12.2. Microemulsions structure

In general terms, MEs are nano-sized dispersions of water phase and oil phase that are stabilized by a surfactant, which usually is conjugated with a cosurfactant (Figure1-6). The rationalization of the thermodynamic activity of the MEs are explained by the ability of a surfactant/cosurfactant mixture to lower the surface-tension of the oil-water interface (87). Different self-assembled types of microemulsions are in existence, according to the composition, ratio among components and arrangements of the component's molecules present: oil-in-water microemulsion (O/W), in which oil droplets (oil phase) are dispersed in water phase; water-in-oil microemulsion (W/O), in which water droplets (aqueous phase) are dispersed in oil; and bi-continuous microemulsion, in which aqueous and oil phases are

intertwined, and both are stabilized by sheet-like surfactant regions in the boundary regions between the two phases (Figure 1-7) (88). The O/W microemulsions are most commonly employed for improving the solubility of poorly water-soluble drugs such as DS and that might enhance its penetration ability through different skin layers (89).

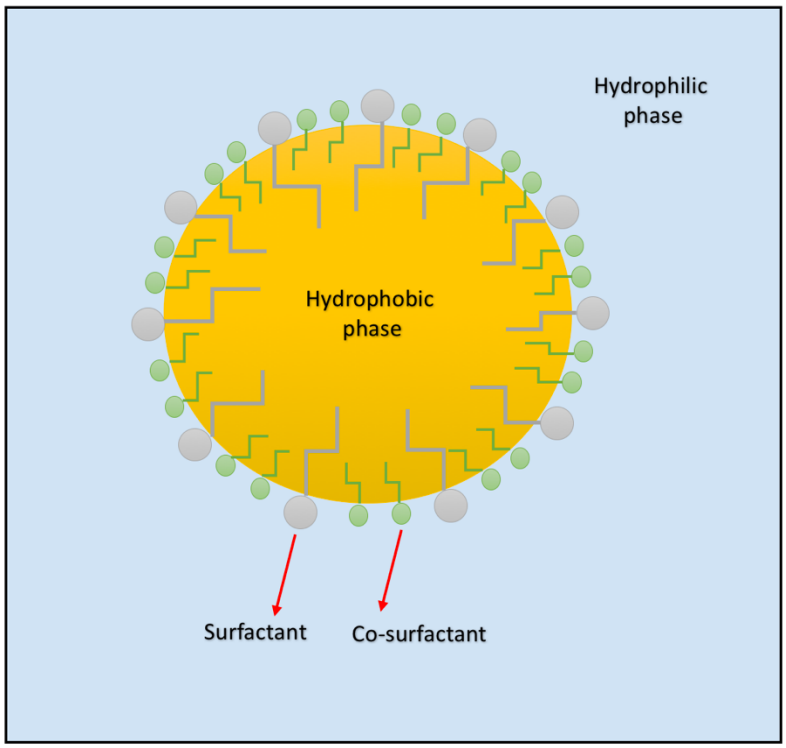


Figure 1-6 Schematic diagram of a surfactant stabilized O/W microemulsion droplet fabricated from oil, water, surfactant, and cosurfactant.

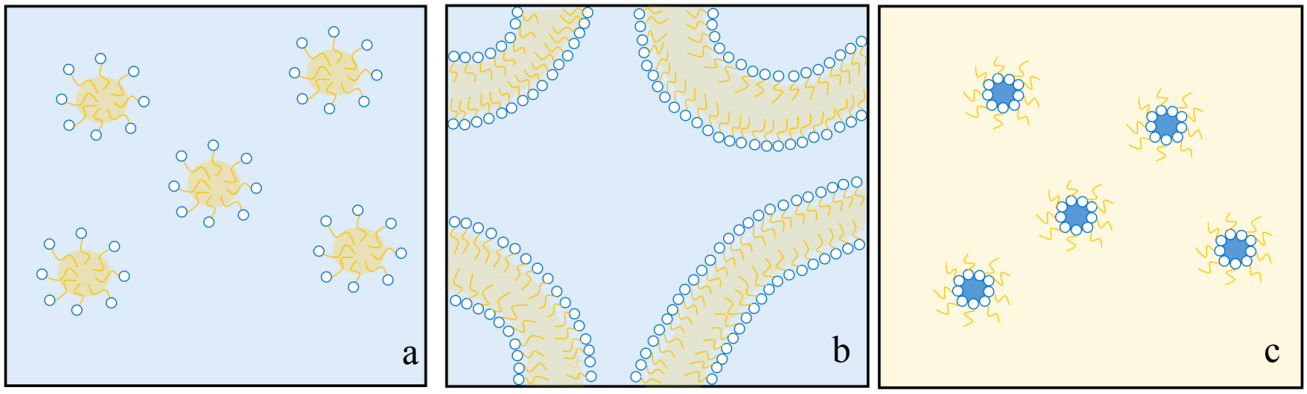


Figure 1-7 Schematic representation of the microemulsion microstructures: (a) oil-in-water ME, (b) bicontinuous ME, and (c) water-in-oil ME.

1.11.1.2. Components of Microemulsion formulations

The most significant issue associated with formulating MEs is the difficulty of selecting the appropriate excipients. Many oils and surfactants are available to be used as components of ME systems for dermal delivery, but their toxicity and irritation potentially limit their use. It must be borne in mind that the selected materials should be biocompatible, clinically acceptable, and their mixture result in MEs that are generally regarded as safe (87). The criteria by which oil phase must be selected for topical MEs depends on several factors, including enhancement of the drug solubility in the vehicle, increasing the drug permeation across skin, and free from rancidity in the water phase (91). Generally, in an ME, when an oil phase is dispersed in nano-droplets, its solubilization capacity and drug permeability is significantly increased. This can be explained by the fact that the drug solubility is often intrinsically related to drug particle size; as particles become smaller, the surface area to volume ratio increases. The larger surface area allows greater interaction with the solvent which causes an increase in solubility. Accordingly, it has been shown that the higher amount of surfactant incorporated in an ME, the lower the droplet size of the produced dispersed

phase. Therefore, the surfactant chosen in an ME must be able to lower the interfacial tension between the oil and aqueous phase to a very small value, facilitate the dispersion process during the ME preparation, and offer a flexible film that can readily surround the droplets (92). In most of the cases, any single chain surfactant alone is incapable of lowering the O/W interfacial tension adequately to form an ME system. However, this problem was solved by the addition of an auxiliary component to the surfactant called the co-surfactant. Usually, the co-surfactant accumulates at the interface layer of the droplet, increasing the fluidity of interfacial film via penetration into the surfactant layer. Ideally, a co-surfactant should not be an irritant to skin, yet, it should disturb the permeation barrier functions to offer more accessibility to a drug for crossing the skin (91). Overall, the ability of the final ME system to enhance drug transportation into and across the skin is largely influenced by the internal structure and type of the ME used, as well as the composition and concentration of its components (88).

1.12.3. Microemulsions role in skin permeation

Several mechanisms have been suggested describing the penetration-enhancing effect of MEs. Most likely, an overall combination of several mechanisms, and not an isolated one, provides a sufficient justification for the superiority of MEs compared to the other conventional formulations. The first property for enhancing cutaneous delivery is the small droplet size and large surface area of the MEs. Several studies have shown the effect of formulation dispersed phase size on their drug transportation ability into/across the skin, which supports the idea that the lower the particle size the larger the surface area and the more drug penetration (88). Furthermore, the individual components involve in ME formulations (oil, surfactant and cosurfactant) offer permeation enhancer effects, which may also improve skin permeation of diclofenac. Certain surfactants, monomers, oil phase

components, and other penetration enhancers combined in MEs can diffuse into the skin and increase the drug's permeation, either by disturbing the lipid structure of the SC, or by increasing the drug solubility in the skin and thus increasing its ability to penetrate the skin. Additionally, MEs have also been proven to increase skin hydration. The presence of water was believed to aid in swelling the corneocytes in the SC. This would mimic the way that swelling bricks in a wall could loosen the mortar. Thus, water existence would enhance the drug permeability by loosening the lipid chains of the SC without causing a direct effect on the lipid ordering; Such effects might contribute to the drug penetration-enhancing ability of MEs formulations. (20,90), therefore, the general mechanism would be; the hydrophilicity of the aqueous phase hydrates the skin, the hydrophobicity of the oil phase enhances the drug penetration across the skin layers, and the presence of surfactant and cosurfactent mixture disrupts the lipid bilayer between cells which enhance the drug penetration.

In such formulation, the drug probably does not have to be released from the vehicle to penetrate the skin in the first place as is the case with conventional formulations. MEs have superiority as a cutaneous drug delivery system over other preparations as their nano-sized particles, the existing hydrophilic/hydrophobic domains, and the presence of penetration enhancers, give the formulations the ability to solubilize higher amounts of a drug, penetrate the SC carrying the drug ,and cross through deeper skin layers.

1.13. *In Vitro* Release Rate Testing

Even with the pharmaceutical advances in topical drug delivery, such as microemulsions, all the new topical products are only of value if their clinical pharmacokinetic profiles produce the appropriate pharmacodynamic responses that is needed for treating patients. For that, it is

essential to assess the percutaneous absorption of drugs after topical application as that might help in understanding and/or predicting the transdermal delivery of active pharmaceutical ingredients throughout the skin. (93).

Typically, most topically applied drug products are designed to exert a local effect following drug application on the skin surface. The objective is to maximize drug concentrations at the site of action with, ideally, a minimal systemic uptake. Thus, systemic availability of a drug may not properly reflect the local cutaneous performance as it does for transdermally applied products which are intended to deliver drug into the systemic circulation (94). Furthermore, the *in vivo* percutaneous absorption techniques described in the literature do not provide methods for measuring the permeability of a substance directly at the site of application and, usually, the distribution and metabolism of the substance does occur prior to measure of serum and/or urine concentrations. Moreover, topical doses tend to be so small (typically 2–5 mg of product/cm^2), they are often undetectable using conventional assay techniques (95). Further complicating factors are that performing an *in vivo* test necessitates an ethical approval in addition to the fact that testing is usually associated with wide biological variability (93). This process can be costly, labor-intensive and time-consuming (96).

Accordingly, *in vitro* release testing (IVRT) has been gaining greater attention as a surrogate test that serves as a predictor of *in vivo* drug performance. The rationale for measuring the percutaneous absorption of a drug through employing *in vitro* techniques is based on the fact that absorption rate of a drug is determined by its passive diffusion through the non-living SC. Based on this fact, the implementation of *in vitro* release studies has become of major importance, not only for assessing drug delivery, but also because they act as valuable tools

for comparing generic formulations with an innovator products, as well as for batch-to-batch quality tests at an industrial scale (93). As a consequence to the issuance of the SUPAC-SS (Guidance for industry for nonsterile semisolid dosage forms) (97), many pharmaceutical manufacturers of topical medicaments have devoted significant resources to develop and validate IVRT throughout the drug development process (98). There have been several recommendations about *in vitro* testing, and some of them have been chosen as guidelines by regulatory entities and committees of interested parties such as the FDA "Guidance for the Industry on Non-Sterile Semisolid Dosage Forms" (99), and more recently the US Pharmacopeia in chapter ⟨1724⟩ entitled "Semisolid Drug Products—Performance Tests" (100). However, as highlighted in an FIP/AAPS position paper, no one standard test protocol can be applied to all topical dosage forms (101). Nevertheless, the most currently publicized method used to conduct IVRT is the Franz Diffusion Cell system, which has been proven to be the standard apparatus for measuring drug permeation through animal skin or synthetic membranes (102).

Even though the Franz diffusion cell system has some limited applicability estimating the complex process of skin permeation, yet, it is considered to be useful as a screening tool for drug release (11). *In vitro* release experiments have drawn much attention due to their advantages over whole-animal or human volunteer experiments. Of these, time, labor and costs saving, less restricted parameter variations, and more replicate measurements can be performed from the same or a number of different subjects, better reproducibility of results and more accurate absorption rates can be determined (1). Even with the popularity of IVRT using Franz diffusion cells, and irrespective to the guidance followed for conducting the test, the techniques used must be carefully considered since several variables in the test settings can influence the outcome and final conclusions (99).

1.13.1. Franz Diffusion Cells Apparatus

The use of the Franz diffusion cell system to assess drug permeability has evolved in the last years into a major research methodology, offering key insights into the relationships between skin, drugs and topical formulations (93). As per the FDA's guidance for industry on Scale Up and Post Approval Changes for Semisolid Dosage Forms (SUPAC-SS), *in vitro* release studies between pre-change and post-change products using vertical diffusion cell procedure are required for approval of SUPAC related changes (97). *In vitro* methods operating Franz diffusion cells are aimed to measure drug penetration into, and subsequent permeation across the skin or a membrane into a fluid reservoir. The process involves an application of the tested drug in an appropriate formulation to the surface of the skin or a synthetic membrane, which is mounted as a barrier between a donor chamber of the diffusion cell and a receptor chamber that are filled with a receiver medium. In this setting, the diffusion of the drug from its vehicle across the membrane is monitored by the analysis of sequentially collected aliquots of medium from the receptor chamber at predetermined time points. After each sampling, fresh medium is added to the receptor chamber to maintain the same volume or the entire medium is replaced to maintain "sink condition". Even though experiments with Franz cells are emerging as a generally accepted methodology for topical drug release, the choice of operational conditions requires careful consideration as the selected methodological parameters will have an influence on the drug release outcomes (93,100,101).

1.13.1.1. *In Vitro* Test Parameters:

1.13.1.1.1.Diffusion cells

Diffusion cells could be of a static or flow-through type. Sampling from static diffusion cells requires replacing the sample periodically with new perfusate at each time point while flow-

through cells are characterized by using a pump to pass perfusate through the receptor chamber, either as an open or closed loop system (103). Diffusion cells of the static type can be sub-classified based on the membrane orientation, which can be placed horizontally or vertically. Several comparative studies found no difference in the permeation profile between the different types of static and flow-through cells (104). However, the majority of *in vitro* release studies are conducted using vertical cells, where the membrane faces the air (Figure 1-8). The diffusion cells must be made from an inert non-adsorbing material with receptor chamber volumes of approximately 0.5-15 ml and surface areas of exposed membranes of around 0.2-2 cm^2 (105). Normally, the test is carried out using a minimum of six diffusion cells (106) .

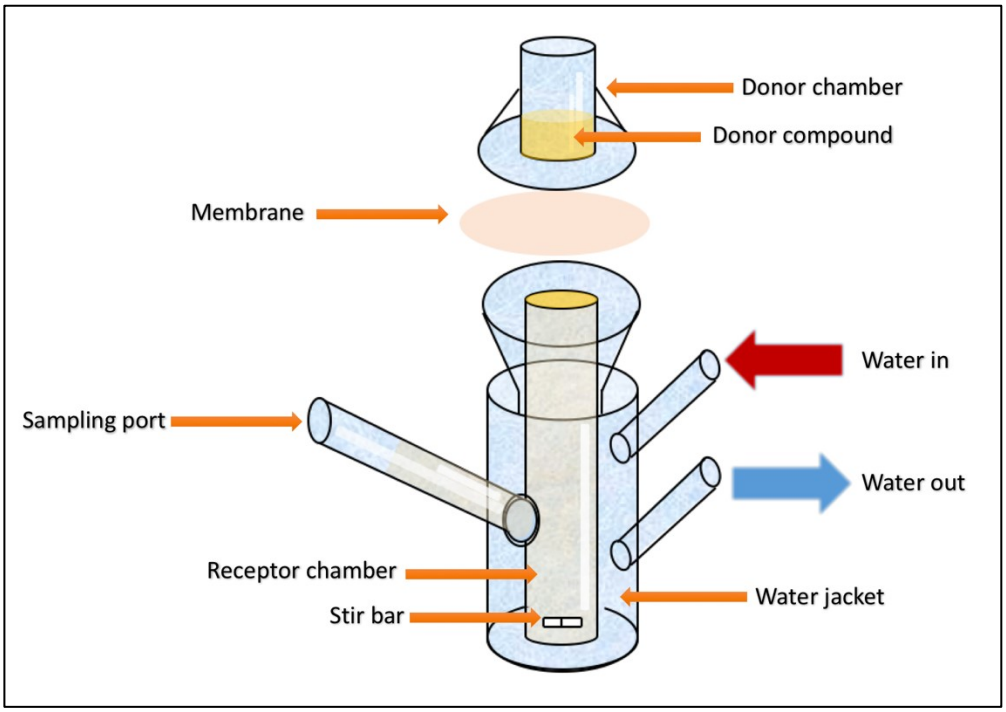


Figure 1-8 Typical diagram of Franz diffusion cell apparatus

1.13.1.1.2.Membrane selection

There are many choices for membranes that can be used for *in vitro*, studies including recently excised tissue, cadaver tissue, or synthetic membranes. The selection of the suitable membrane is influenced by several factors such as compatibility with the tested material, availability, cost, and most importantly, the aim of the experiment itself (107). The choice of skin relies on the purpose of the test and the availability of skin samples. For risk assessment purposes, it is believed that data acquired from *in vitro* using human skin is an adequate representation of living people so human skin is preferred. Typical human IVRT mainly involve using female abdominal or breast skin obtained at autopsy or from cosmetic surgery. Nevertheless, the use of human skin is mainly subjected to national and international ethical considerations (104). Due to their high cost, difficult availability and high variability, animal skin is often used, mainly from rats or mini-pigs. However, it was found that skin structure varies from one species to another, between different strains in the same species, and even within the same species. Thus, skin permeability across the species is dependent on the anatomical site, on the skin condition, and on the hydration state of the skin (11). Animal skin membranes for IVRT are usually prepared from the flank and the back of rats or the flank and ear of pigs. Even though animal skin is relatively less expensive than human skin, drawbacks of animal skin are that they are usually thinner, and have different morphology than human skin resulting in higher drug permeability (108).

Alternatively, when biological skin is not available, *in vitro* diffusion studies are carried out using synthetic membranes: The synthetic membranes employed in Franz diffusion cells studies have two functions to simulate the skin, and for quality control. Membranes with hydrophobic properties simulate the skin since their hydrophobicity act as a rate-limiting step for drug penetration resembling natural skin barriers. On the other hand, synthetic

membranes with hydrophilic moieties are mainly used for quality control as they have minimum diffusion resistance and only act as a support for separating the formulation from the receptor medium (93). There is a wide selection of synthetic membranes, ranging from semisynthetic to synthetic polymers that are currently available in the market. However, according to FDA SUPAC-SS, any "appropriate inert and commercially available synthetic membranes such as polysulfone, cellulose acetate/nitrate mixed ester, or polytetrafluoroethylene membrane of appropriate size to fit the diffusion cell diameter can be employed for IVRT (109). In general, synthetic membranes might be preferred for performing IVRT as they are structurally simpler, easily resourced, less expensive, and exhibit superior permeation data reproducibility as *in vivo* testing variables as skin age, race, and anatomical site are eliminated (110).

1.13.1.1.3.Receptor media selection

For the receiving medium, SUPAC-SS did provide a practical starting point: "appropriate receptor medium such as aqueous buffer for water soluble drugs or a hydro-alcoholic medium for sparingly water-soluble drugs" can be employed (109). Generally, the receptor solution must have an adequate capacity to solubilize the tested compound. In this setting, the sink condition will be maintained throughout the study. Thus, sustaining the rate of absorption would mimic the circulatory system under the *in vivo* conditions. The receptor medium should also not affect the membrane integrity, not interfere with the analytical procedure, and should have a physiological pH (104). Throughout the study, the receptor medium should be well-stirred and must be maintained at the *in vivo* skin temperature of 32 ± 1°C. Generally, the temperature regulated through thermostatically controlled water that circulates through a jacket surrounding the chamber (111).

1.13.1.1.4.Dosing

There are two scenarios that related to the amount of the tested substance applied to the membrane, infinite and finite dosing. In the finite dose regimen, only a limited amount of the tested formulation is applied to the membrane. The application of a finite dose supposedly best resembles the *in vivo* situation, however, with finite dose conditions, donor depletion is likely to be observed due to the evaporation and/or diffusion into and through the barrier during the study. In contrast to finite dose experiments, for infinite dosing, the applied dose is so large, (typically, 300–800 mg), that permanent depletion is negligibly small. Thus, the dose is considered to be constant (104). For that, an infinite dose approach is usually preferred over a finite dose regimen (112) .

1.13.1.1.5.Duration of exposure and sampling time

The exposure time should reflect in-use situations. Typically, for industrial products, the exposure time is suggested to be 6-8 hr (1). In all cases, the frequency of sampling times (at least five time points) must be chosen adequately over an appropriate period to allow the generation of an adequate release profile. (e.g., at 30 min, 1, 2, 4 and 6 hr) are suggested with an aliquot volume between 0.5 ml to 1.5 ml (107). The amount withdrawn must be replaced with fresh medium, so that the lower surface of the membrane remains in contact with the receptor phase over the experiment (109).

1.13.1.2. Evaluation of the results

A suitable method of analysis (usually HPLC or UV) must be defined and validated to determine the drug diffusion data (93). Afterwards, when evaluating drug release data of an

in vitro study mathematically, the terminal procedures are slightly different after infinite and finite dosing experiments. In case of the infinite dose regimen, the permeation can be measured over time and plotted as a cumulative amount permeating versus time or as flux which can be viewed simplistically based on the first Fick's law of diffusion (Equation 1-1)(106).

$$J = -D\frac{\delta C}{\delta x}$$

(Equation 1-1)

where J is the rate of transfer per unit area (flux) (g cm^2h^{-1}), C is the concentration gradient (gcm^{-3}), x is the linear distance travelled (cm), and D is the diffusion coefficient ($cm^2h\text{-}^{1}$) (1).

On the other hand, following a finite dosing trial, the quantity of the tested drug and/or its metabolites must be determined, which requires complete recovery of the tested material (90-110%) (113), or (85-115%) (114). For risk assessments, when the tested substance remains in the skin/or membrane at the end of the study, it may need to be included in the total amount absorbed, as this amount might be considered as a possible reservoir for systemic dose. For that, the amount of recovery determination is relevant to calculation of finite dose and it can be determined using Fick's second law of diffusion (Equation 1-2).

$$J_{ss} = K_p \cdot C_o$$

(Equation 1-2)

Where JSS is the steady state flux per unit area, Kp is the permeability coefficient for a given solute in a given vehicle (cm h^{-1}) and C_O is the concentration of the solute in the donor compartment. Kp can only be used to predict the penetration rate of a chemical at a given concentration from the same vehicle (1).

1.13.2. Topical Diclofenac *In Vitro* Studies

A few *in vitro* studies have been conducted testing the permeation behavior of diclofenac formulated in different marketed formulations. Vasiljevic et al. compared the release profile of 1.16% diclofenac diethylamine (Voltaren emulgel) with different prepared formulations using a dissolution cell covered with cellulose membrane. It was found that the cumulative percent of diclofenac release after 6 h was only 26.12 ± 0.63 % of the applied dose (115). The same product was *in vitro* tested using rat skin by Ozgüney et al. This study compared the release of diclofenac from different topical formulations throughout 8 hours. The results showed a significant difference among all the *in vitro* studied formulations and the least permeation profile was seen from Voltaren diclofenac emulgel (116). Another study employed abdominal skin from human volunteers to compare the *in vitro* skin permeation of diclofenac sodium 4% Spray Gel with that of Voltaren Emulgel (1.16% diclofenac diethylammonium). The study found that within 8hr, up to 2.5% of the diclofenac sodium 4% Spray Gel was absorbed through the skin, while only 0.12% of the diclofenac was permeated from the Emulgel (117). Another few *in vitro* trials have been conducted to test the permeation behavior of compounded diclofenac from different pharmaceutical bases. An *in*

vitro percutaneous absorption study was performed by Dow Pharmaceutical Sciences Inc. using 5% diclofenac formulated in versapro gel, found that only 16% of the diclofenac was released within 24hrs through cadaver skin (118). To our knowledge, no published *in vitro* release study was performed on diclofenac sodium topical solution with DMSO. Regarding PLO's performance, Dreher *et al.* has reported that the partition of diclofenac from the lecithin system seem to be unfavorable, and to get relevant penetration rates, relatively large amounts of diclofenac must be used. Subsequently, a relatively low bioavailability for diclofenac was found by adding higher concentrations of the drug to the gel-system, the gel was destroyed by the drastic loss in viscosity (119). In general, the net result out of the few IVRT that were performed to test the release performance of diclofenac from currently available formulations indicated the need to enhance the diclofenac penetration, which necessitates developing more efficient vehicles for topical delivery of diclofenac (120).

1.14. Rationale, Hypothesis, Study Objectives and plans

1.14.1. Rationale

Based on the previously mentioned studies, some of the investigated topical diclofenac preparations have shown to provide acceptable levels of analgesic efficacy as well as a number of serious systemic toxic side effects (51). The suggested reason for developing side effect after using some of the currently available formulations would be the excessive use of potent chemical penetration enhancers, such as the most commonly used solvent DMSO, that is known to enhance drug penetration at concentration of greater than 60% which usually irritates, injures and harm the hypodermis. Upon long-term exposure, this probably causes the drug to leak into the systemic circulation through nearby capillaries, therefore, it is considered physiologically incompatible (121,122). On the other hand, according to the previously mentioned trials, some of the studied diclofenac formulations, such as some of the compounded preparations, did not cause systemic side effects to their users, yet, they also did not deliver the satisfactory amount of pain relief as seen by the increase of dose. The main reason behind the dose increase was i) the dose was too low ii) the drug failed to be released in the first place from its vehicle and did not penetrate into the skin layers. Based on that, even when physicians and pharmacists increase the amount of drug in the formulations, no increased analgesic effect nor increase in side effects were observed or reported. In both scenarios, most of the diclofenac formulations fail to be successful by dermally delivered as they could not achieve suitable delivery to the skin. Obviously, a complete separation between cutaneous and transdermal delivery is not possible. However, by employing the proper formulation, this problem might be minimized (88).

Currently, Microemulsions are recognized for their ability to improve the delivery of drugs within skin layers. A careful formulation design might allow some adjustment on the cutaneous/transdermal delivery balance to favor the dermal one (88). Typically, the aqueous phase in the MEs hydrates the skin and enhances drug penetration through the SC. Surfactants in the MEs are known to enhance penetration in the dermal tissues but they are not as effective for transdermal delivery, which prevents drug from reaching systemic absorption. On the other hand, the oil phase increases the solubility of the drug in the SC, which increases the partition coefficient of the drug between the SC and the vehicle (88). The oil phase also helps drug to penetrate to deeper skin layers. However, the lower the lipophilicity of the MEs the less the chances of the drug to reach systemic circulation. Therefore, by manipulating the ratios of the component in an ME, the resulted formulation could improve skin penetration with lower systemic exposure (88, 122, 123). Additionally, topical MEs represent an attractive choice as they have been proven to enhance the solubility of lipophilic active pharmaceutical ingredients (API) such as diclofenac. Due to the nano-sized internal phase with an ultra-low interfacial tension, higher drug concentration can be incorporated in an ME which generate an increased concentration gradient towards the skin (91,124). For that, the present dissertation work is to focus only on developing ME adaptable topical delivery systems that are able to enhance the penetration of diclofenac sodium.

1.14.2. Hypothesis

Microemulsion systems can improve the *in vitro* permeability of DS.

1.14.3. Objectives

The study covers the following objectives:

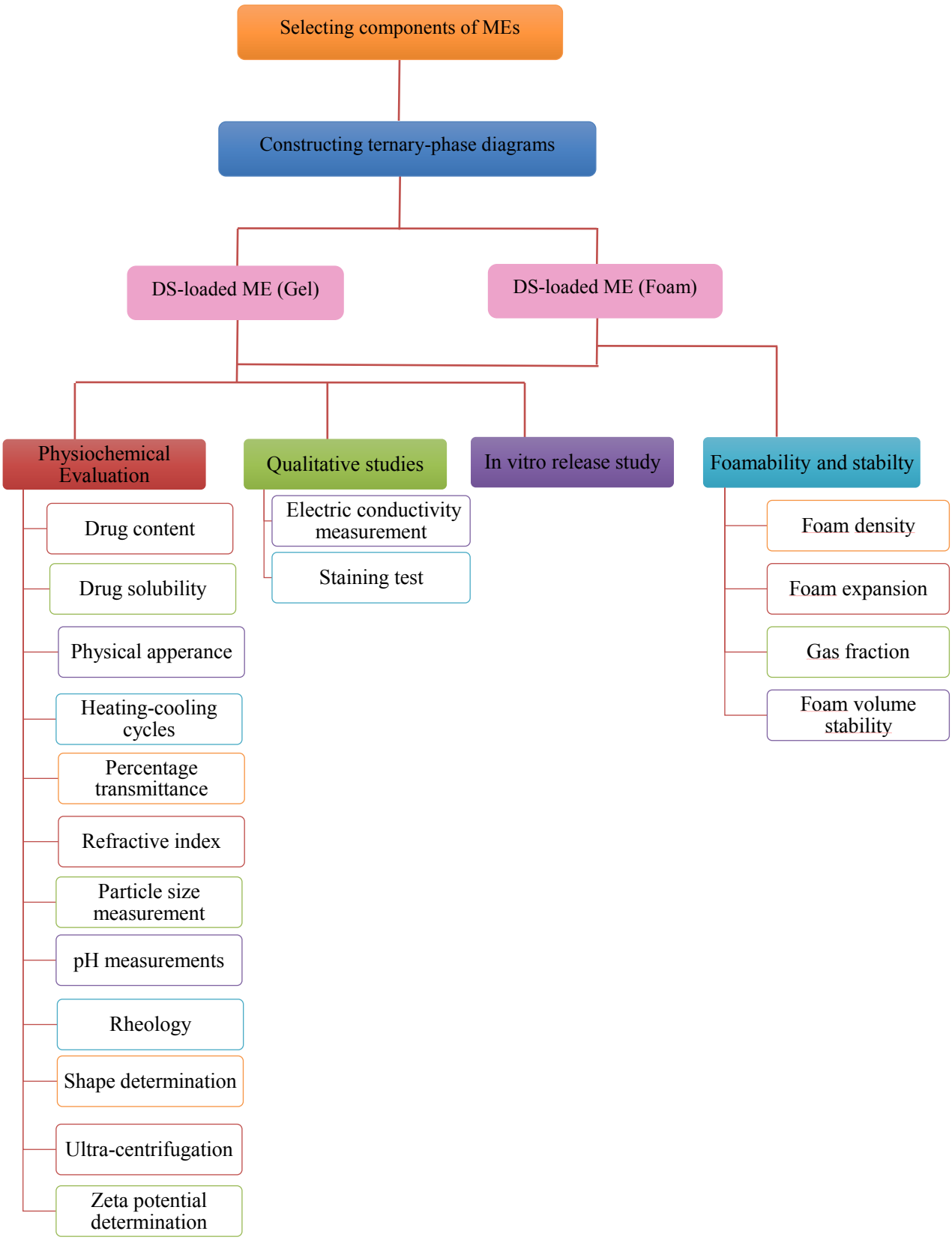
1- To formulate ME-based gel and foam that act as suitable drug carriers for DS.

2- To characterize the properties of the developed ME-based systems.

3- To evaluate the stability of the formulated ME-based systems through performing physiochemical stability testing.

4- To evaluate the *in vitro* drug release performance of DS from the formulated ME-based systems in comparison to different marketed formulations.

1.14.4. Study Plan



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Chapter 2

2. Evaluation of a Microemulsion-Based Gel Formulation for Topical Drug Delivery of Diclofenac Sodium

This study has already been published as Braa Hajjar · Kira-Isabel Zier et al. Evaluation of a microemulsion-based gel formulation for topical drug delivery of diclofenac sodium in Journal of Pharmaceutical Investigation. April 2017.

Abstract

The aim of the current research was to investigate the release of diclofenac sodium, a poorly water-soluble drug from different formulations *in vitro*. A microemulsion (ME) was prepared using caprylocaproyl polyoxyl-8 glycerides, diethylene glycol monoethyl ether, and propylene glycol monolaurate. For enhancing the viscosity, Carbopol was used to form an ME-based gel. The prepared formulations were characterized for physical appearance, droplet size, Zeta potential, refractive index, percentage transmittance, heating-cooling cycles, phase separation, pH, conductivity, viscosity, drug content, staining solubility test, transmission electron microscopy and *in vitro* drug release using Franz diffusion cells. The mean droplets size for ME and ME-based gel-systems were 114.4 ± 0.472 nm, and 178± 2.46 nm respectively, whereas the zeta potential values were -33.3 ± 0.64 mV for the former and -33.0 ± 0.40 mV for the latter. No significant variations in the pH nor physical appearance alterations were observed while stability tests were performed. Further, TEM images for drug-loaded ME and the gel exhibited nano-droplets that were spherical in shape. The release rate of diclofenac sodium formulated as ME or as ME gel had the highest release values (76.67 ± 8.63%) and (69.28 ± 7.14 %) after 6 hrs respectively. This was statistically significant ($p < 0.0001$) compared to the control and different marketed formulations or compounded preparations. The ME-based gel had a higher viscosity suitable for topical administration without dripping. The *in vitro* result suggested that ME systems are powerful topical vehicles for enhanced penetration of diclofenac sodium.

Keywords: Diclofenac sodium, Microemulsion, Gel, Carbopol, Topical delivery, *In vitro* release

2.1. Introduction

Diclofenac Sodium (DS) is a nonsteroidal anti-inflammatory drug (NSAID). It has been broadly used to manage musculoskeletal and inflammatory disorders due to its potent analgesic, antipyretic and anti-inflammatory effects (1). DS is a non-specific cyclooxygenase (Cox 1/2) inhibitor, which binds to certain prostaglandin receptors. The drug causes side effects mainly in the gastrointestinal tract, which results in gastric bleeding, ulceration or intestinal wall perforation (2). More importantly, Bhala et al. stated that diclofenac increases heart and stroke adverse events more than other NSAIDs. Hence, local topical administration of DS has been studied as one way to minimize side effects. Clinical evidence suggests that the topical application allows localizing higher concentration of the drug at the required site (3). This might lower systemic exposure, which can result in fewer or no adverse drug effects (1). The stratum corneum acts as a barrier and limits penetration of most exogenous substances through the skin. Limited skin permeation of DS is due to this barrier. The innovator product monograph states that only 6.6 % of the drug is bioavailable (4). Additionally, transdermal delivery is non-invasive, painless, avoids drug degradation by gastric enzymes, and hepatic first-pass metabolism. Despite the great potential of topical drug delivery, relatively few dermal formulations are commercially available without prescriptions. Recently, there has been an interest to improve dermal formulations to provide higher degrees of skin permeation (5). Therefore, numerous researches investigated vesicular and nano-sized carrier systems with specific attention to microemulsion systems (ME). Such systems are considered promising transdermal drug delivery systems due to their formulation properties, thermodynamic stability, excellent biocompatibility and their ability to enhance penetration of lipophilic and hydrophilic drugs into the skin due to the existence of hydrophilic and lipophilic domains (5,6). MEs are isotropically clear homogeneous systems

that typically consist of an oil phase, aqueous phase, and surfactant/co-surfactant component with a droplet range of 0.1-1.0 μm (7). Several mechanisms have been reported to explain the advantages of MEs as a topical drug delivery system. First, the large amount of a drug that can be incorporated into an ME due to its improved solubilization capacity which can increase the thermodynamic activity of a drug towards the skin. Some surfactants are able to emulsify sebum, thereby enhancing the thermodynamic activity of drugs and allowing it to penetrate cells more efficiently (8) Second, the increase in the thermodynamic activity of a drug might improve its partitioning into the skin (9). Third, the ingredients of MEs might impact the diffusional barrier of the stratum corneum. Formulation ingredients may also increase the rate of drug permeation through the skin as they act as permeation enhancers. Moreover, the permeation ability of formulations might be influenced by the hydration effect of MEs on the stratum corneum (10).

The administration of MEs as a solution onto the skin can be challenging due to its low viscosity. To optimize the ME as a transdermal formulation, different hydrogels such as xanthan gum, carbomer, and carrageenan have been studied to increase the viscosity of MEs (11). The addition of gelling components into MEs produces ME-based hydrogels, which are easier to apply to the skin compared to runny liquid MEs.

Therefore, the aim of this study was to evaluate the *in vitro* performance of DS through preparing and testing a diclofenac-loaded ME solution and a gel formulation. A direct comparison was made with commercially available and pharmacy-compounded products.

2.2. Materials and methods

2.2.1. Materials

Diclofenac sodium USP was purchased from PCCA (London, ON). A commercial Diclofenac-formulation Voltaren (Novartis) purchased from a local pharmacy Batch No.: K00448A, Exp. Date: 01/2018. Diethylene glycol monoethyl ether NF, Caprylocaproyl polyoxyl-8 glycerides NF and propylene glycol monolaurate NF were received as a generous gift from GatteFosse, (Montreal QC). Carbopol 934P NF was from L.V. Lomas Limited (Brampton ON). Compounded topical formulations consisting of different bases (Lipoderm, Versapro gel, and Pluronic Lecithin Organogel PLO gel) were kindly received from a local pharmacy. Double distilled water was used for the MEs preparation. All other solvents and materials used were of analytical grade.

2.2.2. Methods

2.2.2.1. ME and ME based gel preparation

2.2.2.1.1. Diclofenac Sodium Assay

The quantitative determination of Diclofenac Sodium was performed by Milton Roy Spectronic 3000 Array UV spectrophotometry at λmax = 277 nm. A calibration curve was then obtained (Y = 30.174x + 0.0273), in which Y was concentration [µg/mL], X was absorbance, and r^2 was 0.999). The standard plot of DS was performed over the concentration range of 2.5 to 40 µg/mL.

2.2.2.1.2. Selecting components of ME and constructing a pseudo-ternary phase diagram

In order to prepare an optimized ME system, it was of great importance to select an appropriate oil, surfactant and cosurfactant combination that had a good solubilizing capacity of DS. For the preparation of the ME system in this study, propylene glycol monolaurate was selected as an oil phase, caprylocaproyl polyoxyl-8 glycerides as a surfactant and diethylene glycol monoethyl ether as a cosurfactant. These components were chosen on the basis of a solubility study reported by Thakkar et al., who developed w/o MEs using five different cosurfactants, not including caprylocaproyl polyoxyl-8 glycerides, combined with diethylene glycol monoethyl ether as surfactant and Propylene glycol monolaurate as the oil phase (2). The pseudo-ternary phase diagram was constructed to distinguish the ME domains and to detect the possibility of producing MEs with different possible concentrations of oil, surfactant, cosurfactant, and water. The phase diagram was developed at ambient temperature using an aqueous phase titration method. It was prepared at surfactant/cosurfactant ratios (Smix) of 2:1. The oil and Smix were combined in different weight ratios that varied from 1:9 to 9:1. The systems were stirred by a magnetic stirrer during the addition of the aqueous phase to ensure a thorough mixing. Based on visual observation, the end point of the titration was determined when the mixtures became turbid or cloudy. Based on the ME region that determined from the constructed pseudo-ternary phase diagram, five formulations contain different proportions of oil, water, and Smix were mixed in the ratios presented in Table 2-1. The drug was dissolved directly in the formulations to formulate drug loaded MEs. All MEs were then tested for *in vitro* drug release. Further investigations were then conducted on the formulation that provided the highest drug release profile. The areas corresponding to either microemulsions or macro/conventional-emulsions were constructed inside the triangular phase diagram using the Microsoft Excel 2015.

Table 2-1 Composition of microemulsions containing 3% Diclofenac with various amounts of Smix (Caprylocaproyl polyoxyl-8 glycerides /Diethylene glycol monoethyl ether), Propylene glycol monolaurate and water.

Formulation	% Smix (2:1)	% Water	% Propylene glycol monolaurate	Drug solubility µg / mL
F 1	75	0	25	97.31 ± 2.366
F 2	50	37.5	12.5	85.69 ± 0.304
F 3	60	25	15	89.30 ± 0.117
F 4	75	10	15	90.07 ± 0.374
F 5	75	12.5	12.5	85.65 ± 3.584

2.2.2.2. Preparation of Drug-loaded ME

ME formulations were formed spontaneously by mixing caprylocaproyl polyoxyl-8 glycerides as a surfactant with diethylene glycol monoethyl ether as cosurfactant at 2:1 ratio. Water and propylene glycol monolaurate were added directly and mixed gently at room temperature. An amount of 3% w/w DS was added to each ME and the formulations were stirred for 5 min at 600 rpm until a clear ME was formed (12) In this study, 3% w/w DS was used which is within the therapeutic range. According to the FDA-approved prescribing information by the innovator, the recommended dose of topical DS is 2- 4 g of gel for a normal application at one location. The total applied dose should not exceed 32 g per day over all affected areas (13).

2.2.2.1.3. Preparation of plain Carbopol gel base

Carbopol 934P gel base was prepared by dispersing 1.5% (w/w) carbopol into distilled water and mixing it using a magnetic stirrer at 1200 rpm for at least 30 min (14) The dispersion was left for 24 h to equilibrate. After that, sodium hydroxide solution was added dropwise in order to get a suitable gel with appropriate viscosity and a pH between 5-7 (15).

2.2.2.1.4. Preparation of drug-loaded ME based-gel

In order to enhance the viscosity of the formulated ME, the freshly prepared drug-loaded ME was added portion-wise onto the previously prepared plain carbopol gel in a ratio gel: ME (2:1) under continuous stirring (16). The final ME- based gel formulation contained 3% w/w DS (Table 2-2).

Table 2-2 Percentage Composition (%w/w) of the ME and ME based-gel formulations.

Excipients	ME	ME based-gel
Caprylocaproyl polyoxyl-8 glycerides	50	50
Diethylene glycol monoethyl ether	25	25
Propylene glycol monolaurate	12.5	12.5
Diclofenac Sodium	3	3
Carbopol 943P	–	1.5
10 % NaOH	–	1.1
Purified Water	q.s.	q.s.

2.2.2.2. Physiochemical evaluation of the prepared ME and ME based gel

To overcome problems related to metastable and unstable formulations during storage, the physical stability of ME formulations was assessed by the following thermodynamic stability tests.

2.2.2.2.1. Physical appearance

The prepared ME and ME based gel loaded with DS were examined visually for their color, homogeneity, and consistency (17).

2.2.2.2.2. Percentage transmittance and refractive index

The optical clarity of the MEs was determined by measuring the percentage transmittance of the formulations using UV-Visible spectrophotometer (Milton Roy Spectronic 3000 Array UV). The MEs were analyzed at 650 nm against distilled water as a blank solution, and three replicates were performed for each ME (18). The refractive indices of the systems were measured in triplicate at 25°C using K7135, ABBE Refractometer.

2.2.2.2.3. Centrifugation study

The ME based formulations were subjected to centrifugations by Microleter Centrifuge (Heraeus Biofuge Pico) at 10000 rpm with relative centrifugal force (RCF) 8,960 g for 30min at 25℃ and observed for any changes in their homogeneity (19).

2.2.2.2.4. Heating-cooling cycles

Heating-cooling cycles were performed to evaluate the stability of the formulations under thermal conditions. Both systems were kept at 0 °C for 48 h then at 25 °C for 4 h; each cycle was repeated five times (20). At the end of the experiment, both formulations were assessed for physical properties including pH, homogeneity, and consistency.

2.2.2.2.5. Particle size measurement

The particle size determination was performed by Zetasizer Nano-DTS 1060 (Malvern Instruments Ltd, UK) at 25^{o} C and 173^{o} fixed angle. The samples were kept in disposable cuvettes, and observations were performed in triplicate following a proper dilution of the formulations in double distilled water. The polydispersity index (PDI) was used as a quality marker for droplet-size distribution (2).

2.2.2.2.6. Zeta potential determination

The surface charge of DS loaded ME, and ME based gel was determined by the dynamic light scattering method employing a Zetasizer Nano-DTS 1060 (Malvern Instruments Ltd, UK). Analysis time was kept for 50 seconds. The zeta potential was measured using clear zeta dip cells after dilution of all samples with double distilled water. Cuvettes were washed and then rinsed with samples to be measured before each experiment. The zeta potential values were calculated according to Helmholtz-Smoluchowsky equation. All the results were the average of three measurements (20,21).

2.2.2.2.7. Transmission Electron Microscopy (TEM)

The TEM images of drug-free ME, drug-loaded ME and drug-loaded ME based gel were taken to investigate the morphology and structure of the formulations (22). The images were taken by Philips / FEI (Morgagni) Transmission Electron Microscope operated with Gatan Digital Camera. For performing TEM observations, a drop of diluted (1 in 10 dilutions) formulations were directly deposited on a copper grid, and the excess was removed with a filter paper. One drop of 2% aqueous solution of phosphotungstic acid was placed onto the grid and left for 30–60 seconds to allow staining and the excess fluid was removed using a filter paper (16,23).

2.2.2.2.8. Determination of Drug Solubility

For determining the drug solubility in the prepared MEs, an excess amount of DS was added to 5 g of each of the previously prepared MEs and stirred at room temperature for 24 hours with a magnetic stirrer. Afterward, the sample was centrifuged, and the concentration of DS in the supernatant was determined spectrophotometrically at 277 nm. A plain ME without drug was taken as a blank (5).

2.2.2.2.9. pH measurements and drug content

The apparent pH of the tested MEs formulations was determined by a digital pH meter (Accumet XL20, pH meter). All measurements were performed in triplicate at 25◦ C. For determination of drug content, one gram of each formulation was diluted in 100 ml PBS pH 7.4. Then, the resulting solutions were filtered before subjecting it to spectrophotometric

analysis. The concentration of DS was determined at 277 nm. Plain formulations without drug with the same composition were taken to establish a calibration curve (16,19).

2.2.2.2.10. Rheological studies

A Brookfield DV-III Ultra Viscometer was used to measure the viscosity of the prepared microemulsions. The spindle number 21 was rotated at 150 rpm using an interval of 30 s. Samples were allowed to settle at room temperature for 10 minutes before the measurements were conducted. The rheological measurement of the ME based gel was performed using a Malvern Rheometer (Kinexus), equipped with Parallel Plate.

2.2.2.3. Qualitative studies

2.2.2.3.1. Electric conductivity measurement

The conductivity analysis of the drug loaded ME, and the gelled ME was carried out using (Accumet XL20 conductivity meter) that equipped with 1.0 accumet probe. The conductivity meter was calibrated using a 3-point calibration with standard fluids of 23, 447 and 1500 µS/cm and the measurements were carried out in triplicate.

2.2.2.3.2. Staining test

The evaluation of the emulsion type was done by dissolving a water-soluble dye (methylene blue) in the ME and observing its distribution visually after 5 minutes (24). A rapid

dispersion of the water-soluble dye in the system usually appears with an o/w MEs whereas with w/o MEs the dye provides microscopically visible droplets. The reverse happens when using an oil-soluble dye (18).

2.2.2.4. *In vitro* drug release studies

This study was carried out using 6 Franz diffusion cells with an effective diffusion area of 1.79 cm^2 (15.1 mm diameter orifice) to determine the release rate of DS from the MEs, and the drug loaded ME gel. Synthetic 0.22 μm Polyvinylidene Fluoride (PVDF) membranes were first hydrated in phosphate buffer (pH 7.4) at 25˚C for 30 minutes. The membranes were then clamped between the donor and receptor compartments. The receptor compartments were filled with 12 mL of phosphate buffer (pH 7.4). The receiver medium was maintained at 32.0 +/-0.5 °C using a circulating water bath (Haake D2, Germany), the acceptor compartment was magnetically stirred at 600 rpm throughout the experiment (IKA, USA). About 0.5 g of each formulation was accurately weighed and added on the donor compartment. At five time points (0.5, 1, 2, 3, 4 and 6 h), 0.1 mL aliquots were withdrawn through the sampling port and replaced immediately with an equal volume of fresh receptor solution to maintain a constant volume of the receiving solution (25). The samples were then analyzed spectrophotometrically (Milton Roy Spectronic 3000 Array spectrophotometer) at 277 nm against an appropriate reference. Three replicates of each experiment were conducted. The results were plotted as a cumulative percentage of drug release versus time. The release pattern of DS from ME formulations (F1-F5) was tested using hydrophilic PVDF membranes. Then, the formulation with the highest release profile was chosen to be incorporated into carbopol gel. The release rate of DS from ME and ME based gel was examined using hydrophobic PVDF membranes in order to simulate the stratum corneum,

such hydrophobic synthetic membranes generally possess similar rate-limiting permeation properties as skin, thus making them as a suitable choice for predicting drug permeation (26). Release profiles of DS from the formulations were compared with a commercial formulation (Voltaren Emulgel) containing 11.6 mg of diclofenac diethylamine which is equivalent to 1% diclofenac (6) (reference formulation). Three different compounded formulations consisting of commercial or compounding bases (Lipoderm, Versapro gel, and PLO gel) contain 5% DS (commonly prescribed strength) were also tested. The formulations were also compared with carbopol 934P gel contains 3% (w/w) free DS, and vaseline contains 3% (w/w) free DS as controls. % drug release was used for the evaluation of the formulations to account for the different strengths. The different strengths reflect the variety of strengths a patient might use.

2.2.2.2.5. Statistical analysis

All the experiments were performed three times, and data were reported as mean ± SD. Data were analyzed statistically by using one-way analysis of variance (ANOVA) and student t-test. The individual variances between formulations were calculated by non-parametric post hoc test (Tukey's test). $P<0.05$ was considered to be statistically significant at an alpha of 0.05. The Statistical analysis was done using SPSS software (version 19.0), and Microsoft Office Excel 2015. DDSolver 1.0 software was used to compare drug release data using univariate ANOVA and similarity factor f2. Calculations of f2 values, which is a measurement of the similarity in the (%) dissolution between two curves, was performed according to the equation (2-1).

$$f_2 = 50 \bullet \log \{[1+(1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \bullet 100\}$$

(Equation 2-1)

Where *n* is the number of time points, *Rt* is the dissolution value of the reference product at time *t,* and *Tt* stats to the dissolution value of the test product at time *t*. *f*2 values must be greater than 50 (50-100) to ensure sameness or equivalence of two dissolution curves (27).

2.3. Results

2.3.2. The pseudo-ternary phase diagram and ME formation

A microemulsion is formed when the interfacial tension between the water and oil interface is reached an extremely low level, and the interfacial layer is maintained highly flexible and fluid like. Resulting in a spontaneous dispersion of one liquid into the other. This is usually met by a careful and precise selection of surfactants and cosurfactants and their respective proportions (28-30). Moreover, the components used for developing MEs should have high drug solubilization capacity, to ensure maximum solubility of the drug in the resultant system. According to the solubility study reported by Thakkar et al. the selected oil (propylene glycol monolaurate), surfactant (caprylocaproyl polyoxyl-8 glycerides) and cosurfactant (diethylene glycol monoethyl ether) showed a high solubility profile of DS compared to other oils and among the investigated surfactants and cosurfactants (2). Likewise, evaluating the area of ME region in the phase diagram is essential for a successful development of an optimum ME (23). Hence, constructing a pseudo-phase diagram is vital to determine the concentration range of components for the existence range of MEs. The monophasic ME region and biphasic emulsion region were presented in the pseudo-ternary

phase diagram (Figure 2-1). It was observed that the area of ME region increased as the surfactant/ cosurfactant mixture increased. This is probably due to the reduction of the interfacial tension and increased the fluidity of the system (2). The drug solubility was increased with the increase of the propylene glycol monolaurate as shown in Table 2-1. Based on the drug solubility, phase diagram and the *in vitro* drug release of the tested MEs, a microemulsion containing 3% DS was prepared at surfactant to a co-surfactant ratio of 2:1 and then employed for further analysis.

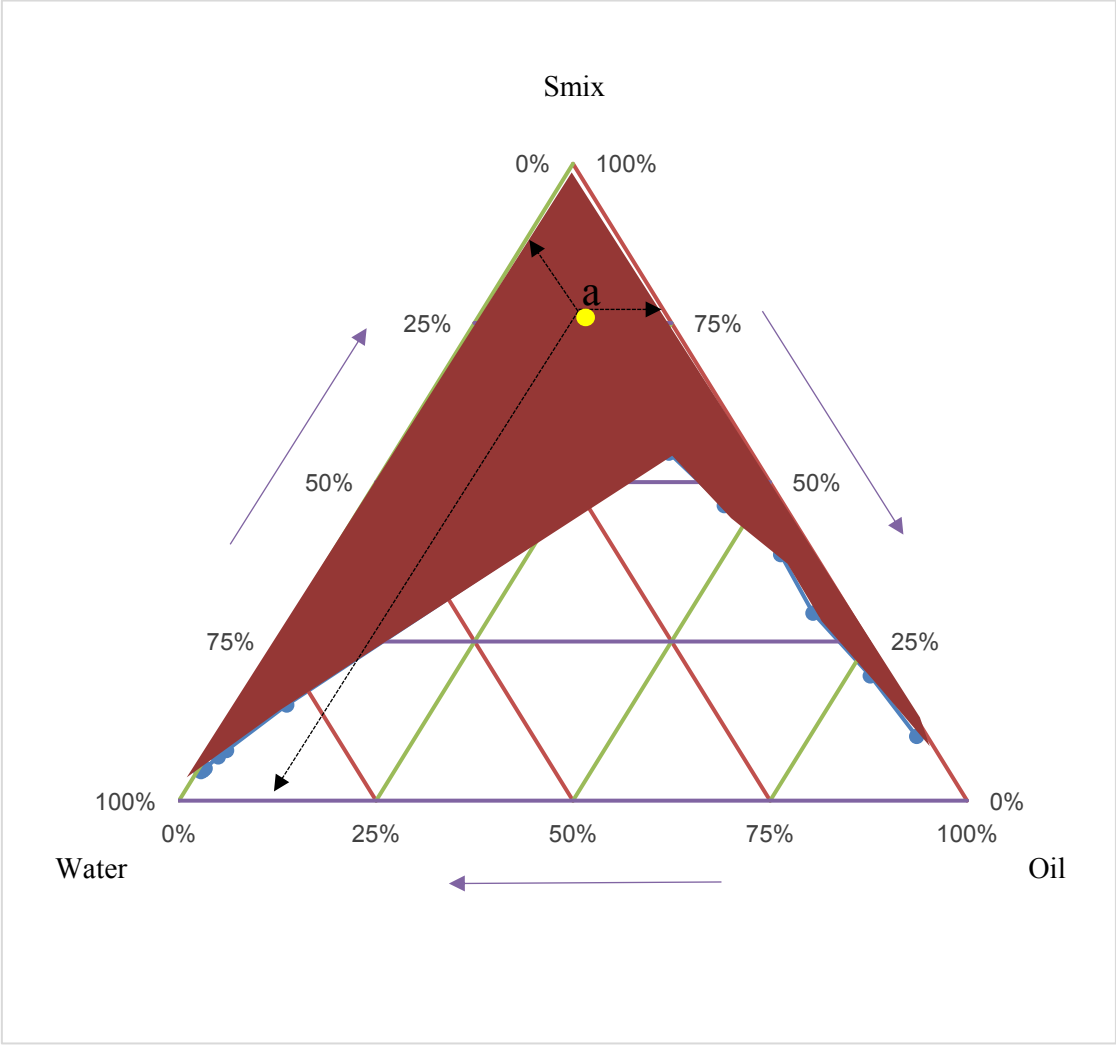

Figure 2-1 Pseudo-ternary phase diagram of propylene glycol monolaurate (oil), caprylocaproyl polyoxyl-8 glycerides / diethylene glycol monoethyl ether (Smix) and water. The shaded area shows the ME region and the point (a) represent the optimized ME system.

2.3.3. Physical appearance

The prepared drug-loaded ME was clear, transparent, liquidly and with homogenous appearance. On the other hand, the ME based gel was of a glossy appearance, and a smooth, homogeneous texture.

2.3.4. Percentage transmittance and refractive index

The percentage transmittance is an essential parameter to determine the transparency of the system. If the value of the percentage transmittance (%T) is close to 100%, this indicates that the selected formulation is clear, transparent and has a particle size in the nanometer range, which indicates that the formulations have a large surface area for drug release (31) It was found that the ME free drug and the ME loaded with diclofenac have transmittance values greater than 98% (Table 2-3), suggesting their clarity, due to the smaller particle size, which increases the transparency of the formulated systems. Moreover, if the refractive index of a system is similar to that of the water (1.333), then a formulation is transparent in nature. The refractive indices of the produced formulations ranged between 1.07-1.35, which indicate the clarity and isotropy of the MEs systems.

Table 2-3 Physiochemical characteristics of the prepared formulations (mean ± SD, n=3).

Formulation	Refractive Index	Transmittance %	Particle size nm	PDI	Zeta potential mV
ME	1.25 ± 0.00	100.33 ± 0.08	134.3 ± 0.351	0.046 ± 0.006	-25.2 ± 2.48
Diclofenac ME	1.077 ± 0.47	99.48 ± 0.24	114.4 ± 0.472	0.282 ± 0.007	-33.3 ± 0.635
Diclofenac ME gel	1.35 ± 0.00	___	178.8 ± 2.464	0.196 ± 0.014	-33 ± 0.404

2.3.5. Phase separation

Emulsions are normally thermodynamically unstable system and may separate when subjected to physical stresses like centrifugation. Though MEs are visually appear homogeneous as a single-phase system, they are in reality emulsion systems, which were confirmed by laser light scattering measurements. Therefore, they were subjected to centrifugation to confirm the absence of phase separation (32). MEs did not show any sign of phase separation nor any precipitations when subjected to centrifugation, which confirms the physical stability of the MEs.

2.3.6. Heating-cooling cycle analysis

After five heating–cooling cycles, the physical appearances of DS loaded ME was unchanged regarding transparency and phase separation. Moreover, drug precipitation was not noticed. The drug-loaded ME gel did not show any sign of creaming, cracking or phase separation. The changes in the pH of both formulations were not significant (P= 0.2824 and P= 0.3624) for drug-loaded ME and drug-loaded ME gel, respectively. Therefore, the studied formulations were considered physically stable.

2.3.7. Particle size analysis

One of the most important characteristics to evaluate ME stability is to measure their particle size. A Zetasizer (DLS) was used to detect the particle size of the drug loaded ME and the gel-based ME. The results of particle size study are listed in Table 2-3. Amongst all, the drug loaded ME showed the lowest mean particle size of 114.4 $\pm$ 0.4726 nm while the highest was

observed for diclofenac ME gel with a particle size of 178.8 ± 2.464 nm. The mean droplet size of ME loaded with diclofenac decreased slightly in comparison to the mean droplet size of the drug-free ME. Currently, the exact mechanism by which the droplet size was decreased is not clear. However, the following two possibilities might be considered. The first is that a certain portion of the undissolved drug could perform as an emulsifying agent by the deposition of drug molecules at the interface of the ME. Secondly, by the deposition of the drug at the interface of the ME. A reduced mobility of surfactant is thought to be the cause of the decreased particle size (33). The increase size of the ME based gel might be related to the addition of carbopol 934P to the DS loaded ME. The particle size distribution of ME and gelled ME is graphically represented in (Figure 2-2). Polydispersity index indicates the uniformity of droplet size within each formulation, and varies from 0 to 1. The closer to zero the polydispersity value is the more homogenous are the particles (19). The polydispersity values of the formulations were very low (< 0.3) which indicate uniform droplet size within the formulations (34).

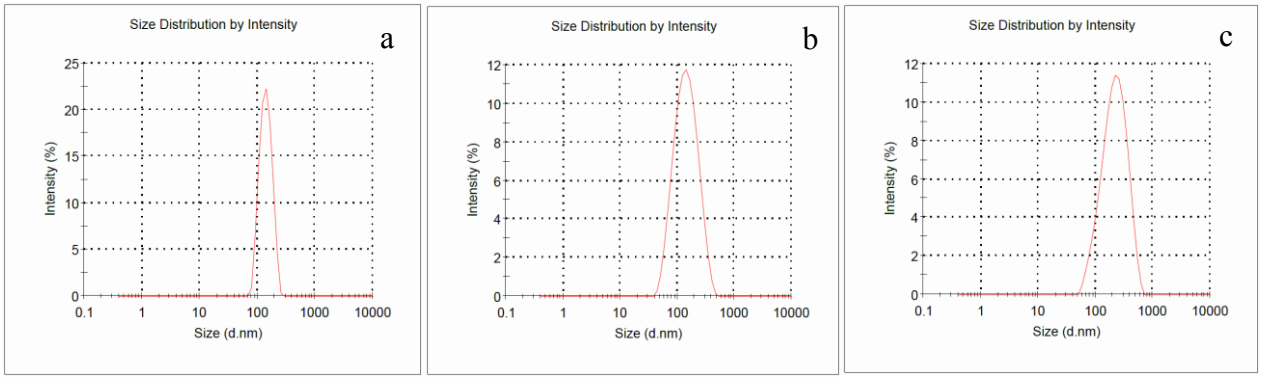

Figure 2-2 Particle size distribution of (a) drug-free ME (134.3 ± 0.351nm), (b) DS-loaded ME (114.4 ± 0.4726 nm) and, (c) DS-loaded ME based gel (178.8 ± 2.464 nm).

2.3.8. Zeta potential analysis

Zeta potential is the measurement of particle charge and/or electrostatic repulsion (20). The physical stability of any disperses systems said to increase with the increase in the electrostatic repulsion energy, which is directly proportional to the particle surface charge and the thickness of the diffusion layer (35). The negative zeta potential of MEs usually produces steric repulsive forces of hydrocarbon chains which protrude into the oil phase and subsequently hindering aggregation with neighboring oil droplets. Hence, negative zeta potential is imparting stability of a MEs systems (36). The tested MEs and ME gel based formulations showed physical stability due to their zeta potential between (-25.2 and -33.3 mV) as shown in Table 2-3. These values indicated that the prepared formulations have sufficient charge and mobility to inhibit particles aggregation.

2.3.9. Transmission Electron Microscopy (TEM) Analysis

TEM is one of the most important technique to study the microstructures of MEs. Usually, it captures any coexistent structure and produces direct high-resolution images (37). The TEM images revealed that the particle sizes for all formulations were in the nanometer range, which was confirmed by Zetasizer, and that the particles had approximately spherical morphology as shown in (Figure 2-3).

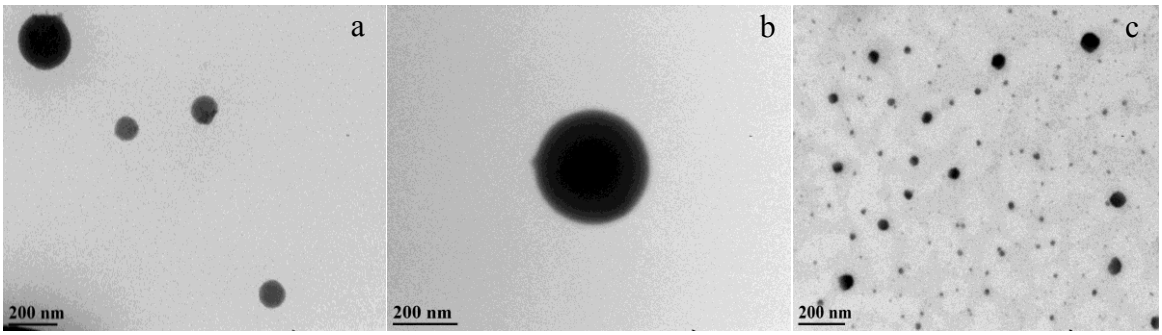

Figure 2-3 TEM images of (a) o/w drug-free ME droplets (Magnification 36,000X), (b) DS-loaded ME (Magnification 52,000X) and (c) DS-loaded ME based gel (Magnification 11,000X).

Correction for Figure 2-3: Figure (b), the size of the bar is 100 nm; Figure (c) the size of the bar is 0.5 µm

2.3.10. pH measurement analysis

The ME drug-free formulation had an observed pH value of 4.94 ± 0.078. Incorporation of DS did significantly affect the observed pH value of the ME (P = 0.0047) (Table 2-4). However, gelling the DS loaded ME with carbopol significantly reduced the pH to 5.47 ± 0.02 (P< 0.01) which is a suitable pH value for topical applications close to the pH of the skin (38).

2.3.11. Rheological studies analysis

It has been observed that the viscosity of formulations can differ (Table 2-4), after the addition of diclofenac or adding carbopol gel. The ME containing diclofenac had higher viscosity value relative to the drug-free ME. Nevertheless, both exhibited Newtonian flow behavior. The studied diclofenac ME-based gel showed a shear thinning behavior with a viscosity in the range of 108.30 ± 24.74 cP, which is significantly higher compared to the other MEs formulations (P< 0.01).

2.3.12. Qualitative studies analysis

An electrical conductivity meter was used to assess the conductivity of ME samples. Due to the conductivity behavior of aqueous phase, o/w MEs express higher conductivity values than the w/o MEs (19). It was found that the formulations had average conductivity ranged between 0.0082 mS/cm and 0.0477 mS /cm, which depict o/w ME structure (Table 2-4). The microemulsion conductivity was influenced significantly ($p < 0.05$) by the addition of the drug. The conductivity values for the drug-loaded ME increased in comparison to the drug-free ME. However, the added drug did not influence the stability or the optical texture of the formulation. Additionally, the dye solubility test confirm an o/w system.

Table 2-4 pH, drug content, viscosity and conductivity measurements of the prepared formulations (mean ± SD, n=3).

Formulation	PH	Drug content %	Viscosity cP	Conductivity mS/cm
ME	4.94 ± 0.078	___	34.00 ± 0.24	0.0082 ±0.000563
Diclofenac ME	8.166 ± 0.282	99.496 ± 0.992	36.70 ± 0.21	0.0477±0.00133
Diclofenac ME gel	5.466 ± 0.020	99.093 ± 0.671	108.30 ± 24.74	0.0163±0.000153

2.3.13. *In vitro* drug release studies

In vitro drug release from all formulations are illustrated in Figure 2-4 (F1-F5 MEs) and Figure 2-5 (semisolid dosage forms). From the data obtained, it was observed that the lowest drug release of DS through the hydrophilic membrane was out of formulation 4 (33 ± 7.1 %)

with 10% water content. Formulation 2 and 3 exhibited similar release rates at 6 hours of about (44 ± 2.8%). Both formulations differ in their Smix and water concentrations but have similar oil concentrations. Based on the conducted release study, it was found that about 52% of the drug was released from formulation 1. The formulation with the highest diffusion rate was Formulation 5 with (83 ± 3,6 %) drug release through the hydrophilic membrane. This formulation has a 1:1 ratio of water and oil phase. The ME with the highest diffusion capability (formulation 5) was gelled with carbopol. Both un-gelled and gelled formulations were then tested against each other and commercial/compounded formulations/preparations using a hydrophobic membrane to simulate the stratum corneum. A carbopol gel loaded with 3 % DS and DS in vaseline were used as controls. The latter showed no release while the gel-drug formulation released only (4.37 ± 2.52 %). The ME showed as expected a higher drug release compared to its gel form where (76.67 ± 9.46 %) of DS was release from the ME versus (69.28 ± 7.14 %) from the gel form. This is presumably due to the increased viscosity. Other authors postulated that carbopol might hinder the drug release by entrapping the drug into its structure or by producing chemical interactions with the drug (39).The release of DS from the commercial formulation and the PLO gel was about (35.50 ± 1.77 %) and (36.60 ± 1.67 %) respectively. This is nearly half of the release of the drug loaded ME and its gel after six hours. Only, 17.04 ± 3.30 % of the DS was released from the lipoderm based formulation, and less than 5 % was released from the versapro gel. From the current *in vitro* release study, it was observed that the DS loaded ME, and its gel form had significantly higher drug releases as compared to marketed formulations and the controls. *f*2 comparison of all dissolution profiles indicated no similarity at ($p < 0.0001$) as shown in (Table 2-5). Conversely, no significant differences were observed in the release rates of DS from ME, and ME based gel ($P = 0.1711$). The *f*2 comparison showed similarity despite the difference in their viscosity and total amount released. Possible explanations for the high drug release from

the ME and the ME based gel could be for two reasons. First, the high solubility profile of DS in the ME based formulations might be a significant factor in increasing the drug release rate since only the dissolved fraction of a drug in a vehicle can cross the membrane. Second, the MEs structural organization may play a major role in enhancing drug transport across the membrane where small droplet size coupled with low interfacial tension due to high surfactant/cosurfactant concentration could potentially improve drug permeation across the membrane (39).

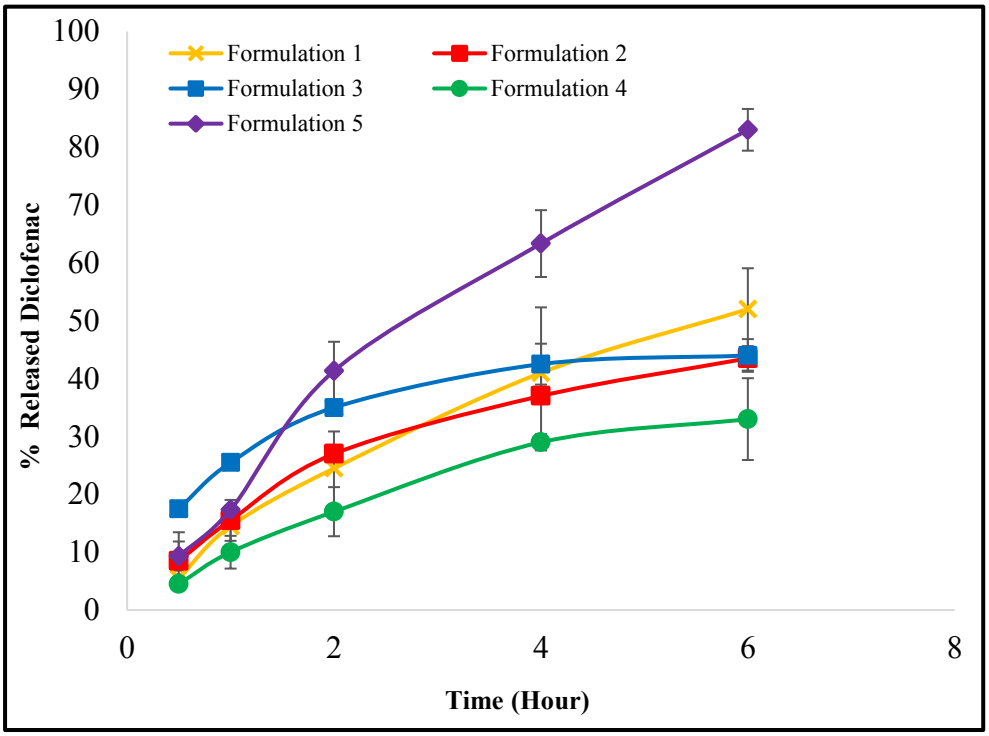

Figure 2-4 *In vitro* release profiles of DS through Hydrophilic PVDF membranes from F1-F5 microemulsions that were prepared with different ratios of Caprylocaproyl polyoxyl-8 glycerides / Diethylene glycol monoethyl ether 75 % (2:1), Propylene glycol monolaurate and water.

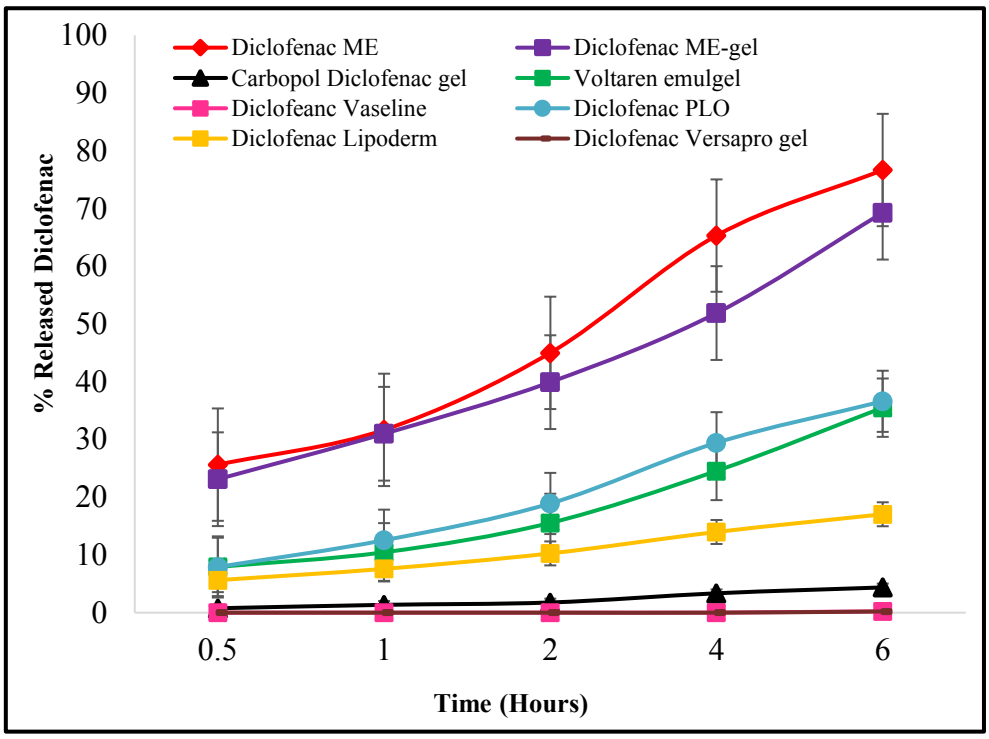

Figure 2-5 *In vitro* release profiles of DS through Hydrophobic PVDF membranes from the ME, ME based gel, different marketed formulations, carpopol gel and Vaseline with free drug as controls.

Table 2-5 Results of similarity factor (*f2*) for the dissolution profile comparison of DS between the ME, ME-based gel, different marketed formulations and the controls. Bold values indicate similarity

Formulation	Similarity factor (*f2*)						
	Diclofenac ME	Voltaren emulgel	PLO gel	Lipoderm gel	Varsapro gel	Carbopol gel	Vaseline base
Diclofenac ME	—	25.02	26.74	19.39	13.39	15.07	14.01
Diclofenac ME gel	**56.59**	30.05	32.14	23.19	16.91	18.14	16.92

2.4. Discussion

The rationale for developing topical NSAIDs is to have a localized delivery method that decreases systemic absorption and potentially limits toxicity without compromising local effect and therapeutic benefits (40). However, topical NSAIDs have been skeptically reviewed since their approval to treat pain under arthritic conditions and soft tissue injuries. Topical diclofenac has however been the most widely studied drug in regards to musculoskeletal disorders (45). In the majority of studies evaluated topical diclofenac formulations (sodium lotion, lecithin, sodium gel or epolamine gel, patch or plaster) were found to be superior to placebo formulations and as effective as oral diclofenac formulations in relieving pain, improving physical function, and enhancing patient's overall pain assessment (40,42-44). Yet, over the years compounding pharmacies started to provide non-commercially available strengths of diclofenac in different semisolid bases. Since then, physicians began to increase the drug amounts and prescribed 3%, 5% and in some cases 10%, or more DS (45-48). The anecdotal reason for this increase was, the belief that higher doses would work better or the prescribed dose did not effectively relieve pain, and more drug was needed. However, scientifically one must have the side effects of NSAID in mind. It is known that diclofenac increases the chance of a heart attack or stroke that can lead to death. Tugwell et al. compared 1.5% (w/w) topical diclofenac solution versus oral diclofenac in patients with osteoarthritis in their hands. Safety analyses revealed that some patients treated with topical diclofenac had developed gastrointestinal (GI) adverse events, including dyspepsia, abdominal pain, diarrhea, and nausea and number of patients developed abnormal liver function tests (involving clinically significant elevation), hemoglobin, and creatinine clearance (45). Shainhouse et al. reported the use of topical diclofenac with DMSO for treating osteoarthritis in knees. They found that 12% of patients had developed GI events that

included gastrointestinal reflux, dyspepsia, nausea, diarrhea, abdominal pain, liver function test abnormalities, and GI bleeding (49). Cardiovascular events were reported for 9.1% of patients and included angina, myocardial infarction, arrhythmia, palpitations, venous thrombosis, and hypertension. In most clinical trials employing 1-2 % (w/w) topical diclofenac solution gastrointestinal or cardiovascular adverse events were reported (45). This raises the question of why physicians and compounding pharmacies keep elevating the amount of drug in the topical formulations? One answer, as supported by this study is, that the prepared formulations did not deliver the drug to the site of action. Otherwise, an increase in the side effects should be observed when the dose was increased. An essential requirement for a successful topical therapy is the ability of a drug to be carried by a vehicle to penetrates through the skin in sufficient amounts and at an adequate rate (50). Therefore, this study aimed to develop and test a topical formulation that improves the topical delivery of diclofenac sodium and to compare the drug release from different formulations including compounded ones. Microemulsions are promising drug delivery systems that improve the absorption of poorly absorbable compounds. However, their development and stability depend highly on the selection of suitable excipients (51). From the above findings, it was seen that the high solubility profile of diclofenac in the propylene glycol monolaurate, caprylocaproyl polyoxyl-8 glycerides, and diethylene glycol monoethyl ether made these excipients to suitable candidates as oil, surfactant, and cosurfactant system, respectively. The results of the physicochemical property assessments of the drug-loaded ME and its gel indicated the physiochemical stability of both formulations. Following a 6-hour period of *in vitro* permeation studies, it was found that the drug loaded ME and its gel based formulations delivered more DS to the receptor media relative to the other bases. By comparing the drug release data using similarity factor *f*2 and univariate ANOVA, it was found that permeation of diclofenac from the tested formulations was significantly higher than the commercial or

the compounded formulations. The low permeation profile of diclofenac from the versapro gel is probably due to the different study duration and conditions performed by MEDISCA where they found that 16% of the diclofenac was released within 24h through cadaver skin (52). Similarly, the release from the commercially available gel was also significantly lower than that from the ME formulations. This result is partially consistent with a published study employing DS in w/o ME systems. In this study, the permeability rates of DS from MEs were the highest among different formulations while emugel showed the lowest release (5). No published *in vitro* release study was performed on Lipoderm loaded with DS yet. However, it was found that the permeation of Ketoprofen, another hydrophobic drug, from Lipoderm was about 14% which is close to that of DS from this study (53). PLO gel of DS was reported in many studies to provide short-term pain relief (54-55). However, no *in vitro* study was published to measure the permeation of DS from this type of base. Nevertheless, Richards compared the permeation of Ketoprofen with different pharmaceutical agents from PLO gels. It was found that substantial amounts of Ketoprofen retained on the skin surface and only low amounts permeate the skin (56). The findings from this study were consistent with previous studies that improved the permeation of topical medicaments over marketed formulations by employing topical ME based preparation (57-59).

2.5. Conclusion

This study demonstrated that microemulsions can be used as topical delivery system for DS. The *in vitro* diffusion rate and the amount of diffusion was significantly increased in comparison to a marketed formulation and different compounded preparations. The optimum formulation of the ME consisted of caprylocaproyl polyoxyl-8 glycerides/ diethylene glycol monoethyl ether 75% (2:1), propylene glycol monolaurate 12.5%, and water 12.5%. An ME-based gel was successfully prepared by incorporation of carbopol 1.5% as a gelling agent. Carbopol was used to increase viscosity which increases the ease of topical administration and potentially residence time. Considering *in vitro* release and physicochemical property tests, the gel base may be preferable for patients due to better handling while administering the formulation to the skin. Therefore, it can be concluded that the prepared ME-based gel with carbopol has a great potential for the delivery of DS via the topical or transdermal route of administration.

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Chapter 3

3. Formulation, Characterization and Evaluation of a Microemulsion-based Foam as a Topical Drug Delivery vehicle for Diclofenac Sodium

Abstract

The aim of the current study was to develop, evaluate and compare the *in vitro* transdermal potential of a microemulsion-based foam for improving the transdermal delivery of diclofenac sodium (DS). Microemulsions (ME) were prepared using caprylocaproyl polyoxyl-8 glycerides, polyglyceryl-3 dioleate, caprylic capric triglycerides, and water. The prepared formulations were assessed for physiochemical stability, *in vitro* drug release using Franz diffusion cells in comparison to different compounded and marketed preparations, and foam foamability and foam stability. Mean droplets size for the ME-based foam systems were less than 48 nm, whereas the zeta potential values were approximate to -34 mV. No significant changes in the pH nor physical appearance alterations were detected after employing stability testing. Additionally, TEM images for drug-loaded ME and its free form exhibited nano-droplets that were almost spherical. The release rate of DS formulated as foamable ME was statistically significant and the highest among the tested formulations (75.586 ± 9.074 % after 6 hr) where the *f*2 comparison of all release profiles showed no similarity at ($p < 0.0001$) except between a liquid and a foam of the same ME. Here the *f*2 of the two formulations was similar. The foam produced from the drug-loaded ME was classified as "1" whereas "2" for the foam generated from the drug-free ME on Abram and Hunt's foam quality scale. According to the percentage of foam expansion, foam volume stability and foam liquid stability, the foam produced from the DS-loaded ME had a higher stability profile at room temperature and 32°C in comparison to the drug-free foam. The results suggested that the DS-loaded ME- based foam system is a potential vehicle for enhancing the topical penetration of diclofenac sodium.

3.1. Introduction

The paramount aim of pharmaceutical product development is to produce effective products based on state-of-the-art active pharmaceutical ingredients with enhanced patient compliance and usability. The drug delivery system that is used to deliver topical therapeutics can considerably influence their performance (1). The vehicle can have a direct impact on the skin condition as a barrier, as it can improve or retard the delivery of the active substance to the site of action. Additionally, it can affect the physical appearance and sensory properties of the skin, and that might influence patient compliance (2). Users of topical formulations apply a wide spectrum of preparations. These preparations range from semi-solid compositions, such as creams, lotions, gels, and ointments to liquid such as topical solutions (3). The current markets are mostly dominated by conventional topical products. However, developing new forms are desirable, to achieve enhanced control of the drug release pattern, improved skin absorption, and to improve patient compliance (2).

Despite the lack of comparison to traditional formulations, the analysis of patent literature indicates that more and more interest is being shown towards foams (4). Foams are becoming a prominent drug delivery system for topical therapeutics. This platform acts as an innovative, easy to apply, contemporary alternative to creams, gels, and ointments (2). The marketing potential of foam vehicles is not the only reason behind the growing interest of the pharmaceutical industry, but also thanks to their significant advantages which include, but are not limited to their ability to spread easily on large skin areas and minimize the rubbing that is often needed in traditional topical dosage forms. Furthermore, after application, they usually do not leave a greasy or oily layer on the skin and do not impart a greasy feeling upon and after each application. An additional benefit of incorporating foams in metered-dose

topical transdermal dispensers is that they deliver a precisely controlled quantity of drug on each activation which may improve patient compliance and enhance safety profiles (2,4).

The first reported use of a foam in dermatology was in 1977 by Woodward and Berry, who compared the therapeutic effect of Betamethasone benzoate foam with an equivalent semisolid dosage form (5). The activity of the foam was found to be similar to the ointment but better than a cream. Since then, many studies have been conducted, and many comprehensive reviews were written about topical pharmaceutical foams. One of which is a study that was performed by Tamarkin et al., to evaluate the usability profile of foams versus a cream as control. This trial based on 120 patients' opinions and it was concluded that the foam was significantly better than the control with regarding the ease of application, uniform spreading, greasy feeling, stickiness, and appearance (6). The efficacy of topical foams has been reported in several studies. A randomized, double-blind trial regrading clobetasol propionate foam showed that the foam is more effective than a control for treating psoriasis (7). Similarly, another study reported that a foamed vehicle delivers more clobetasol than other corresponding formulations (cream, lotion, and solution) using human skin as an *in vitro* permeation model (8). The success of foams as topical drug delivery is not limited to corticosteroids. The penetration of ketoconazole from foamed vehicles across a silicone membrane was found to be 11-fold higher than that from creams (8). Currently, only a few pharmaceutical foam products are available in the market. Luxiq Foam contains 0.12% betamethasone valerate, and Olux Foam, contains 0.05% clobetasol propionate, both are thermolabile (temperature-sensitive) steroid hydroethanolic foams. EpiFoam, which is indicated to relieve corticosteroid-responsive dermatoses manifestations, contains hydrocortisone acetate 1% and pramoxine hydrochloride 1% (2). Evoclin is a hydroethanolic foam, comprising 1% clindamycin, which is prescribed for acne (9).

As foams are highly dynamic systems, and wide-ranging methods can be used to produce them, it is not surprising that various new methods have been developed to generate pharmaceutical foams for multiple medications. Diclofenac Sodium (DS) is one of the most widely used drugs in the treatment of pain and inflammation (10). Yet, it seriously associated with dose-dependent gastrointestinal, renal and, cardiovascular adverse reactions. Consequently, different DS- containing drug formulations have been developed with the goal of enhancing efficacy, tolerability, and patient convenience. Notably, research has focused on developing topical DS formulations to enable local treatment of pain and inflammation with minimizing systemic absorption of the drug (11).

Therefore, this study aimed to both (1) develop a foamable DS formulation as an alternative dosage form for improving topical delivery of DS, (2) and to evaluate the physiochemical stability and to compare the *in vitro* performance of the prepared formulation with commercial and pharmacy compounded formulations. For that, a microemulsion system was employed as a base for the production of foam for its clarity, thermodynamic stability, and the ability to enhance the transdermal absorption of drug molecules through increasing drug solubility (12,13).

3.1.1. Foam Definition:

Despite the wide use of the term (Foams), the definition of foam is ambiguous in regard to topical application. Weaire and Hutzler defined it as a “two-phase system in which gas cells are enclosed by liquid” (14). Purdon et al. defined pharmaceutical foams as “pressurized dosage forms, containing one or more active ingredients that, on valve actuation, emit a fine

dispersion of liquid and/or solid materials in a gaseous medium" (3). The European Pharmacopeia published a monograph called "Medicated Foams", which defines foam as "formulation, consisting of large volumes of gas dispersed in a liquid generally containing one or more active substances and a surfactant to ensure their formation" (15). The US Pharmacopoeia (General Chapters: 1151), basically lists "foam aerosol" as a part of the aerosol section. Nevertheless, pharmaceutical foams should be considered as a transition state (16). Hence, if a foamable formulation in the aerosol container is an emulsion, the foam would be evolved upon release from the can. After being applied to the skin as a foam, it would then, return to an emulsion state at the skin (17). A schematic representation of the topical foam application is shown in (Figure 3-1).

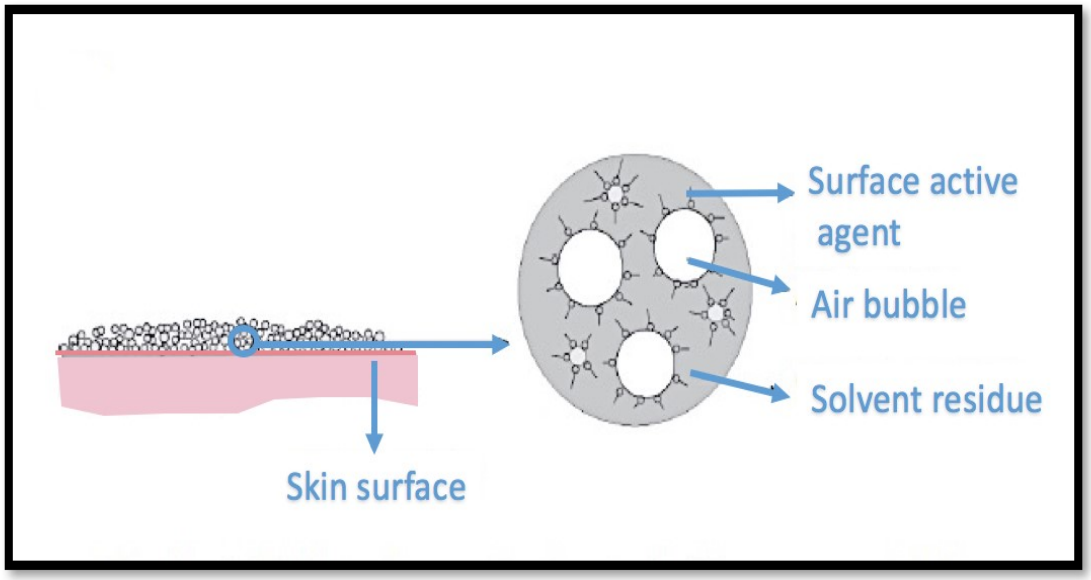


Figure 3-1 topical foam structure after dose application to the skin surface where the apparent air bubbles that dispersed in the solvent residue are stabilized by the surrounding surface active agent. Reproduced from ref (4).

3.1.2. Foam structure

Bikerman et al. has described the bubbles in foam as more or less homogeneously dispersed. They can be diverse in size and shape, ranging from almost spherical to irregular polyhedral bubbles, depending on the used excipients and on the foam generating method. The structure of the foam can also be affected by different parameters, such as the nature and the concentration of the employed foaming agent, the viscosity of the liquid phase, the temperature, and the pH of the system. Additionally, foam generation conditions can have a direct impact on the foam appearance and, consequently, the stability of foam bubbles (18). Bubbles usually disperse as uniform packed spheres in the liquid phase at moderate gas phase volumes. However, at higher phase volumes, normally higher than 0.7, the neighboring air bubbles start to deform themselves to form polyhedral-shape bubbles with partly plane faces (19). The thin film of the continuous liquid phase that separates between two adjacent polyhedral bubbles is known as lamellae (10 nm and 1 μm), while the place where three lamellae meet, the thicker channel, is called the plateau border (20). The air bubble size is typically proportional to the plateau borders length. The liquid in lamellae is fixed by the foaming agent molecules; this fixation is very critical for foam stability otherwise the liquid drains immediately, driven by gravitational forces. Friberg and Saito et al. reported that the presence of an equilibrium between a liquid crystalline phase and an aqueous solution, of surfactant, enhances the foams stability generated from the surfactant solution. Despite the firm fixation, the liquid in a foam tends to drain into plateau channels. This is due to the fact that the pressure within the plateau region is lower than in the lamellae and air bubble regions. This process forces the lamellae to become thinner where their surface area becomes too large for their volume. Consequently, this makes the lamellae unstable and eventually rupture (17,18,21). This is schematically shown in (Figure 3-2).

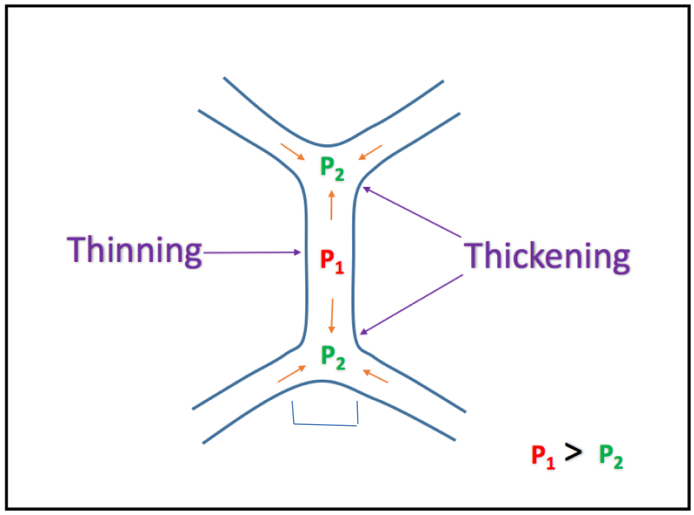


Figure 3-2 the difference of the pressure across the curved surfaces of lamellae in foam, and liquid flow towards the plateau borders. Reproduced from ref (22).

3.1.3. Effect of the formulation on foam foamability

Foams are not thermodynamically stable since they are generated from the dispersion of a gas phase in a second immiscible liquid phase, and hence are typically stabilized using foaming agents which are mostly surface active agents (23). Exceptions to certain proteins and particles that are also capable of producing foams (24). Foaming agents are amphiphilic substances that the hydrophilic part in it is responsible for their solubility in water. When a foam is generated, the hydrophobic parts of the foaming agent adjust their orientation in a way to lower the interfacial tension by minimizing the area of contact with water, and the success of that correlates to the foam stability (Figure 3-3) (17,23).

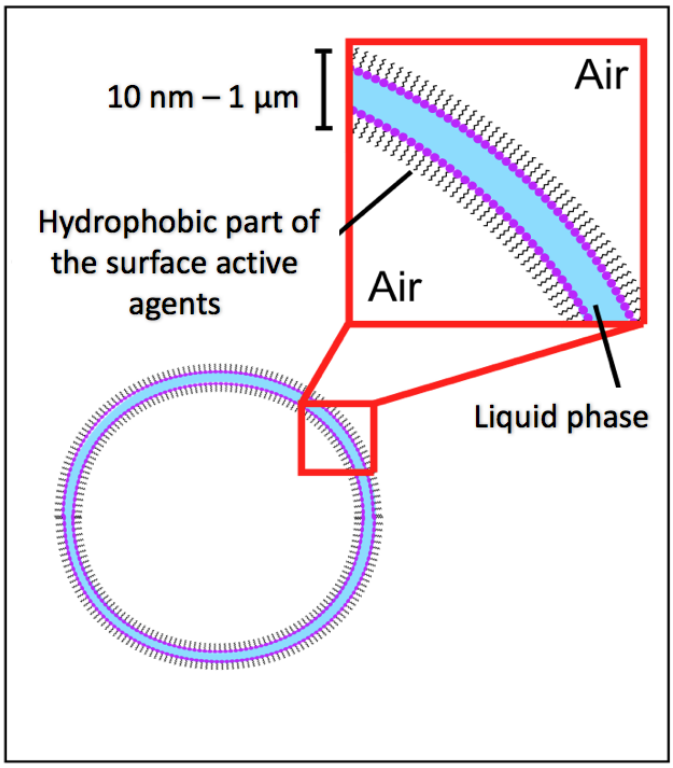


Figure 3-3 Schematic illustration of an air bubble where the surface active (foaming) agents are arranged between the liquid/air interface in a way that the hydrophobic part towards the air and the hydrophilic part towards the liquid phase.

During foam generation a rapid adsorption of the foaming agent is favorable. The rate in which the foaming agent adsorb depends on its diffusion rate. The diffusion rate of a foaming agent is given by the Fick's law of diffusion (Equation 3-1) and the Einstein-Sutherland equation (Equation 3-2), respectively.

$$\frac{dm}{dt} = -D \,.\, A \,.\, \frac{dc}{dx}$$

(Equation 3-1)

Where

$\frac{dm}{dt}$, diffusion rate $\left(\frac{mol}{s}\right)$
D , diffusion coefficient $\left(\frac{mol}{s}\right)$
A , diffusion area (mol)
$\frac{dc}{dx}$, concentration gradient $\left(\frac{mol}{L\,.m}\right)$

$$D = \frac{R\,.T}{6\,.\,\pi\,.\,\eta\,.\,r.\,N}$$

(Equation 3-2)

Where

R, gas constant 8.31 $\left(\frac{Joule}{mol\,.k}\right)$
T , absolute temperature, (K)
η , dynamic viscosity of the solvent$(mPa\,.s)$
r , hydrodynamic radius of molecule, (m)
N , Avogadro number$(6.02\,.10^{23}mol^{-1})$

The diffusion rate of foaming agent molecules towards the interface is determined by the thickness of the solution layer that can provide the surfactant to be adsorbed to a surface and on the foaming agent concentration in the liquid bulk. The latter is critical for foam formation. During foam formation, the foaming agent concentration in the bulk phase decreases with the increase of the formed surface area. This reduction leads to the decrease of the concentration gradient and, consequently, diffusion rate. Therefore, high concentrations of foaming agents and a low viscosity liquid phase are needed to guarantee a fast diffusion of

a foaming agent to the surface (17). In pharmaceutical application, surfactants are the most commonly used as foaming agents. Both ionic and non-ionic surfactants can be employed. However, the former are known for their skin irritancy, and hence non-ionic surfactants are preferred, especially when the targeted area is infected or inflamed (6).

3.1.4. Foam Stability

The stability of topical foams involves consideration of two factors: formulation stability before application (inside the container) and foam stability post-dose application (outside the container). However, evaluating the foam stability inside the canister is often neglected. On the other hand, the foam stability outside the container is well known and is mainly associated with three factors: disproportionation (Ostwald ripening), gravitational separation (liquid drainage) and film rupture (17). These processes occur simultaneously, enhance each other and lead to many possible intermediates from uniform dispersion to two completely separated systems. Ostwald ripening involves gas transportation between foam bubbles of different sizes, which triggers the growth of bubbles، and this process can be explained by the Laplace equation (Equation 3-3).

$$P = P_a + 2\gamma/R$$

(Equation 3-3)

Where

P , $pressure\ in\ a\ gas\ bubble\ \ (psi)$
P_a , $pressure\ of\ the\ atmosphere\ \ (psi)$
γ, $the\ surface\ tension\ (\frac{dynes}{cm})$
R , $the\ bubble\ radius\ (mm)$

From the equation, it is safe to say that the pressure in the foam bubbles is greater than atmospheric pressure and that the smaller the bubble radius, the greater the pressure in the bubbles. Therefore, the air diffuses from small bubbles through the liquid film into larger ones. Foam drainage is complicated and not a fully understood process (17). However, it is known as the flow of liquid through individual channels between the bubbles. It is usually driven by surface tension and resisted by viscous forces. The thickness of the channels can be reduced by foam drainage، which in result can accelerate Ostwald ripening and film rupture. Rupture of the lamellae leads to the coalescence of the bubbles and thereof foam collapse (4). Nevertheless, there are many foam stabilization actions that can be employed to enhance foam stability. One of which is the presence of higher concentrations of surfactants at the interfaces, leading to a higher elasticity of interfacial film around the foam bubbles. Thus, retarding coalescence when the bubbles became contacted. Such interfacial surfactant films might form a diffusion barrier, leading to a low gas permeability which reduces the Ostwald ripening effect. Additionally, the presence of higher gas volume fraction normally delayed the liquid bulk derange, and that leads to more stable foam (17). The rate of liquid drainage can be affected by temperature which can alter the viscosity of liquid bulk. Higher viscosities, in turn, can delay the phase break-up. Foam stabilization can also be explained by having a charged surface film, which can result in the repulsion of the neighboring air bubbles (4). Electrostatic or steric foam stabilization can be achieved by using macromolecules. The macromolecule particles can arrange themselves at the surface to provide a steric stabilization that hinders the air bubbles from coalescing (25).

3.1.5. Production of foams

There are three stages to generate a foam, first the liquid phase of foaming agent, where no air is incorporated. The formation of the emulsion of gas is the second stage where the solution starts to incorporate air. At the lower volume fractions, air bubbles do not have contact with each other, and there is no influence on bubble geometry. The third stage is the formation of polyhedral foam where the air bubbles contact each other through lamellae, and their spherical geometry is disturbed (17).

Foams can be generated by various methods. Typical approaches include (1) whipping, which can be carried out with different mechanically by agitating a liquid which may form an interface with the gas phase. In this method, the volume of the air incorporated into the foam normally increases with an increase of beating intensity. (2) Bubbling, by injecting a stream of gas or liquid, or the mixture, into a liquid. This method is reproducible and provides uniform bubble sizes (17). (3) In situ gas generation, in this method, the gas which is needed can be generated in situ as in vaginal and rectal foams by means of an effervescent formulation composition. Through the contact with mucosal secretions, the gas is generated, leading to a foam production (25). (4) Sudden pressure reduction, which rapidly actuates the valve of pressurized systems (solution or emulsion or suspension) (26).

Foams for pharmaceutical or topical use are often generated in situ employ a method called ‘pressure-fill,’ where all foam ingredients (liquid phase), e.g., active agents, foaming agents, etc., except propellants (gas phase), are measured into open canisters in a premix. Then, the containers are sealed, and the propellant is forced, under pressure to fill the final container (4). Another type is air pump foam dispensers that create foam without incorporating gas

propellants. This technology allows mixing of liquid phase and air, resulting in foam generation. In the stationary position the liquid dosing chamber is filled with the formulation. However, In the operating position the air in the dosing chamber is compressed by a piston, and the formulation and air are transferred through an uptake tube and dispensed as a foam (27).

3.1.6. Classes of foams

Foams can be classified into various groups according to their ingredients, external phase, and practical application. There are several classes of pharmaceutical foams marketed and more under development, which are different from each other in their composition and functionality. It is crucial for pharmaceutical scientists as well as for physicians to differentiate between foam classes to be able to pick the appropriate formulation for a given clinical condition. Some of these classes are oil-based foams and ointment-based foams, which consist of 6 to 75% oil as the main component for the former and up to 90% petrolatum as the main ingredient for the latter. They operate to keep medications in sustained contact with the skin and in the absence of water, protect water sensitive ingredients. However, oil-based foams mimic oil solutions or suspensions while ointment-based foams are for hydrophobic ointments. The ointment-based foam is being developed using a small amount of foam adjuvants, nonionic surfactants and hydrocarbon propellant. Another class are aqueous foams that are made from water, gelling agents and surfactants and it alternate non-greasy gels. Water and alcohol are the main ingredients for hydroethanolic-foams that are mainly used to solubilize drugs to increase their bioavailability, and also serve as suitable vehicles for oily skin areas (17). Suspended active pharmaceutical substances are usually incorporated in suspension-based foams that considered analogues topical suspensions. Emulation-based foams mainly consist of an O/W emulation or W/O emulsion

and act as a parallel to creams. Microemulsion-based foams are mostly consisting of O/W microemulsion and it improve solubility and skin delivery of active agents.

3.1.7. Characterization of foams

Macroscopic processes of foam destabilization and the observed changes in the appearance correspond directly to the microscopic changes described above. The collapse of foam bubbles leads to shrinkage of the foam volume, basically through gas loss. The problem whith measuring foam stability is the insufficient description of these processes. Conversely, it is easy to measure the increased drained liquid volume. There are different methods that can be employed to characterize foams. Some are difficult to reproduce in a laboratory and others seem irrelevant or outdated (15). Some of the commonly applied methods for foam assessment were defined in the European pharmacopeia in the monograph "Medicated foam". The monograph highlighted two essential characteristics regarding foam evaluation. The foamability of the foam and the stability of the generated foam (15). Where foamability is generally defined as the capacity of the surfactants to produce foam irrespective of the special foam properties, whereas foam stability describes the changes of foam height or volume versus time, immediately after foam generation (28). Foamability and foam stability are usually correlated where the more stable the foam films, the greater is the system’s foamability.

3.2. Materials and methods

3.2.1. Materials

Diclofenac sodium USP was purchased from PCCA (London, ON). A commercial Diclofenac-formulation Voltaren (Novartis) purchased from a local pharmacy Batch No.: K00448A, Exp. Date: 01/2018. Caprylocaproyl polyoxyl-8 glycerides NF, Polyglyceryl-3 dioleate NF and Caprylic capric triglycerides, were received as a generous gift from GatteFosse, (Montreal QC). Compounded topical formulations consisting of different bases (Lipoderm, Versapro gel, and Pluronic Lecithin Organogel PLO gel) were kindly received from a local pharmacy. Double distilled water was used for the MEs preparation. All other solvents and materials used were of analytical grade.

3.2.2. Methods

All the physiochemical experiments that was performed on the foamable ME were previously mentioned in chapter 2.

3.2.2.1. Selecting components of ME and constructing a pseudo-ternary phase diagram

The key for preparing an optimized fomable ME system is to select an oil, surfactant and cosurfactant combination with good DS solubilizing capacity. In this study, caprylic capric triglycerides was selected as an oil phase, caprylocaproyl polyoxyl-8 glycerides as a surfactant and Polyglyceryl-3 dioleate NF as a cosurfactant. DS was found to have a high

solubility profile in these components, accordingly, they were chosen for producing the foamable ME (Table 3-1). The pseudo-ternary phase diagram was developed for detecting the ME domains using different possible concentrations of oil, surfactant, cosurfactant and water. The phase diagram was constructed using an aqueous phase titration method at ambient temperature. It was prepared at surfactant/cosurfactant ratios (Smix) of 6:1. Then, the oil and Smix were combined in different weight ratios that ranged from 1:9 to 9:1. The systems were stirred during the addition of the water to ensure a thorough mixing. The end-point of the titration was when the mixtures became turbid or cloudy. Based on the ME region, the selected ME system had the highest water content (> 75%) in the ratio presented in Table 3-2. The areas corresponding to either microemulsions or macro/conventional-emulsions were constructed inside the triangular phase diagram using Microsoft Excel 2015.

Table 3-1 Diclofenac sodium solubility in oil phase, surfactant and cosurfactant.

Phase type	Excipient	Drug Solubility
Oil	Labrafac Lipophile WL1349 (Caprylic capric triglycerides)	28.96 ± 1.80 mcg/mg
Surfactant	Labrasol (Caprylocaproyl polyoxyl-8 glycerides NF)	10.47± 0.33 mg/mg
Cosurfactant	Plurol Oleique CC (Polyglyceryl-3 dioleate NF)	91.846 ± 1.90 mcg/mg

3.2.2.2. Preparation of Drug-loaded ME

ME formulations were formed spontaneously by mixing caprylocaproyl polyoxyl-8 glycerides NF as a surfactant with polyglyceryl-3 dioleate NF as cosurfactant at a 6:1 ratio. Water and caprylic capric triglycerides were added directly and mixed gently at room temperature. An amount of 3% w/w DS was added directly to the foamable ME and the formulation was stirred for 5 min at 600 rpm until a clear ME was formed (Table 3-2) (29)

Table 3-2 Percentage Composition (% w/w) of the drug-free ME and the drug-loaded foamable ME formulations

Excipients	ME	Drug-loaded ME
Labrasol (Caprylocaproyl polyoxyl-8 glycerides NF)	18	18
Plurol Oleique CC (Polyglyceryl-3 dioleate NF)	3	3
LabrafacLipophile WL1349 (Caprylic capric triglycerides)	0.5	0.5
Diclofenac Sodium	-	3
Purified Water	q.s.	q.s.

2.1.1.

3.2.2.3. Evaluation of the prepared foamable ME

The stability of the foamable ME formulation was assessed by different thermodynamic stability tests, to overcome any problems related to metastable and unstable formulations during storage. In addition, the produced foam was evaluated to detect its stability profile.

3.2.2.3.1. Physical appearance

The prepared ME loaded with DS was examined visually for its color, homogeneity and consistency (30) Foam also was assessed macroscopically, with the determination of such characteristics as being fine pored or coarsely porous foam, viscous or runny foam (17).

3.2.2.3.2. Percentage transmittance and refractive index

The optical clarity of the foamable ME with and without drug was detected by measuring the percentage transmittance of the formulations using a UV-Visible spectrophotometer (Milton Roy Spectronic 3000 Array UV). The MEs were analyzed at 650 nm against distilled water as a blank solution and three replicates were performed for each ME (31). The Refractive indices of the formulations were measured in triplicate at 25°C using K7135, ABBE Refractometer.

3.2.2.3.3. Centrifugation study

The foamable ME based formulations were subjected to be centrifuged by Microleter Centrifuge (Heraeus Biofuge Pico) at 10000 rpm with relative centrifugal force (RCF) 8,960 g for 30min at 25℃ and spotted for any alterations in their homogeneity (32).

3.2.2.3.4. Heating-cooling cycle

The heating-cooling cycle was carried out to assess the stability of the ME based formulations under extreme conditions. Both the drug-free ME and the drug-loaded ME were kept at 0 °C for 48 h then at 25 °C for 4 h, each cycle was repeated five times (33). At the end of the experiment, both formulations were evaluated for any changes in their pH,

homogeneity, and consistency. This experiment was performed using stability chamber (Sanyo Electric c., Japan).

3.2.2.3.5. Particle size measurement

The foamable MEs particle size determination was done using Zetasizer Nano-DTS 1060 (Malvern Instruments Ltd, UK) at 25^{o} C and 173^{o} fixed angle. The samples were kept in disposable cuvettes, and observations were performed in triplicate following a proper dilution of the formulations in double distilled water. The samples polydispersity index (PDI) was used as the size distribution parameter (34).

3.2.2.3.6. Zeta potential determination

The surface charge of the foamable drug-loaded ME and drug-free ME was determined by the dynamic light scattering method employing a Zetasizer Nano-DTS 1060 (Malvern Instruments Ltd, UK). Analysis time was kept for 50 seconds. The zeta potential was measured by clear zeta dip cells after dilution of all samples with double distilled water. All the results were the average of three measurements (33,35).

3.2.2.3.7. Transmission Electron Microscopy (TEM)

To inspect the morphology and structure of the drug-free and the drug-loaded foamable MEs, TEM was used for imaging the formulations (36). The images were taken by Philips / FEI (Morgagni) Transmission Electron Microscope operated with Gatan Digital Camera. To perform TEM observations, a drop of diluted (1 in 10 dilutions) MEs in distilled water

directly deposited on a copper grid, the excess was removed, and one drop of 2% aqueous solution of phosphotungstic acid was placed onto the grid and left for 30–60 seconds to allow staining, the excess was then removed (12,36) .

3.2.2.3.8. Determination of Drug Solubility

To measure DS solubility in the oil phase, surfactant, cosurfactant, and foamable MEs, an excess amount of DS was added in 5 g of the previously prepared ME and stirred for 24 hours at room temperature. Later, the formulation was centrifuged at 4000 rpm for 10 min and the concentration of the drug in the supernatant was then determined spectrophotometrically at 277 nm versus plain ME as a blank (13). The solubility profile is shown in (Table 3-1).

3.2.2.3.9. pH measurements and drug content

A digital pH meter (Accumet XL20 pH meter) was employed to measure the apparent pH of the ME formulations. All the measurements were performed in triplicate at 25°C. For determination of drug content, 1 g of the drug-free and drug loaded MEs was diluted in 100 ml PBS pH 7.4. Then, the resulting solution was filtered before subjecting it to spectrophotometric analysis. The concentration of DS was determined at 277 nm. Plain formulation without drug with the same composition was used as a blank (12).

3.2.2.4. Qualitative studies

3.2.2.4.1. Electric conductivity measurement

The conductivity analysis of the foamable MEs with and without drug was carried out using an Accumet XL20 conductivity meter. All measurements were carried out in triplicate.

3.2.2.4.2. Staining test

To evaluate the type of foamable ME, a water-soluble dye (methylene blue) was used. The dye was sparkled on the surface of the prepared ME formulations and then observed visually after 5 minutes (37). A rapid dispersion of the water-soluble dye in the system usually indicates an O/W ME system whereas for W/O MEs the dye provides microscopically visible clumps. The reverse happens using an oil-soluble dye (30).

3.2.2.5. *In Vitro* Drug release studies

This experiment was performed using 6 Franz diffusion cells with an effective diffusion area of 1.7906 cm^2 (15.1 mm diameter orifice) to determine the release rate of DS from the MEs and the drug loaded ME gel. A synthetic 0.22 µm Polyvinylidene Fluoride (PVDF) membranes were first hydrated in phosphate buffer (pH 7.4) at 25˚C for 30 minutes. The membranes were then clamped between the donor and receptor compartments. The receptor compartments were filled with 12 mL of phosphate buffer (pH 7.4). The receiver medium was maintained at 32.0 +/-0.5 °C using a circulating water-bath (Haakel D2, Germany) and magnetically stirred at 600 rpm throughout the experiment (IKA, USA). About 0.5 g of the formulations were accurately weighed and applied in the donor compartments. At five time intervals (0.5, 1, 2, 3, 4 and 6 h), 0.1 mL aliquots were withdrawn through the sampling port

and replaced immediately with an equal volume of fresh receptor solution to maintain a constant volume of the receiving solution. The samples were then analyzed spectrophotometrically (Milton Roy Spectronic 3000 Array spectrophotometer) at 277 nm against blank samples. Three replicates of each experiment were conducted. The results were plotted as a cumulative percentage of drug release versus time. The release pattern of DS from the foamable ME was examined using Hydrophobic PVDF membranes. The drug-loaded ME was examined in liquid form and again in foam form to test the difference in the release profile between the two phases. The release profiles of DS from the formulations were compared with a commercial formulation (Voltaren Emulgel as a reference formulation) containing 11.6 mg of diclofenac diethylamine, which is equal to 1% diclofenac sodium (53). Three different compounded preparations consisting of commercial or compounding bases (Lipoderm, Versapro gel, and PLO gel) each containing 5% DS, which is a commonly prescribed strength, were also tested. The foamable ME was also compared to vaseline containing 3% (w/w) free DS as a control. The percentage of drug release was calculated to evaluate the formulations to account for the different strengths. The different strengths imitate the variety of strengths a patient might apply.

3.2.2.6. Foam quality

Foam quality was evaluated using the Abram and Hunt ranking of 0–5 to assess pharmaceutical foams (Figure 3-4); where the lower the value, the more stable the foam “0” representing full, fine and stable bubble foams and “5” representing coarse, large bubble foams or foams that immediately breaking to large bubbles (38).

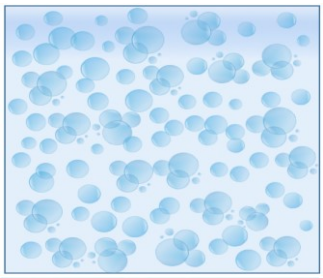
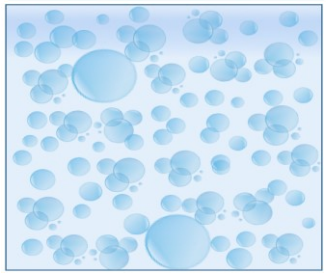
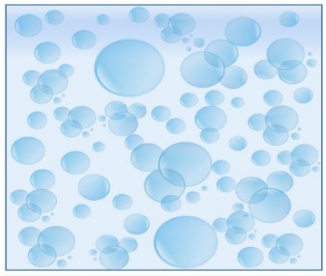
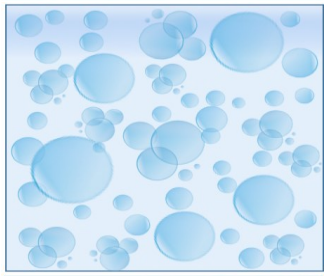
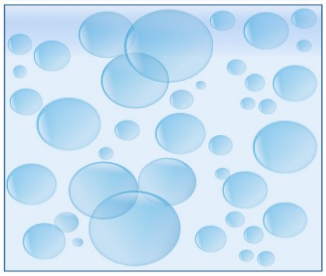
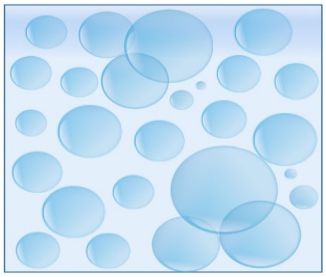
Scale	Structure	Description
0		Full, fine, stable (holds structure or only a very slow, small collapse over 30-60 sec).
1		Mostly fine with a couple of coarser bubbles on surface than stable, or fine then slightly coarser over time.
2		Slightly coarse initially but reasonably stable, or fine (possibly some slight dimples) with a couple of larger bubbles appearing on surface, or flat but and reasonably stable.
3		Slightly coarse bubbles then growing larger throughout, or very coarse but stable, or fine (possibly with dimples) then many larger bubbles appearing on surface, or fine then quick collapse.
4		Coarse bubble quickly grows to larger throughout, or fine with many larger bubbles immediately on surface.
5		Out as large bubbles, or immediate break to large bubbles.

Figure 3-4 Abram and Hunt‘s visual aid that can be used for evaluating foam structures. Adapted from ref (38).

3.2.2.7. Generation of foam from foamable MEs

Foams are generated via the bubbling method by injecting formulations and gas through a narrow opening. This foaming technique is reproducible and gives uniform bubble sizes. Two 10 ml syringes with a luer-lok™ tip attached with a Baxter sterile Rapid-Fill™ connector luer lock-to-luer lock is used (Figure 3-5). This Foaming technique is simple, where the bubbling of the MEs is done by placing 2ml of formulation and 4ml of ambient air in one syringe while the second syringe contains 8ml of ambient air. The solutions and the ambient air are pushed from one syringe to the other, and after few gas-liquid transfers, foams are produced.

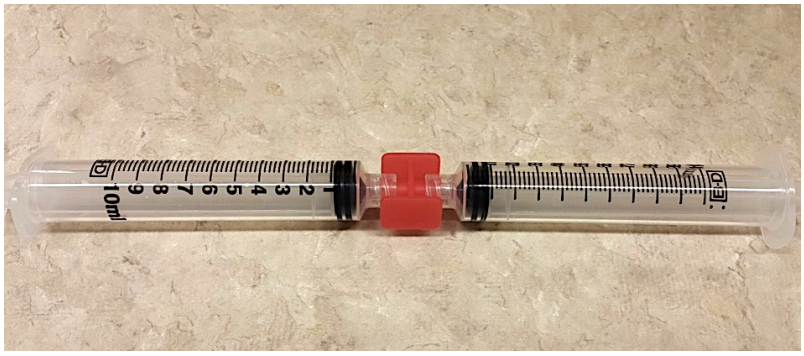


Figure 3-5 Bubbling technique for foam production

3.2.2.8. Foamability evaluation

The relative foam density (FD) which estimates the foam firmness, is one of the parameters that acts as a function of foamability. The lower relative density of foam the better the foamability profile, and it can be determined by weighing a predefined volume of foam compared to the weight of the same volume of water (Equation 3-4).

$$FD = \frac{m(Foam)}{m(water)}$$

(Equation 3-4)

Where m(foam) mass of foam per volume unit (g); m(water) mass of water per volume unit (g).

Foam expansion (FE (%)) is another parameter that has been used to assess the foamability, where higher values of foam expansion mean more foamable the formulation is. To measure FE(%), The foam is discharged into a glass cylinder, and the initial volume of foam, the volume of aged foam, and the volume of drained liquid are recorded after defined time intervals. (Equation 3-5).

$$FE(\%) = \frac{V(Foam) - V(Formulation)}{V(Formulation)} * 100$$

(Equation 3-5)

Where V(foam) volume of the produced foam and v(formulation) volume of the formulation to produce v(foam) ml.

3.2.2.9. Foam stability evaluation:

Foam stability is a vital parameter for foam evaluation that can be assessed by the determination of foam drainage rate, and collapse time by discharging foams into a measuring cylinder. The gas fraction (GF) of the foam can be detected by calculating the difference between the initial foam volume and volume of the expanded formulation. This parameter can also provide information regarding the stability where the higher the GF values, the better is the stability (Equation 3-6).

$$\mathrm{GF} = V(Foam) - V(Formulation)$$

(Equation 3-6)

Where V(foam) volume of the produced foam and v(formulation) volume of the formulation to produce v(foam) ml.

The foam volume stability FVS (%) (Equation 3-7) compares the initial foam volume with the aged foam volume at different time points. The higher the FVS values, the more stable is the generated foam. Similarly, the foam stability can be assessed by measuring the foam liquid stability (FLS%) which compares the volume of the drained liquid at different time intervals with the volume of formulation to produce the foam, the lower the FLS, the more stable is the produced foam is (Equation 3-8).

$$FVS(\%) = \frac{V(t)}{V(Foam)} * 100$$

(Equation 3-7)

Where V(t) volume of foam at a single time point (ml) and v(foam) volume of the produced foam (ml).

$$FLS(\%) = \frac{V(t)}{V(Formulation)} * 100$$

(Equation 3-8)

Where V(t) volume of liquid drained at a particular time point (ml) and v(formulation) volume of the formulation to produce the foam (ml).

3.2.2.9.1. Cylinder method

The cylinder method was used to assess the foamability and the stability of the foams generated from the ME free drug and the diclofenac-loaded ME. The produced foams were placed directly into a cylinder, and the changes of the foam's volume were measured over time. In this experiment, the foams were assessed at two different temperatures. At room temperature and at 32°C, which resembles skin temperature. At room temperature, the generated foams were placed directly in a graduate glass cylinder, and the foam volume was measured at different time intervals. However, to measure the foam volume at higher temperatures, the Franz diffusion cells were used to control the heat via a water-bath (Haake® D8-L) (Figure 3-6). The thermostatically controlled water can keep the temperature in the Franz diffusion cells constant. To reach a temperature that is corresponding to the skin temperature, the thermostatic water bath was set to 33 +/- 0.5°C. The experiment was

performed by filling a glass graduated cylinder that is compatible with a Franz cell system with a specific amount of water to gain certain height. The amount of water is defined by the volume of the graduated cylinder in contact with the thermostatically controlled water of the Franz cell system. Then, the generated foams were added directly to the water inside each cylinder, and the volume of the foams were measured at time 0. The volume of foam, the volume of drained liquid and the total volume were measured at 0, 5, 10, 15, 20, 25 and 30 minutes. FE (%), GF, FLS (%) and FVS (%) were then calculated to analyze the foamability and the stability of the foams produced from the drug-free and the drug-loaded MEs at two different temperatures (room temperature and 32 °C). Each experiment was performed three times all calculations were done according to the previously mentioned equations.

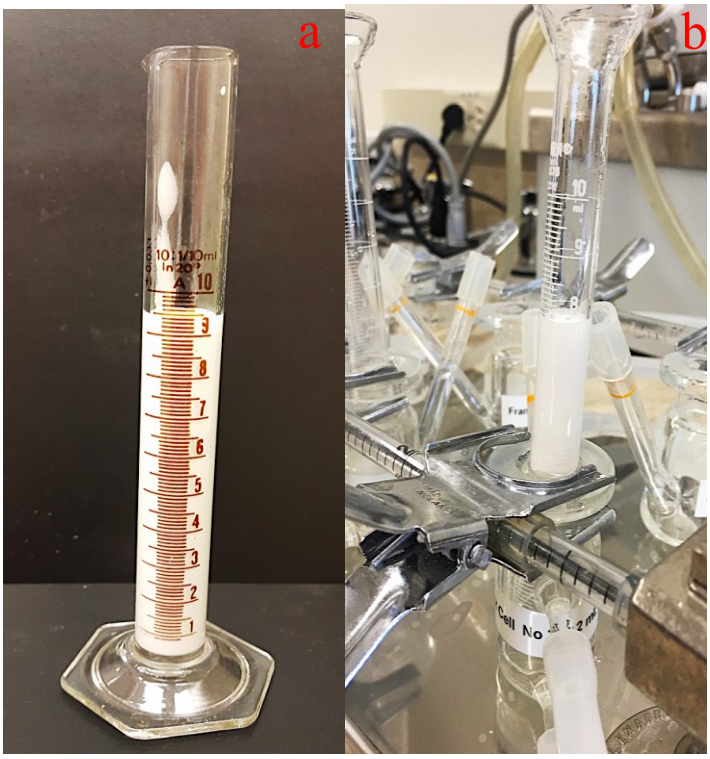


Figure 3-6 Cylinder method used to measure FE (%), GF, FLS (%) and FVS (%) of the formulated foams, (a) measuring foams volume at room temperature, (b) measuring foams volume at 32 +/- 0.5°C using Franz diffusion cells apparatus.

3.2.2.10. Compound Light Microscope

The produced foams were evaluated microscopically to characterize the process of destabilization. This was determined using a Compound Light Microscope (ZEISS AXIO) equipped with an Optronics MacroFire Digital Camera (Bright field Imaging). Foam samples were placed on glass slides and examined directly under the microscope.

3.2.2.11. Air foam pump dispenser

An air foam dispenser was employed to produce a precisely measured amount of drug-loaded foam. For that, DS-loaded ME was incorporated in the foam dispenser and stored at room temperature. Foam quality, pH, and, particle size of the formulation were analyzed after 1, 2, and 3 months.

3.2.2.12. Statistical analysis

All experiments were replicated three times and data stated as mean ± SD. Data were analyzed statistically by one-way analysis of variance (ANOVA) and the student t-test. The individual variances between formulations were calculated by non-parametric post hoc test (Tukey's test). Statistical significance was considered at an alpha of $p>0.05$. Statistical analysis was performen by SPSS software (version 19.0), and Microsoft Office Excel 2015. DDSolver 1.0 software was employed to compare different drug release profiles through univariate ANOVA and similarity factor *f2*. Calculations of f2 values was done according to the equation (Equation 3-9).

$$f_2 = 50 \bullet \log \{[1+(1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \bullet 100\}$$

(Equation 3-9)

Where *n* is the number of time points, *Rt* is the dissolution value of the reference product at time *t,* and *Tt* stats to the dissolution value of the test product at time *t*. F2 values must be greater than 50 (50-100) to ensure sameness or equivalence of two dissolution curves.

3.3. Results and Discussion

3.3.1. The pseudo-ternary phase diagram and ME formation

A microemulsion system is usually produced when an oil phase is introduced to an aqueous media and the interfacial tension between them is reached to an extremely low level due to the adsorption of surfactant and co-surfactant at the interface. This reduces the interfacial energy and the interfacial layer is maintained highly flexible and fluid-like. Improving the thermodynamic stability of the formulation and resulting in a spontaneous dispersion of one liquid into the other. This is usually met by a careful and precise selection of surfactants and cosurfactants and their respective proportions (39-41). In addition, the high solubility of the drug in the oil phase is crucial in designing a stable ME formulation. The drug should possess suitable solubility, so it can avoid precipitation during the shelf life of the formulation (42). Accordingly**,** caprylic capric triglycerides was selected as an oil phase, caprylocaproyl polyoxyl-8 glycerides as a surfactant and polyglyceryl-3 dioleate as a cosurfactant as they showed a high solubility profile of DS (Table 3-1). To develop an optimum ME, it is of great importance to evaluate the area of ME region in the phase diagram(36). Consequently, it is vital to create a pseudo-phase diagram for determining the concentration range of

components for the existence range of MEs. The microemulsion and the macroemulsion regions were shown in the pseudo-ternary phase diagram (Figure 3-7). It was observed in this study that as the surfactant/ cosurfactant mixture increases, the area of ME region increases, this is due to the interfacial tension reduction and ME fluidity enhancement (33). Based on the constructed phase diagram, microemulsions containing surfactant/cosurfactant mixture, oil phase, and aqueous phase were prepared at amount that presented in Table 3-1 and then employed for further analysis.

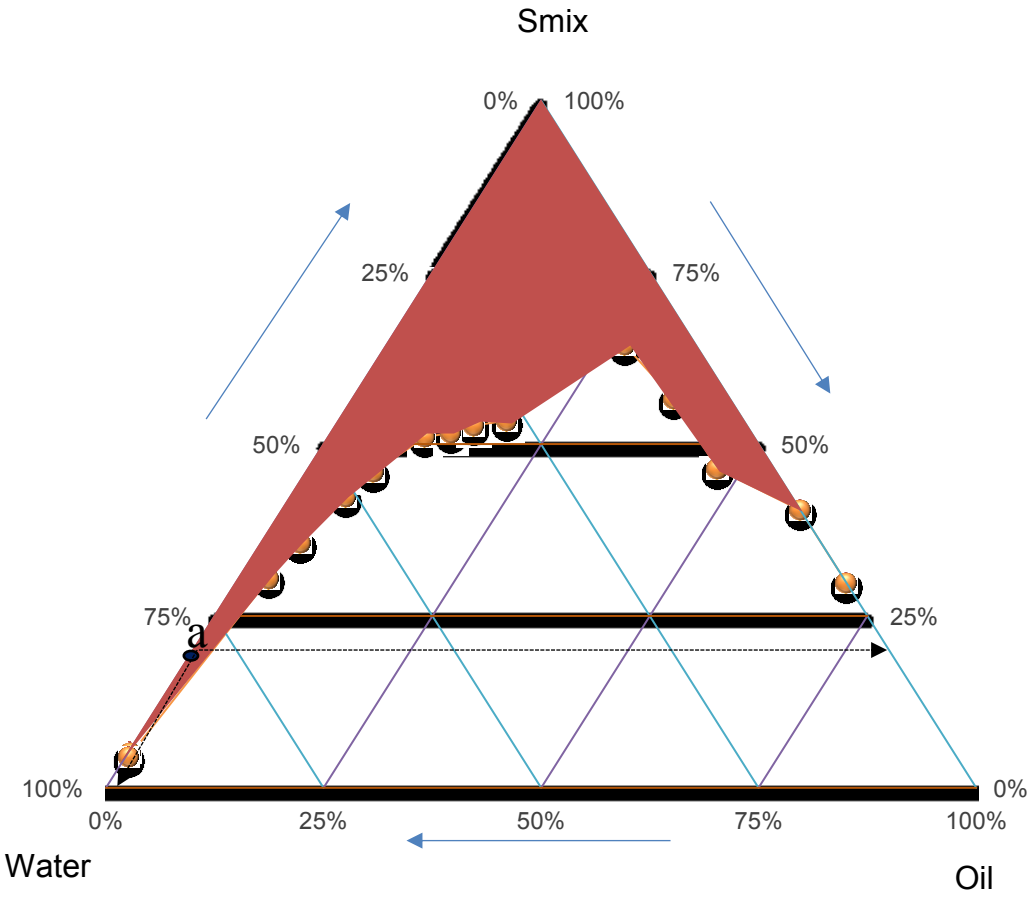


Figure 3-7 Pseudo-ternary phase diagram of the foamable ME system consists of caprylic capric triglycerides (oil), caprylocaproyl polyoxyl-8 glycerides / polyglyceryl-3 dioleate (Smix) and water. The shaded area shows the ME. The point (a) represents the composition of the optimized ME.

3.3.2. Physical appearance

The prepared drug-loaded and drug-free foamable MEs were yellow-colored, clear, transparent liquid with homogenous appearance. The foams as dispensed were viscous in appearance with typically white to off-white color and with a fine bubbles surface structure.

3.3.3. Percentage transmittance and refractive index

The percentage transmittance is one of the fundamental parameters for determining the transparency of a system. When a value of %T is closer to 100%, this signifies the clearance, the transparency and the nanometric ranged globule size of the formulation, which indicates that the formulations have a large surface area for drug release (43). The transmittance values of the foamable MEs with and without drug were greater than 98.5 % (Table 3-3), the smaller particle size might be the reason behind the clarity and transparency of the formulated systems. Likewise, if the refractive index of a system is similar to that of the water (1.333), subsequently, the formulation is transparent in nature. The refractive indices of the produced foamable formulations was around 1.36, which suggests clarity and isotropy of the MEs systems.

Table 3-3 Physicochemical characteristics of the prepared formulations (mean ± SD, n=3)

Formulation	Refractive Index	Transmittance %	Particle size nm	PDI	Zeta potential mV
ME	1.36 ± 0.471	99.26 ± 0.412	47.64 ± 0.2689	0.148 ± 0.003	-34.2 ± 0.1
Diclofenac ME	1.36 ± 0.00	98.89 ± 0.672	22.73 ± 0.1447	0.21 ± 0.013	-34.4 ± 2.26

3.3.4. Phase separation

Unlike emulsions, it is believed that MEs are thermodynamically stable systems even after an application of physical stresses like centrifugation (44) Even though the homogeneous single-phased MEs were subjected to centrifugation to confirm the absence of phase separation, they did not show any sign of phase separation, nor any precipitations when they were subjected to centrifugation, confirming their thermodynamics and physical stability.

3.3.5. Heating-cooling cycle analysis

The physical appearances of the drug-free and the drug-loaded foamable MEs were unchanged after five heating–cooling cycles. In terms of their transparency, drug precipitation was not noticed, and no sign of any phase separation was observed. The change in the pH values of both formulations was not significant (P= 0.6778 and P= 0.7676) for the drug-free and the drug-loaded foamable MEs, respectively. Consequently, the studied foamable MEs formulations were considered physically stable (Figure 3-8).

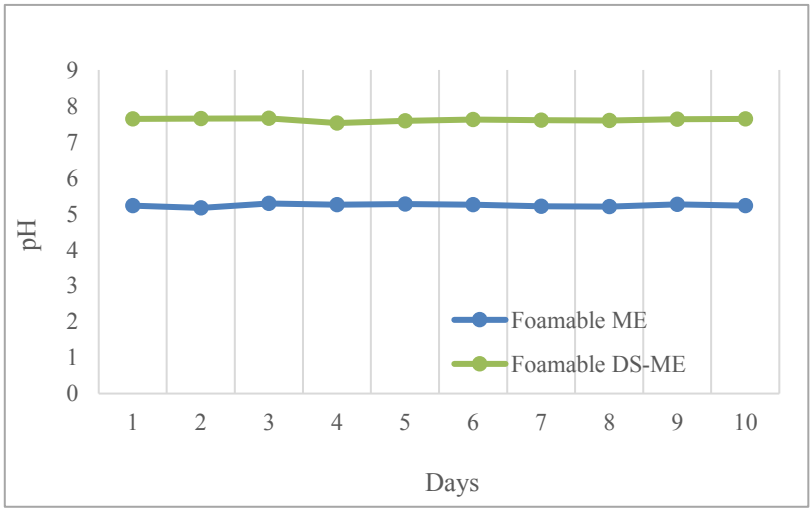


Figure 3-8 Representing the change in pH values of the drug-free and drug-loaded MEs during the heating-cooling cycles.

3.3.6. Particle size analysis

Microemulsion particle size is one of the most essential characteristics to be measured for the evaluation of any ME stability. Zetasizer (DLS) was used to measure the particle size of the foamable free and drug loaded ME systems. The results are shown in (Table 3-3). The droplet size of the ME shrank significantly ($p < 0.05$) after the addition of DS to the system, where the drug-loaded ME had a mean droplet size of 22.73 ± 0.14 nm in comparison to the mean droplet size of the drug-free ME at 47.64 ± 0.26 nm.

Currently, it is not clear what is the exact mechanism by which the droplet size was reduced. Nevertheless, the following two probabilities might be considered. First, is that a certain portion of the undissolved drug might performed as an emulsifying agent by the deposition of drug particles at the interface of the ME. Second, by dispositioning the drug at the interface of the ME, the reduced mobility of surfactant is assumed to be the reason behind decreasing the particle size of drug-loaded ME (45). The particle size distribution of the foamable MEs is graphically represented in Figure 3-9.

The polydispersity index indicates the droplet size uniformity and it varies from 0 to 1 where the closer to zero the more homogenous are the particles. The polydispersity values of the formulations were very low (< 0.22) which suggest droplet size uniformity within the ME formulations (46).

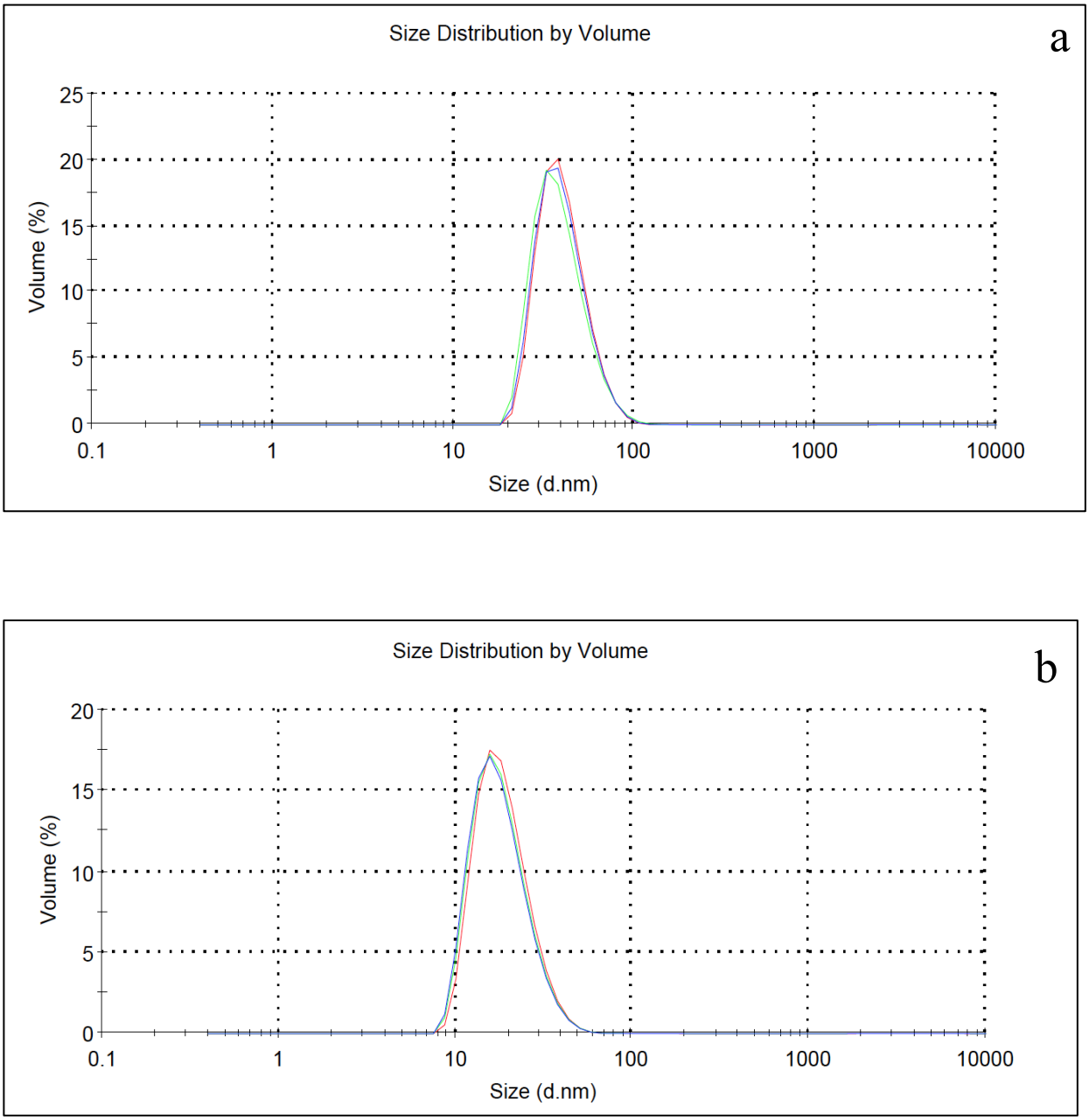


Figure 3-9 Particle size distribution of (a) drug-free foamable ME (47.64 ± 0.26 nm), and (b) DS- loaded foamable ME (22.73 ± 0.14 nm).

3.3.7. Zeta potential analysis

Investigating the zeta potential, which is a measurement of electrostatic repulsion and/or particle charge, is very crucial in case of ME systems (32). It is believed that the physical stability of any disperse system increases with increased the electrostatic repulsion energy, and this is directly proportional to the particle charge and the thickness diffuse layer (47). The presence of a negative zeta potential in MEs usually creates steric repulsive forces of hydrocarbon chains which protrude into the oil phase and hinder the aggregation with neighboring oil droplets. Consequently, negative zeta potential is an indication of the MEs system stability (30).

The tested MEs have zeta potential ranged between (-34.2 and -34.4 mV) as shown in Table 3-3. These values imply that the prepared formulations have sufficient charge and mobility to prevent particle aggregation and therefore they have the potential for physical stability (48) .

3.3.8. Transmission Electron Microscopy (TEM) Analysis

TEM is one of the most critical techniques to inspect the microstructures of any ME system. Typically, it captures any coexistent structure and yields directly high-resolution images (49). From the TEM images, it has been revealed that the particle sizes for the foamable ME formulations were in the nanometer range and that the particles were approximately spherical in their morphology as presented in Figure 3-10.

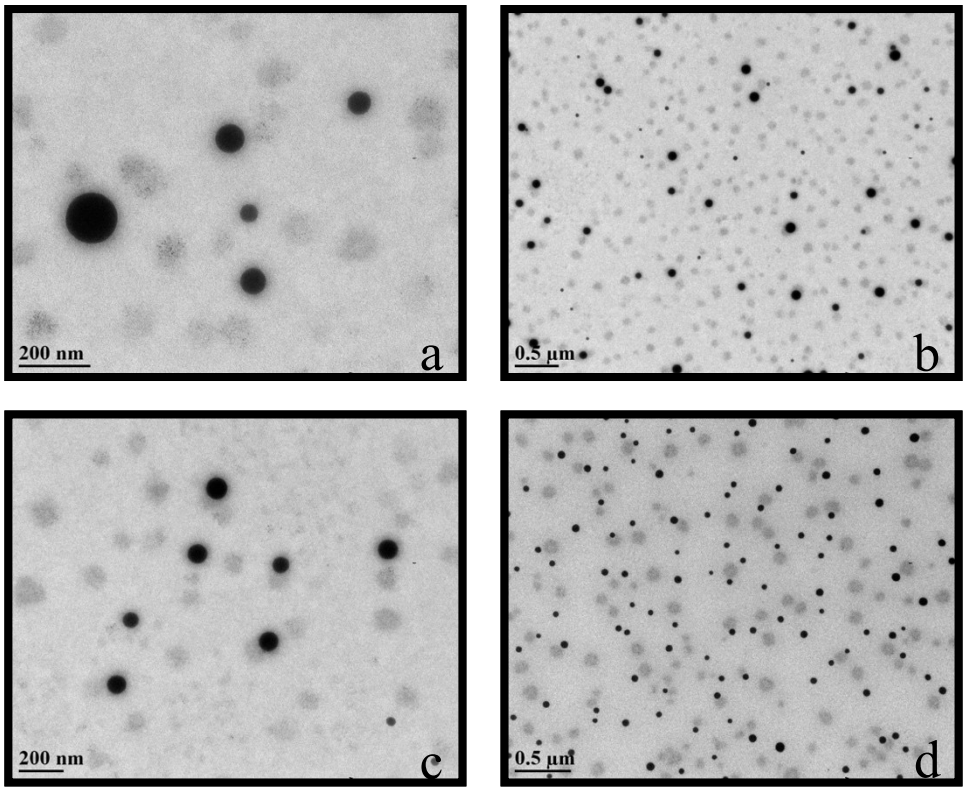


Figure 3-10 TEM images of (a) and (b) of Foamable drug-free ME droplets (magnification 38,000X and 16,000X, respectively), (c) and (d) Foamable DS-loaded ME (magnification 43,000X and 15,000X, respectively).

3.3.9. pH measurement analysis

The ME drug-free formulation had an observed pH value of 5.22 ± 0.001. Incorporation of DS significantly affected the observed pH value of the ME (7.64 ± 0.198) ($P < 0.0001$) (Table 3-3).

3.3.10. Rheological studies analysis

Generally, it has been observed that the viscosity of the foamable ME formulation changes with the addition of DS (Table 3-4), where the ME containing DS was found to have

significantly higher viscosity value (9.09 ± 0.244 cP) relative to the drug-free ME (6.76 ± 0.004 cP), (P< 0.0001). Nevertheless, both formulations exhibited Newtonian flow behavior.

3.3.11. Qualitative studies analysis

Typically, o/w MEs express higher conductivity values than w/o MEs, and this is due to the conductivity behavior of the aqueous phase (31). In this study, the foamable MEs were found to have average conductivity values ranged between 2 μS/cm and 86.9 μS/cm, which illustrates o/w ME structures (Table 3-4). The conductivity of the ME was influenced significantly ($p < 0.05$) by the addition of the drug. Even though the conductivity values for the drug-loaded ME increased yet, the addition of DS did not cause any changes in the stability or the optical clarity of the formulation. Furthermore, o/w structure of MEs was confirmed using the dye solubility test.

Table 3-4 pH, drug content, viscosity and conductivity measurements of the prepared formulations (mean ± SD, n=3)

Formulation	PH	Drug content %	Drug Solubility mcg/mg	Viscosity cP	Conductivity μS/cm
ME	5.22 ± 0.001	___	___	6.76 ± 0.004	2 ±0.02
Diclofenac ME	7.64 ± 0.198	98.71 ± 0.160	163.2 ± 5.136	9.09 ± 0.244	86.9 ± 2.36

3.3.12. Foam quality

Foams can vary according to their quality. Foam quality is an evaluation of the foam's physical appearance (50) Therefore, Abram and Hunt's scale was used for comparison purposes which includes foam bubble structure and stability over time (38). As shown in Figure 3-11, the foam produced from the drug-free ME was fine with some sight dimples, and a couple of large bubbles on the surface and this classifies the foam as "2". On the other hand, the drug loaded ME generated a stable, mostly fine foam with a couple of coarser bubbles on the surface, which ranks the foam at "1". It should be noted that the foam quality is one of the criteria for an acceptable foaming composition in developing foams, good manufacturing practice (GMP) and quality control (QC) (38).

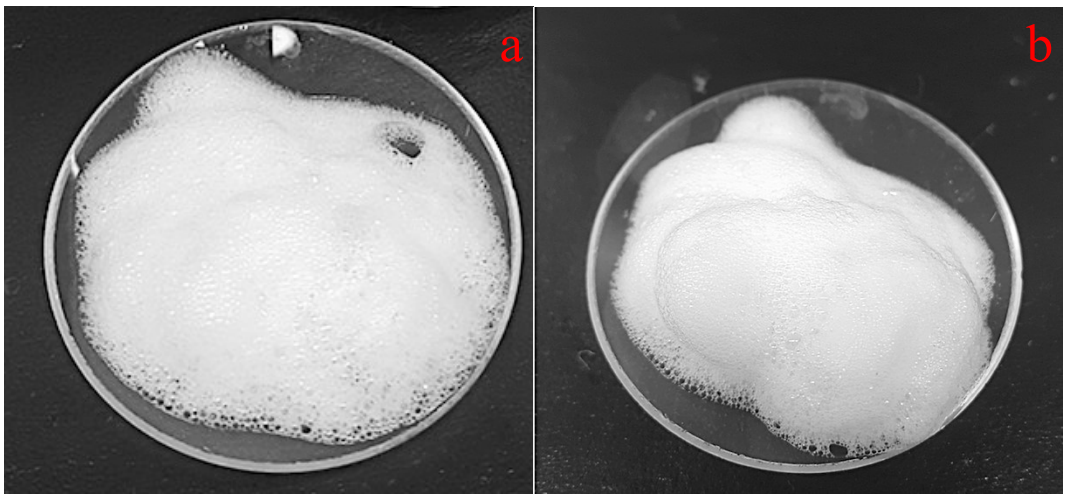


Figure 3-11 Macroscopic images of (a) foam generated from drug-free ME, and (b) foam generated from DS-loaded ME.

3.3.13. *In Vitro* Drug release studies

(The release profiles except for the foams were adapted from chapter 2). From the *In vitro* drug release shown in Figure 3-12, it was seen that the lowest drug release of DS through the hydrophobic membrane was out of the Versapro gel (0.341± 0.603%). The release of DS from the Lipoderm based formulation was 17.04 ± 3.30 %. Surprisingly, only 35.50 ± 1.77 % of the drug was released from the commercial formulation and about 36.60 ± 1.67 % from the PLO gel. This is nearly half of the release of the drug from the foamable ME after six hours. The foamable DS-loaded ME formulation exhibited the highest release with 75.586 ± 9.074 % of drug release through the membrane. This formulation has been applied to the membrane in the liquid form. Upon applying the foam form on the membrane, the drug release was marginally slower than the liquid form in the first hour. This is probably due to the time needed for braking down the foam into its liquid phase. From the current *in vitro* release study, it was observed that the foamable DS loaded ME had a significantly higher drug release among the compared control, compounded, and marketed formulations.

*f*2 comparison of all dissolution profiles showed no similarity $p < 0.0001$ except between the liquid and foam forms of the same ME where the *f*2 of the two forms were similar (Table 3-5). The high solubility profile of DS in the foamable ME formulations could be the main reason behind the increase in the percentage of drug release, since it is known that only the dissolved segment of a drug in can cross the membrane. Furthermore, the small droplet size and the low interfacial tension of the foamable ME might cause a significant improve in drug permeation across the membrane (51).

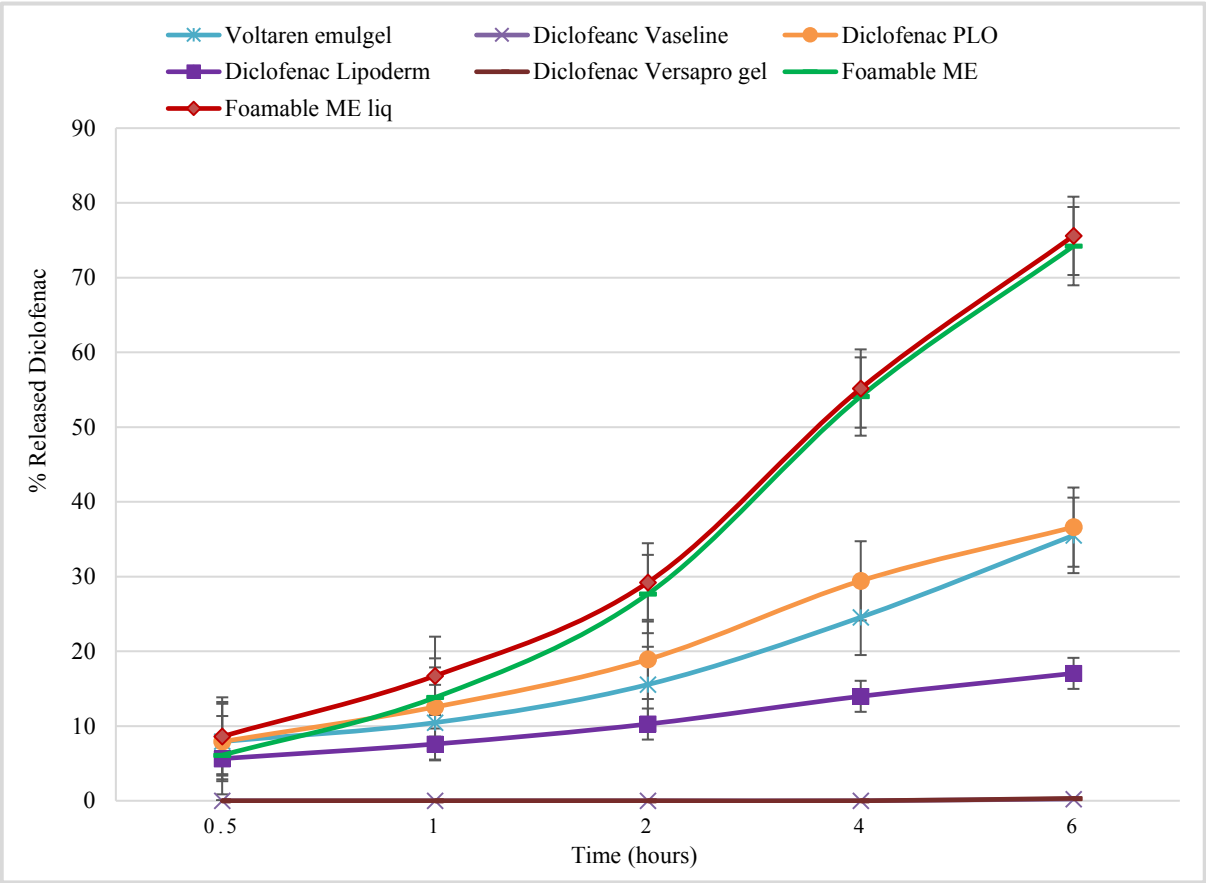


Figure 3-12 ***In vitro*** **release profiles of DS through Hydrophobic PVDF membranes from the drug-loaded ME and its foam form in comparison to different marketed formulations and Vaseline with free drug as a control.**

Table 3-5 Results of similarity factor (*f2*) for the dissolution profile comparison of DS between the foamable ME, its liquid form, different marketed formulations, and the controls.

Formulation	Similarity factor (*f2*)						
	Foamable ME Liquid	Voltaren emulgel	PLO gel	Lipoderm gel	Varsapro gel	Carbopol gel	Vaseline base
Foamable ME	96.86	32.37	34.38	24.52	18.17	15.07	18.19

3.3.14. MEs foamability and foam stability analysis

For the determination of foam foamability, the European Pharmacopoeia in the monograph "Medicated foams" have stated the relative foam density as an indication of the foam firmness, which is an indication of bubble solidity, where the lower relative density the better is the foamability. The foam expansion time was also considered in the monograph as a function of formulation foamability, where the higher the foam expansion the more foamable is the formulation (52). It was observed that the addition of DS to the foamable ME had a significant reduction on the foam firmness (figure 3-13). The relative density was increased from 0.334 ± 0.0014 to 0.581 ± 0.0060 for the drug-free and the drug-loaded MEs, respectively.

Similarly, the foam expansion showed the same trend where the values decreased for the drug loaded with DS in comparison to the drug-free ME. At room temperature, the foam expansion was significantly influenced by the addition of DS, where the values significantly decreased from 226.6± 12.583% for the drug-free ME to 158.33 ± 7.637 % for the drug loaded ME (figure 3-14). On the contrary, the incorporation of the drug to the foamable ME did not have a significant reduction on the change in the foam expansion in both temperatures. From the foam density and foam expansion measurements, it is clear that, the addition of DS did cause a reduction in the foamability behavior of the foamable ME. Nevertheless, the measurement of foam expansion time of the drug-free ME was 0 % after 10 min at both temperatures, while the values after 30 min for the drug loaded ME foam at room temperature and at 32°C were 16.667 ± 2.886 % for the former and 6.667 ± 2.88 % for the latter. This is an indication that the ME alone has an initial better foamability profile, yet the foam breakdowns faster than the DS loaded ME.

Foam stability is normally reflected by the initial volume of the foam and subsequent measurements of the foam volume as a foam ages. Gas fraction, foam volume stability, and foam liquid stability are the three parameters that are assessed to characterize foam stability. To assess the effect of temperature on the ME foam stability, all three foam stability parameters were also investigated at room temperature and at 32°C. The gas fraction parameter is desired to be high as it implies a high amount of gas incorporated into the foam and, thus, lowers the velocity of foam destabilization mechanisms due to gravitational force and the velocity of foam destabilization is also directly related to the viscosity of the system. Considering the gas fraction of the produced foams, specific trends through addition of DS to the ME was observed (Figure 3-15). Similar to the foam expansion, at room temperature, the gas fraction values were significantly influenced after incorporating the drug. The measured amount of gas in the drug-free ME at room temperature was about 4.533 ± 0.251 mL, this value decreased in the ME loaded with DS to 3.166 ± 0.152 mL. Contrarily, the addition of DS did not significantly influence the reduction in the amount of gas fraction in the MEs where the values ranged between 2.466 ± 0.152 mL and 2.633 ± 0.208 mL for the drug-free ME and the drug loaded ME, respectively. Through measuring foam gas fraction over the time, it was found that no gas fraction was detected from the drug-free ME at both temperatures after 5 min, yet about 0.533 ± 0.416 mL and 0.333 ± 0.152 mL of gas was detected after 10 min in the DS loaded ME at 25°C and at 32°C, respectively.

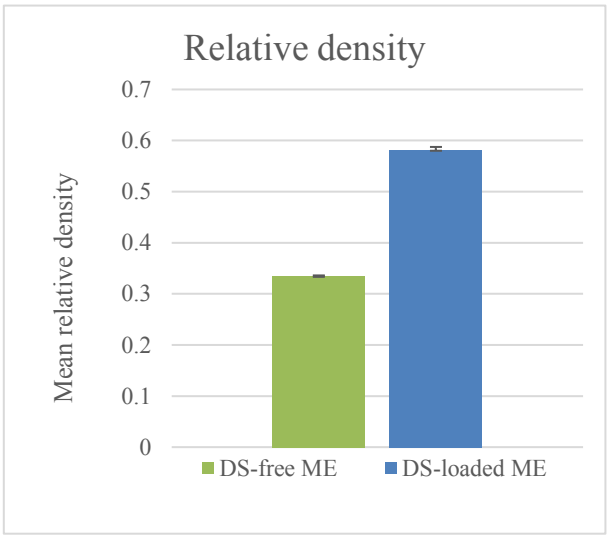


Figure 3-13 Represent the mean relative density of foam generated from the drug-free microemulsion and from the drug-loaded microemulsion at room temperature.

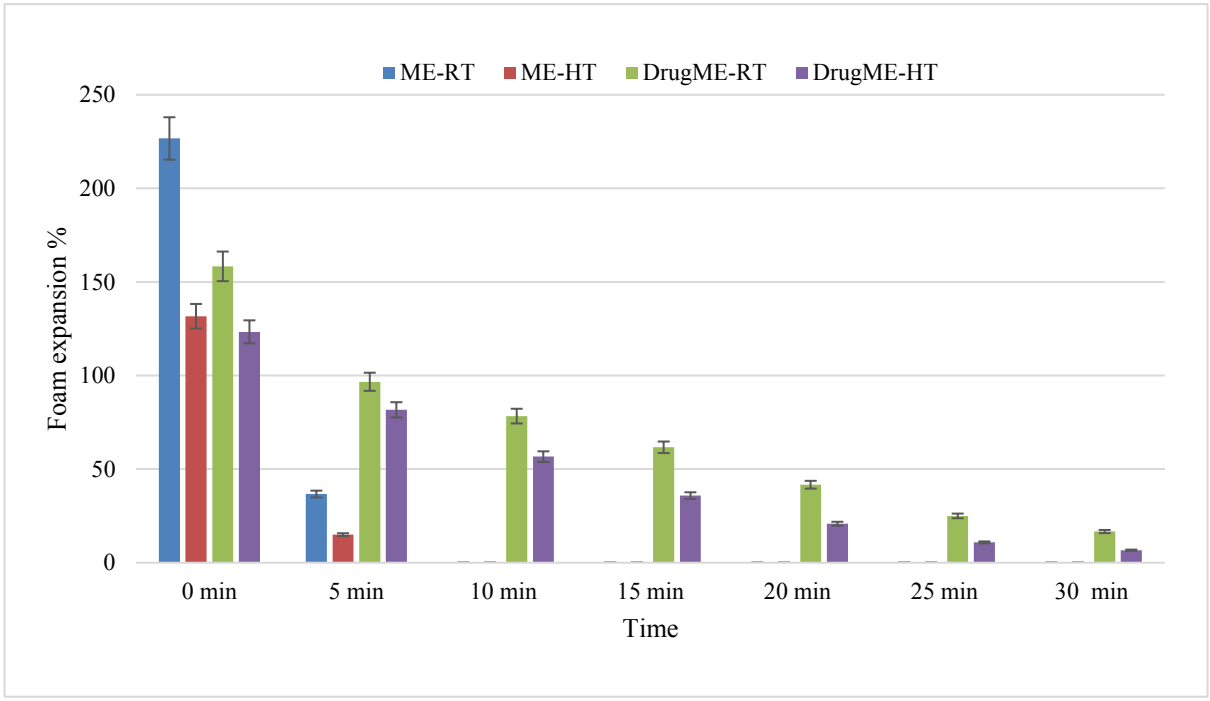


Figure 3-14 Represent the percentage of foam expansion where, RT-ME is the drug-free microemulsion at room temperature, HT-ME is the drug-free microemulsion at 32°C, Drug ME-RT Is the drug-loaded microemulsion at room temperature and, Drug ME-HT Is the drug-loaded microemulsion at 32°C.

Higher values for foam expansion in both formulations were observed at room temperature, which indicates the presence of temperature impact on the foam production. These findings illustrate that the drug-free ME initially has a higher gas fraction but collapses faster than the drug loaded ME, which has the ability to remain in a contacted structure for a longer time, even at higher temperatures.

Higher values for foam volume stability (FVS %), another parameter for foam stability, are reflected as a slowdown in the foam destabilization mechanisms. Typically, (FVS %) defines the process of air bubble coalescence and, consequently, the reduction in the height of the foam column. On the other hand, Foam liquid stability (FLS %) is a parameter which imitates the intensity of liquid drainage. Low (FLS %) values indicate a low volume of the liquid phase which is separated during the foam aging process, which indicates a better foam stability profile.

Considering the data obtained from the cylinder method, it was found that over the time, the foam produced from the ME loaded with DS has higher FVS% and lower FLS% values compared to the foam produced from the drug-free ME at both temperatures (figure 3-16). The rate of air bubble coalescence and liquid drainage was quicker in the drug-free ME, which caused a complete foam collapse before 10 min and yielded 0% FVS and 100 % FLS values with no significant deference was found at different temperatures. Contrarily, the addition of DS to the system seems to delay the air bubbles coalescence which decreases the liquid drainage over time, and that caused an improvement in the foam stability. The application of higher temperature caused a slight reduction in the foam stability, where higher

temperatures appear to accelerate air bubbles closeness and liquid drainage of the foam. At 32°C, the measured FVS% in the drug loaded ME foam was found to be 10% less than at room temperature and the FLS% was 6% more. From measuring the FE%, FVS% and FLS%, it is seen that the drug loaded ME produces more stable foam than the drug-free ME.

Considering foamability parameters (foam expansion) and foam stability parameters (gas fraction, foam volume stability, and foam liquid stability), the main explanation of the current difference in fomability and foam stability profiles of both MEs is thought to be the solution viscosity. Lower values for foam density indicate a high foam expansion values in the foam, and the higher the foam expansion the higher the gas volume fraction and the smaller the air bubbles, the larger the created surface area. The reduction in the viscosity of the bulk solution leads to an increase in the diffusion rate of foaming agent. Therefore, low viscosity of the liquid phase is needed to assure a rapid diffusion of a foaming agent to the surface. This mainly explains the high fomability profile of the drug-free ME that exhibited lower viscosity values in comparison to the drug-loaded one (52). On the other hand, the most obvious cause of foam stability was seen by Plateau as surface viscosity. Normally, liquid drains into the plateau border region from the lamellae, and this process causes the lamellae to become thinner, unstable, and rupture. Plateau's idea is that each film is stratified and composed of a sandwich-like structure, where the inner layer of the film has a viscosity of the liquid in bulk solution but the two exterior layers that adjacent to the gas phase are much more viscous (18). Thus, the velocity of foam destabilization is directly related to the viscosity of solutions. In this case, liquid drainage is delayed where the foam drainage depends on the solution viscosity. Therefore, higher viscosities could lead to a delay of phase break-up (52). This explains the high stability profile of the foam produced by DS loaded ME over the one that produced from the drug-free ME. It has also been reported that temperature affects the rate of

drainage through altering the liquid bulk viscosity. This could be the primary reason behind the reduction in foam stability at higher temperatures.

pH was assumed to have a direct effect on the foam stability. It has been reported in many studies that foam stability increases as pH increases (54,55). This might explain the stability of the foam generated from the DS-loaded ME compared to the drug-free one, as the addition of the acidic drug increased the pH value of the ME from 5.22 to approximately 7.64. It is believed that the surface potential is a function of the pH of the solution, and when the surface film is charged, this can cause a repulsion between the air bubbles coming near to each other, which may cause the formation of a stable foam (17,54). Therefore, the amount of gas volume fraction in the foam, the viscosity the temperature and the pH could have a direct impact on foam foamability and stability.

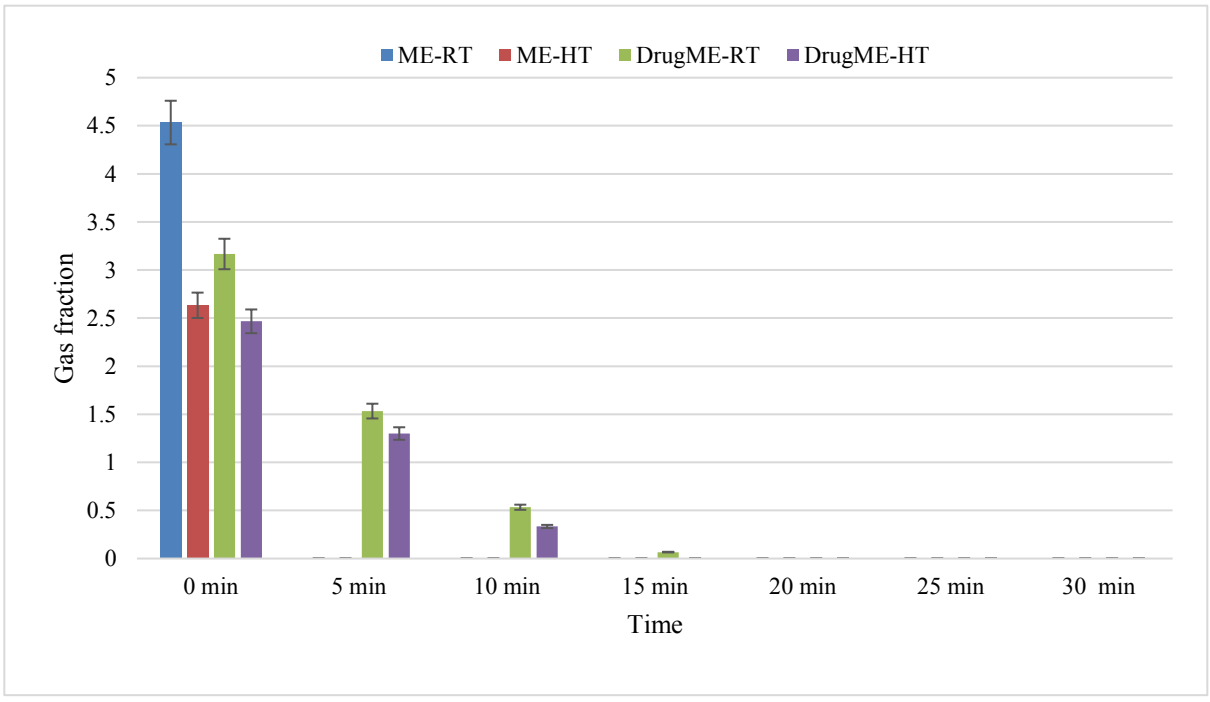


Figure 3-15 Represents the foam gas fraction where, RT-ME is the drug-free microemulsion at room temperature, HT-ME is the drug-free microemulsion at 32°C, Drug ME-RT Is the drug-loaded microemulsion at room temperature and, Drug ME-HT Is the drug- loaded microemulsion at 32°C.

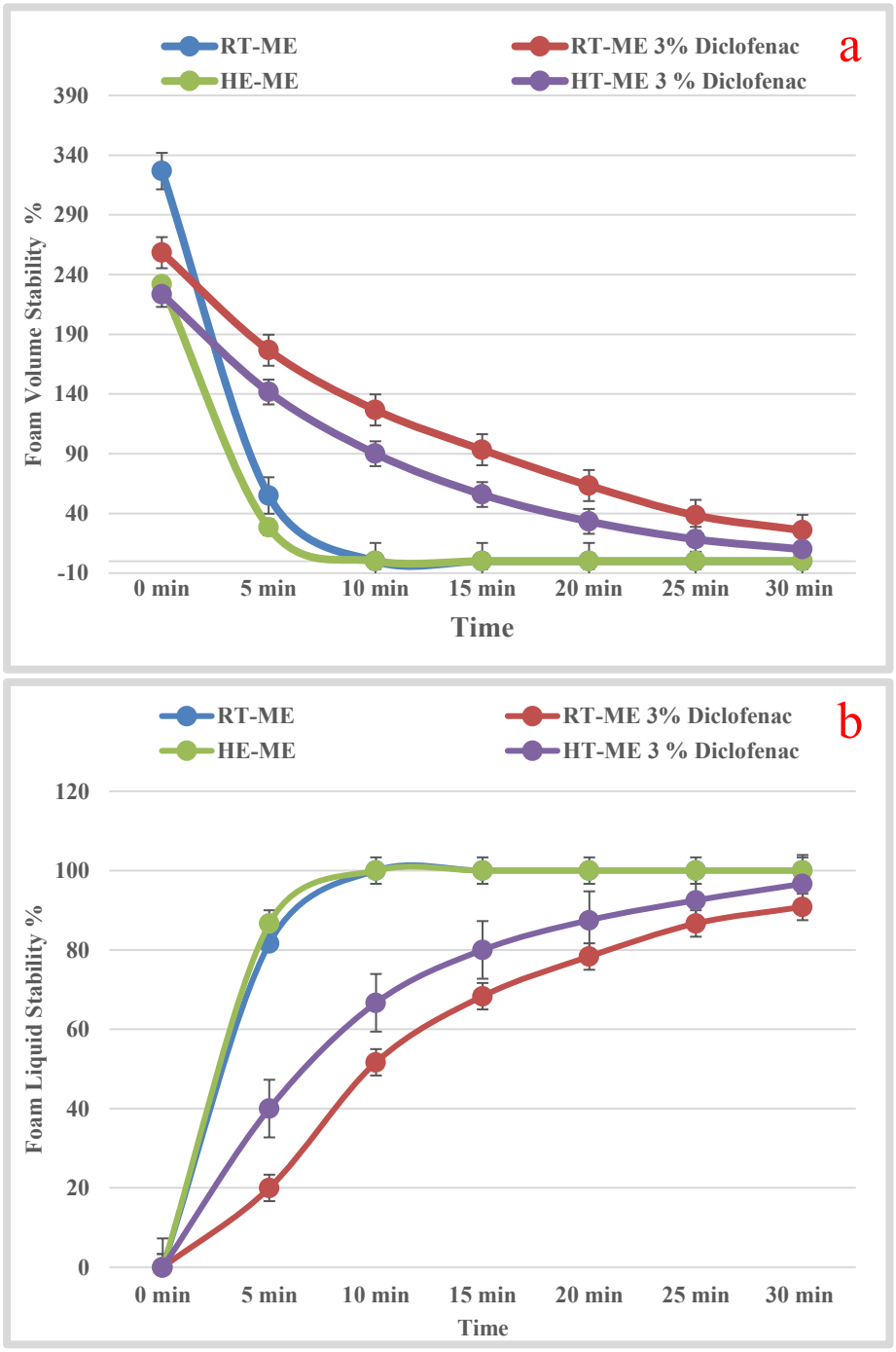


Figure 3-16 (a) represent the percentage of foam volume stability (b) represent the percentage of foam liquid stability where, RT-ME is the drug-free microemulsion at room temperature, HT-ME is the drug-free microemulsion at 32°C, RT-Diclofenac ME Is the drug-loaded microemulsion at room temperature and, HT-Diclofenac ME Is the drug- loaded microemulsion at 32°C.

3.3.15. Microscopic analysis

The produced foam was assessed microscopically to follow foam destabilization mechanisms. As soon as foam bubbles are formed, several changes start to appear and different processes were identified in the breakdown of generated foams (Figure 3-17). The destabilization processes do not occur individually but, to a considerable extent, simultaneously for both foams. Disproportionation (Ostwald ripening) was shown where the smaller air bubbles dissolved while the bigger ones grew. This is mainly due to the fact that the pressure and the dispersed air phase is greater in smaller bubbles, which causes gas diffusion from small air bubbles to larger ones, or to the bulk liquid (56). Gravitational separation (creaming) was also detected and this happen mainly because of the difference in the density between the phases, which causes the continuous liquid phase to flow around the dispersed air bubbles, and this is reflected by moving the air bubbles towards the top, while the liquid to drains within the foam lamellae (17). Further, film rupture was observed were lamellae appear to be Ruptured and that caused the bubbles coalescence and foam collapse. The reason for that is believed to be the fall in the elasticity of the foam surface film, which means no sufficient liquid with the foaming agent to be transported to the place of the possible rupture to prevent film rupture. The same three foam destabilization mechanisms were detected in both foams. However, it started faster in foam generated from drug-free ME and the rate of the occurring was quicker than the foam produced from the drug-loaded ME. This is an indication that the foam produced from the drug-loaded ME was more stable. This is probably due to the viscosity of the DS-ME that delayed the creaming and foam drainage, which elongated the phase break-up (17).

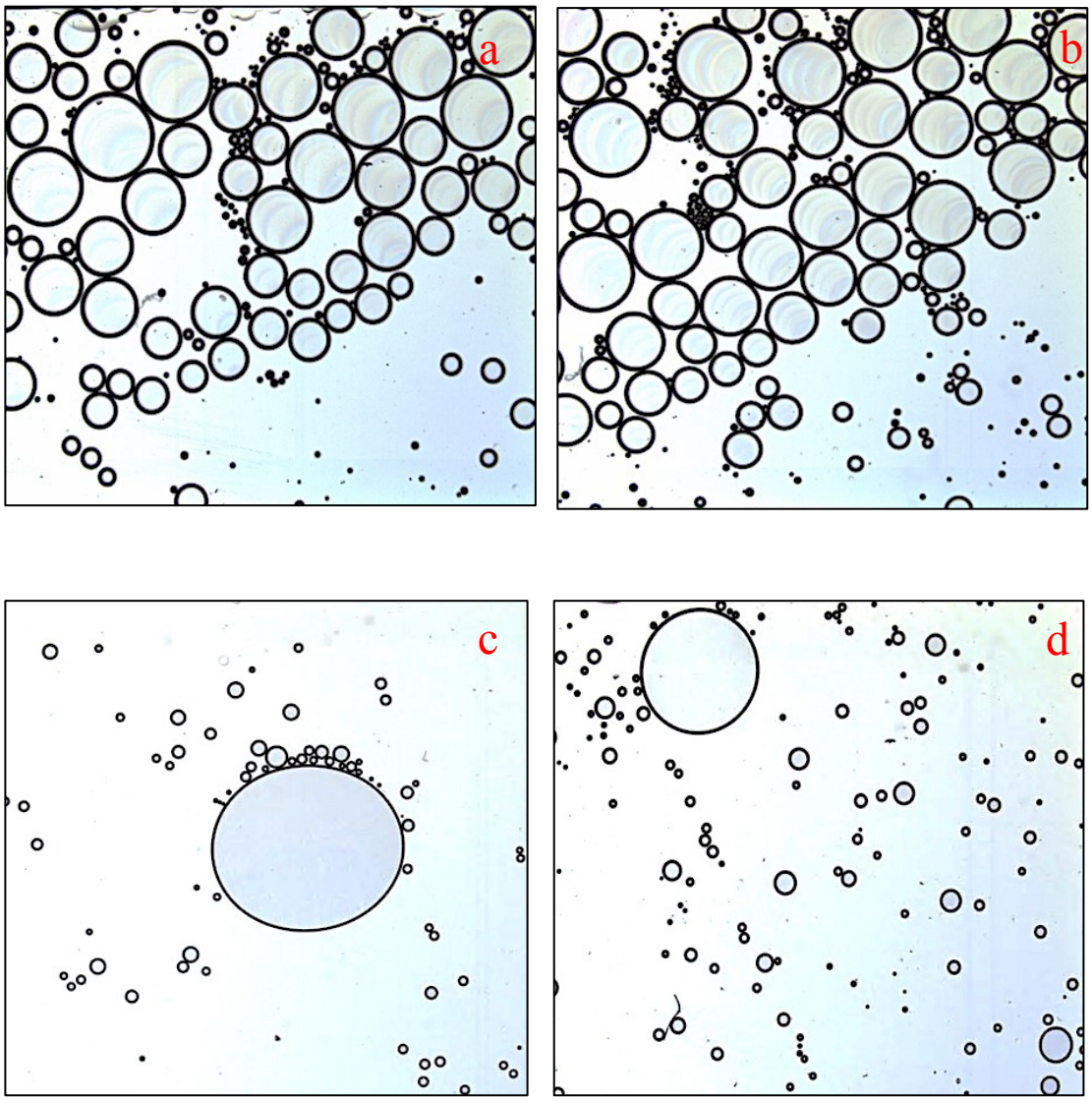


Figure 3-17 Compound Light Microscope images of drug-free ME and drug-loaded ME. (a) and (b) represent the gravitational separation of the foam produced from the ME and DS-ME, respectively (Magnification 400X). (c) and (d) represent the Ostwald ripening effect on the foam produced of the ME and DS-ME, respectively (Magnification 100X).

3.3.16. Air foam pump dispenser

2.1.2.

The formulated foamable DS-loaded ME was fused in an air foam dispenser. This unique technology allows generating a high-quality foam without using propellant, resulting in a precise dose with each activation. The foam generated from the air foam dispenser was with same quality as from the bubbling method, and it was also considered as class "1" on Abram and Hunt's scale (Figure 3-18). The statistical analysis of the stability data obtained during the period of 3 months (Table 3-6) showed that the DS-loaded ME did not undergo any significant changes regarding foam quality and particle size, which indicates its stability in the foam dispenser.

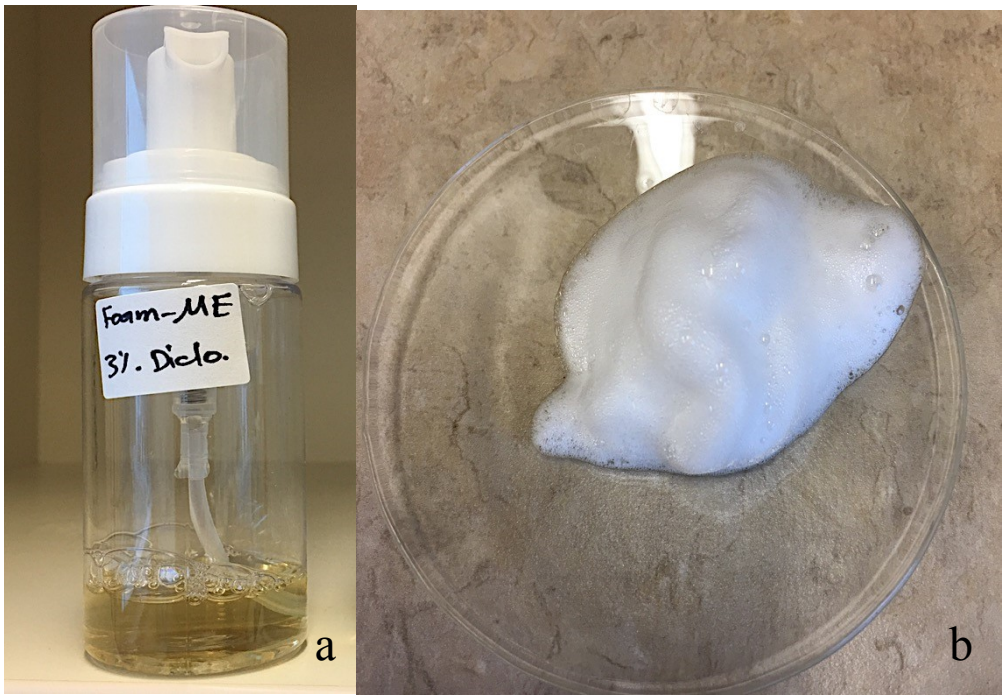


Figure 3-18 Macroscopic images of (a) DS-loaded ME in an air foam pump dispenser and (b) DS-loaded ME foam generated from the foam dispenser.

Table 3-6 pH, particle size and foam quality analysis of the prepared foamable DS-loaded ME stored in a foam dispenser for a period 0, 30, 60 and 90 days (mean ± SD, n=3).

Time	pH	Particle size	Foam quality
0 day	7.54 ± 0.173	21.51 ±1.185	Stable-fine foam with a couple of coarser bubbles
30 days	7.63 ± 0.152	21.48 ± 1.143	Stable-fine foam with a couple of coarser bubbles
60 days	7.72 ± 0.041	21.3 3± 0.068	Stable-fine foam with a couple of coarser bubbles
90 days	7.71 ± 0.096	20.7 ± 0.558	Stable-fine foam with a couple of coarser bubbles

3.4. Conclusion

This study proved that foamable microemulsion based formulations could be employed as an alternative dosage form for improving the solubility and *in vitro* permeability of DS. The successfully prepared foamable ME consisted of caprylocaproyl polyoxyl-8 glycerides / polyglyceryl-3 dioleate (6:1) ratio, caprylic capric triglycerides, and water. These new foamable formulations proved their physicochemical stability, and after a series of *in vitro* studies, the foamable drug-loaded ME formulation has demonstrated its ability to deliver DS at an increased rate compared to other vehicles. Therefore, it can be concluded that the prepared DS-loaded ME-based foam has a great potential as a topical dosage form for enhancing the drug delivery of DS.

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Chapter 4

4. General Conclusion and Future Directions

4.1. General conclusion

Diclofenac preparations are among the most frequently prescribed medicament for relieving pain in many musculoskeletal disorders. For that, countless efforts have been made to enhance the therapeutic efficacy of topical diclofenac formulations. One of the most popular techniques is the use of higher concentrations of penetration enhancers. Yet, the potency of the most used enhancers has limited their applications due to the several dermatological side effects. Another approach was to incorporate higher doses of diclofenac in several commercially available pharmaceutical bases. Nevertheless, not much data has been published regarding the safety and efficacy of these formulations. Yet, the large amount of diclofenac in these dosage forms could lead to an accidental systemic toxicity. It has been widely believed that for any topical drug to be effective, it must be able to penetrate the skin first, and only when the drug enters the lower layers of the skin it can be absorbed by local blood supply, or penetrate deeper into areas where inflammation occurs. Therefore, the rate and amount of a drug to be topically absorbed relies on the penetration ability of its vehicle. Accordingly, much attention has been made towards modifying the formulation of the vehicle, with a specific focus on nano-particulate carrier systems. Recently, microemulsions have been recognized as promising vehicles for the percutaneous absorption of drugs as they can solubilize large amounts of drugs in their domains. In addition to their ability to be relatively remain stable for a long period of time, ease of preparation and penetration-enhancing properties.

Correspondingly, this thesis has explored the idea that the topical penetration of DS might be enhanced through using an optimized DS-loaded ME formulations. The first study was aimed

at developing an *in vitro* evaluation of an ME and an ME-based gel system as a topical drug delivery for DS. The data obtained from this study indicated that the developed drug-loaded ME and it's gelled form are physiochemically stable. The optimized spherical o/w nano-droplet systems consisted of caprylocaproyl polyoxyl-8 glycerides / diethylene glycol monoethyl ether 75 % (2:1) as surfactant/cosurfactant, propylene glycol monolaurate 12.5 % as the oil phase, and water 12.5 % as the aqueous phase. Carbopol polymer was successfully incorporated into the ME system as a gelling agent to produce DS-loaded ME gel, the produced gel form was assumed to enhance the viscosity of the system to ease the topical administration and potentially residence time. Results from the *in vitro* release testing demonstrated that both the non-gelled and the gelled MEs systems exhibited the highest release values ($p< 0.0001$) compared to the controls and the different marketed formulations. The results of this study illustrated that ME formulations are promising vehicles for topical delivery of DS. However, considering the viscosity, the gel base might be preferable by patients for better handling and administration.

The objective of the second study was to produce and investigate the stability and the *in vitro* performance of a ME-based foam as a potential drug delivery for improving the topical penetration of DS. This study revealed that successfully constructed o/w microemulsion-based foams, DS-free and DS-loaded, which were stable after subjecting them to stability testing. The optimized foamable MEs were prepared using caprylocaproyl polyoxyl-8 glycerides / polyglyceryl-3 dioleate (6:1) ratio, caprylic capric triglycerides, and water in quantities of 18, 3, 0.5 and 78.5 %, respectively. In-vitro drug release study using Franz diffusion cells indicated that the release rate of DS formulated as a foamable ME was significantly the highest among the tested formulations ($p< 0.0001$). Results obtained from measuring the percentage of foam expansion, foam volume stability, and foam liquid stability

at different temperatures showed that the foam generated from the DS-loaded ME was more stable than the drug-free foam. The results suggested that the foamable DS-loaded ME system is a potential vehicle for generating a stable nano-foam for enhancing the topical penetration of DS.

The above studies concluded that ME based systems are potential vehicles for enhancing the penetration of lipophilic compounds such as DS. This study also showed that for DS topical preparation in general, the choice of components and the ratio between them can dramatically alter the system characteristics and affect its ability to penetrate membranes. This explains way many compounded and commercially available preparations might have difficulties to penetrate the membrane and therefore, achieving an efficient drug delivery. Additionally, this thesis demonstrated how crucial is to perform an IVRT to assess the in vitro drug release performance of topically applied vehicles, which might mimic the in vivo performance of these compounds.

4.2. Future perspectives

- The current study was executed using IVRT as a preliminary step to test the developed topical formulations. However, further *in vivo* studies will be needed to further evaluate the findings exhibited with the existing *in vitro* model.

- The safety of the formulated ME-based systems is required to be evaluated *in vivo* through measuring the amount of drug reaching the systemic circulation, and accumulating in the tissues and by a careful assessment to any side effects.

- This research highlighted the stability and the efficacy of the formulated ME formulations as topical vehicles for DS. However, the efficiency of these formulations can be further validated through incorporating different pharmaceutical active agents.

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Appendix

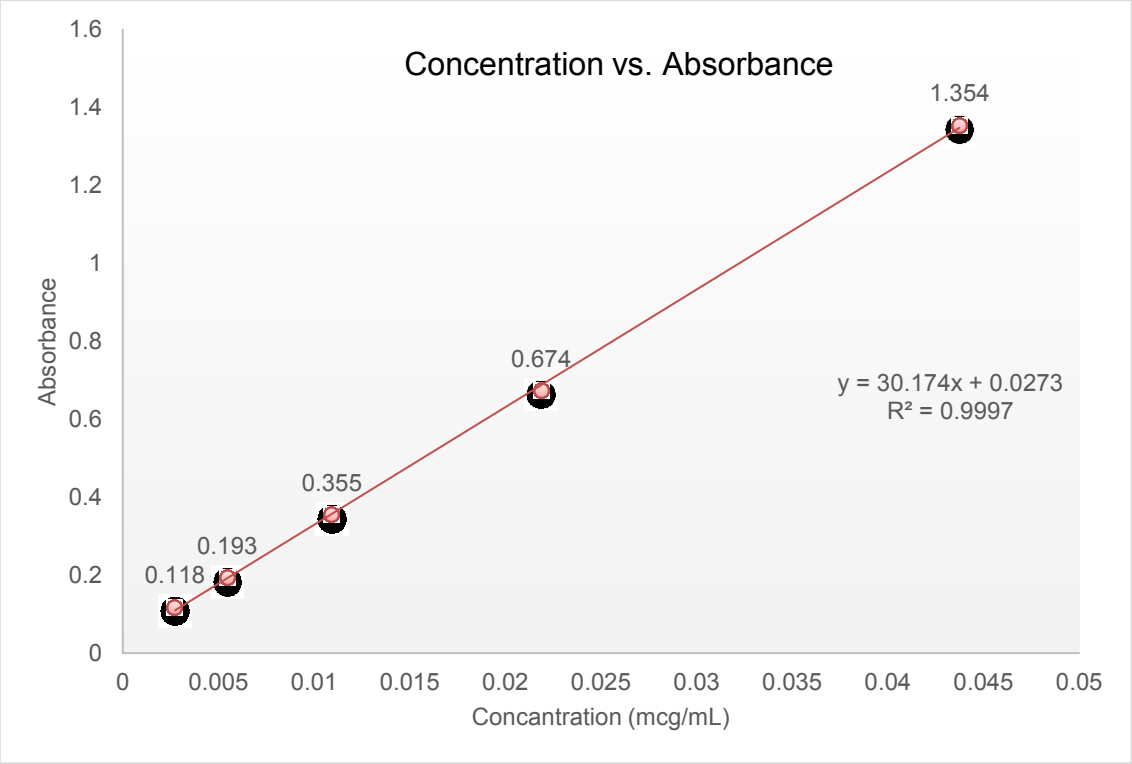

Figure A.1. Calibration curve used for the quantitative determination of Diclofenac Sodium.

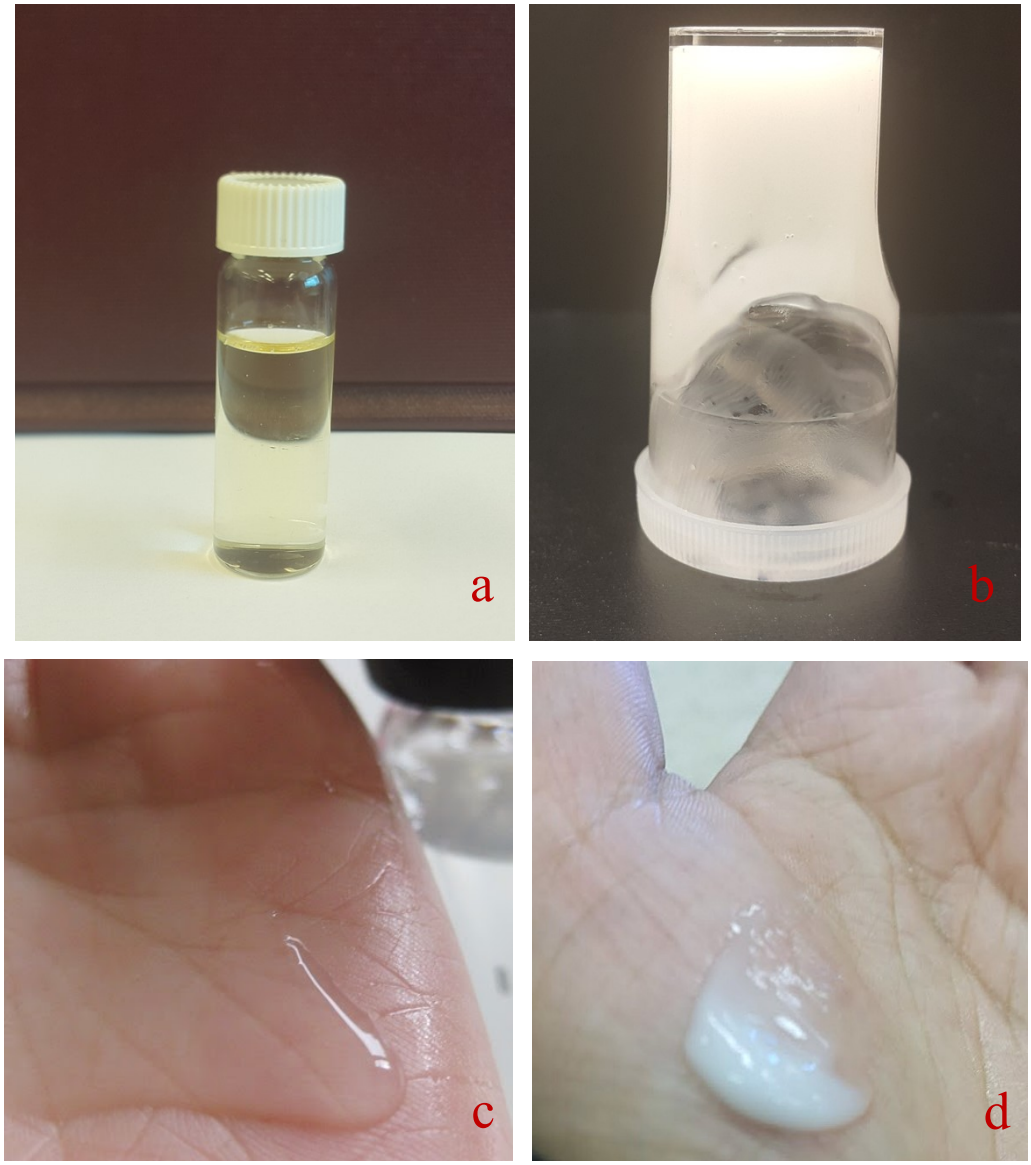

Figure A.2. (a) and (c) DS-loaded ME system, (b) and (d) DS-loaded ME based gel form (in carbopol gel).

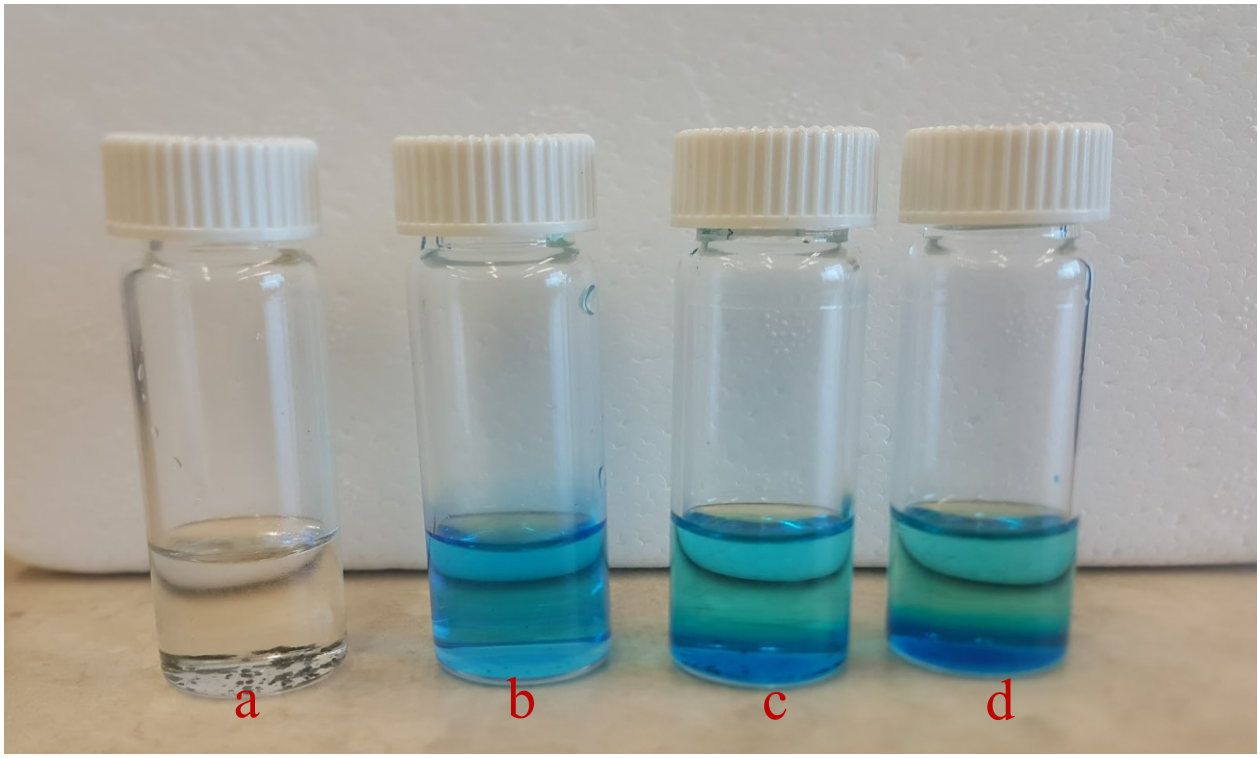

Figure A.3. Evaluation of the microemulsion type by using dye solubility tests (a) blank oil phase (Propylene glycol monolaurate) (b) blank water (c) Drug-free ME (D) diclofenac-loaded ME.

Table A.1. The release profile of 3% DS from diferent MEs Formulations (1-5) (Mean, n=3)

	F1	F2	F3	F4	F5
Time	Release 1	Release 2	Rlease 3	Release 4	Releae 5
0.5	4.5	9.333	17.5	4.5	9.333
1	17	17.333	25.5	10	17.333
2	24.5	27	35	17	41.333
4	42.5	37	42.5	29	63.333
6	52	44	44	33	83

Table A.2. The release profile of 5% DS from PLO gel (Mean, n=3)

PLO gel 5 % Diclofenac						
	cell1	cell2	cell3	cell4	cell5	cell6
1/2 hr	8.68	7.361	8.17	8.25	7.60	7.44
1 hr	12.67	11.38	12.85	13.67	13.05	11.62
2 hr	18.99	18.56	19.62	17.36	18.70	20.24
4 hr	28.18	27.29	32.31	27.68	30.13	30.96
6 hr	35.84	33.66	38.29	36.72	37.53	37.61

Table A.3. The release profile of 5% DS from Lipoderm gel (Mean, n=3)

Lipoderm 5 % diclofenac						
	cell1	cell2	cell3	cell4	cell5	cell6
1/2 hr	6.36	6.31	5.60	4.66	4.83	6.04
1 hr	8.98	8.12	6.70	5.67	6.33	9.73
2 hr	11.08	11.45	9.54	7.38	9.14	12.99
4 hr	14.63	15.44	14.07	9.46	11.83	18.41
6 hr	18.25	19.21	16.78	12.62	13.96	21.43

Table A.4. The release profile of 5% DS from Versparo gel (Mean, n=3)

Versparo gel 5 % diclofenac						
	cell1	cell2	cell3	cell4	cell5	cell6
1/2 hr	0	0	0	0	0	0
1 hr	0	0	0	0	0	0
2 hr	0	0	0	0	0	0
4 hr	0	0	0	0	0	0.24
6 hr	0	0	0.05	0.49	0	1.51

Table A.5. The release profile of 3% DS from the foamable ME system (Mean, n=3)

	Foamable Diclofenac ME					
Time	cell1	cell2	cell3	cell4	cell5	cell6
0.5	11.35	9.89	6.84	3.39	2.42	2.69
1	17.49	12.54	22.48	4.59	4.72	21.06
2	35.11	20.40	59.94	12.32	10.42	27.78
4	83.53	31.45	92.19	58.48	26.94	31.93
6	84.86	62.55	87.46	69.44	67.89	73.06

Table A.6. The release profile of 3% DS from the drug-loaded ME-based gel (Mean, n=3)

	Diclofenac -loaded ME gel					
Time	cell1	cell2	cell3	cell4	cell5	cell6
0.5	20.16	24.19	25.31	23.24	20.64	25.15
1	35.13	32.11	31.68	28.65	27.43	30.94
2	37.52	36.09	39.91	39.49	38.58	47.98
4	47.61	50.85	53.08	47.56	50.16	62.10
6	78.83	67.84	56.31	74.21	72.46	66.03

Table A.6. The release profile of 3% DS from the drug-loaded ME (Mean, n=3)

	Diclofenac -loaded ME					
Time	cell1	cell2	cell3	cell4	cell5	cell6
0.5	32.19	21.74274039	26.26	23.38	24.80	25.45
1	35.71	31.68205824	40.52	29.31	26.63	26.10
2	63.60	37.80762301	66.45	38.35	35.24	28.55
4	69.99	78.5220139	62.46	61.41	62.94	56.58
6	73.56	93.60032719	77.21	75.18	76.00	64.49

Table A.7. The release profile of 3% DS from the drug-loaded plan Carbopol gel (Mean, n=3)

	Carbopol gel loaded with free Diclofenac					
Time	cell1	cell2	cell3	cell4	cell5	cell6
0.5	0.16	0.06	0.14	0.25	0.819	3.14
1	0.41	0.28	0.27	1.31	0.96	4.89
2	0.73	0.48	0.75	1.46	1.80	5.35
4	1.103	2.43	5.70	1.77	2.51	6.61
6	1.85	3.43	8.35	2.53	2.68	7.39

Table A.8. The release profile of 3% DS from the drug-loaded ME-based gel (Mean, n=3)

	Voltarin emulgel 1.16 % Diclofenac					
Time	cell1	cell2	cell3	cell4	cell5	cell6
0.5	8.42	11.63	7.23	6.11	7.54	6.62
1	11.47	12.66	8.89	9.11	12.53	8.07
2	15.74	18.76	13.93	16.13	14.12	14.62
4	28.17	25.59	25.62	23.13	21.94	22.86
6	34.79	37.44	36.67	36.46	31.98	35.69

Table A.9. The release profile of 3% DS from the drug-loaded plan Vaseline gel (Mean, n=3)

	Vaseline-loaded with 3 % diclofenac					
Time	cell1	cell2	cell3	cell4	cell5	cell6
0.5	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
4	0	0	0	0	0.35	0
6	0.23	0.32	0.41	0.27	0.93	0.45

Table A.11. The calculation of the titration method used for constructing the Pseudo-ternary phase diagram of propylene glycol monolaurate (oil), caprylocaproyl polyoxyl-8 glycerides / diethylene glycol monoethyl ether (Smix) and water.

oil	Smax	water	Total	x%	y%	z%	x'	y'
0.1035	0.9149	18.9414	19.96	0.5%	4.6%	94.9%	0.03	0.04
0.1155	0.8033	15.9476	16.87	0.7%	4.8%	94.6%	0.03	0.04
0.1126	0.7085	13.149	13.97	0.8%	5.1%	94.1%	0.03	0.04
0.1159	0.5121	6.819	7.45	1.6%	6.9%	91.6%	0.05	0.06
0.1098	0.4111	4.6915	5.21	2.1%	7.9%	90.0%	0.06	0.07
0.1228	0.2996	1.5698	1.99	6.2%	15.0%	78.8%	0.14	0.13
0.3959	0.6202	0.1197	1.14	34.9%	54.6%	10.5%	0.62	0.47
0.5086	0.5118	0.0843	1.10	46.0%	46.3%	7.6%	0.69	0.40
0.6128	0.4151	0.0463	1.07	57.0%	38.6%	4.3%	0.76	0.33
0.7089	0.318	0.0519	1.08	65.7%	29.5%	4.8%	0.80	0.26
0.8108	0.2038	0.0258	1.04	77.9%	19.6%	2.5%	0.88	0.17
0.9367	0.1072	0.0142	1.06	88.5%	10.1%	1.3%	0.94	0.09

Table A.11. The average concentration of diclofenac / dose from the generated foam out of the air foam pump dispenser.

measurements	mg	DS conc.
1	236	7.08
2	250.1	7.503
3	246	7.38
4	238.7	7.161
5	210.6	6.318
6	246.1	7.383
7	277.1	8.313
8	247.3	7.419
9	271.8	8.154
10	245.4	7.362
average	246.91 ± 17.46	7.4073± 0.52

Table A.12. The calculation of the titration method used for constructing the Pseudo-ternar y phase diagram of the foamable ME system consists of caprylic capric triglycerides (oil), caprylocaproyl polyoxyl-8 glycerides / polyglyceryl-3 dioleate (Smix) and water.

oil	Smax	water	Total	x%	y%	z%	x'	y'
0.0271	0.978	17.75	18.76	0.1%	5.2%	94.6%	0.03	0.05
0.0498	0.96	17.83	18.84	0.3%	5.1%	94.6%	0.03	0.04
0.0768	0.925	19.5	20.50	0.4%	4.5%	95.1%	0.03	0.04
0.1058	0.9138	1.98	3.00	3.5%	30.5%	66.0%	0.19	0.26
0.1024	0.8053	1.35	2.26	4.5%	35.7%	59.8%	0.22	0.31
0.107	0.701	0.85	1.66	6.5%	42.3%	51.3%	0.28	0.37
0.1023	0.6045	0.61	1.32	7.8%	45.9%	46.3%	0.31	0.40
0.1125	0.5133	0.38	1.01	11.2%	51.0%	37.8%	0.37	0.44
0.1122	0.4193	0.28	0.81	13.8%	51.7%	34.5%	0.40	0.45
0.1065	0.3531	0.21	0.67	15.9%	52.7%	31.4%	0.42	0.46
0.1143	0.3144	0.16	0.59	19.4%	53.4%	27.2%	0.46	0.46
0.3017	0.71	0.09	1.10	27.4%	64.4%	8.2%	0.60	0.56
0.3994	0.6123	0.07	1.08	36.9%	56.6%	6.5%	0.65	0.49
0.5052	0.4977	0.07	1.07	47.1%	46.4%	6.5%	0.70	0.40
0.593	0.4018	0	0.99	59.6%	40.4%	0.0%	0.80	0.35
0.719	0.3053	0	1.02	70.2%	29.8%	0.0%	0.85	0.26

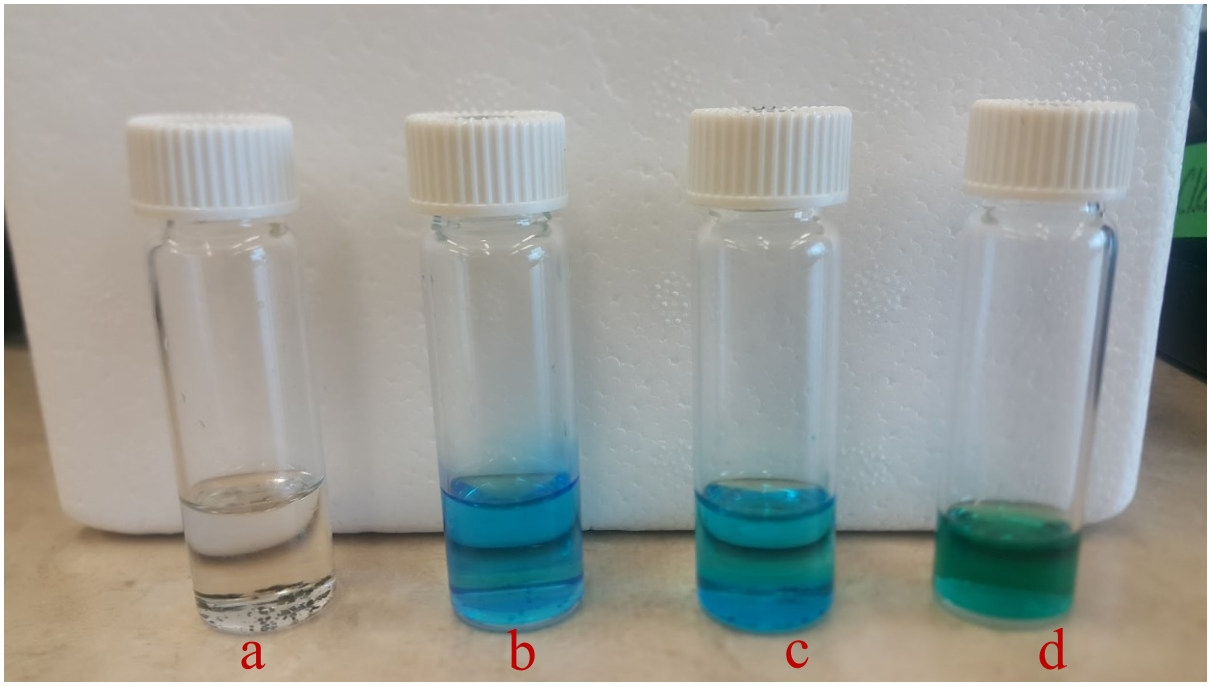

Figure A.4. Evaluation of the microemulsion type by using dye solubility tests (a) blank oil phase (Caprylic capric triglycerides) (b) blank water (c) drug-free ME (D) diclofenac-loaded ME