Veterinary Compounding: *In vitro* Assessment of Methimazole-Based Foam for Feline Hyperthyroidism

By

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Abstract

Veterinary compounding: In vitro assessment of Methimazole-based foam for feline hyperthyroidism

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Objective: Hyperthyroidism is one of the most common feline endocrine disorders due to excess production of active thyroid hormone in middle-aged cats. The management involves oral or transdermal antithyroid drug delivery. The use of transdermal medications in cats has become popular in veterinary medicine due to the ease of administration compared to oral medications. Our hypothesis is that microemulsion-based system can improve the in vitro flux of Methimazole using a Franz cell model.

Method: A concentrations of 2.5% of Methimazole were incorporated into Labrafac-based microemulsion formulations with Labrasol as surfactant and Plurol Oleique as cosurfactant to be used for transdermal delivery of Methimazole. The in vitro studies were carried out using Franz cell apparatus with a diffusional surface area of 1.79 cm^2 and synthetic membranes. A direct comparison of release profiles using Franz diffusion cells between Methimazole-loaded microemulsion and commercial formulations of transdermal Methimazole were performed. Purified water was used as the receptor fluid and the temperature

maintained at 32 ± 0.5°C. The withdrawn samples were appropriately diluted and calculated at different time points 30 min, 1, 2, 4, and 6hrs using HPLC.

Result: The obtained result of *in vitro* study indicated that the foamable microemulsion system might be a candidate carrier for transdermal delivery of Methimazole. Cumulative drug percentage release through hydrophobic synthetic membranes into the receptor media were found to be 84.64% in Methimazole-loaded microemulsion compared to 47.86%, 33.53%, 33.08% in Lipoderm, Versapro, PLO vehicle, respectively, $p < 0.05$.

Conclusion: Hence the microemulsion system is one of the promising tools for percutaneous delivery of Methimazole. The release profiles obtained from in vitro permeability tests might be used for predicting the in vivo permeability of the formulation. Findings from the current research work evidenced that foam-based microemulsion formulation was superior to cream-based formulations; thus, ME based foam might be a potential vehicle for enhancing the transdermal penetration of Methimazole.

Preface

This thesis is an original work by Areej Mohammad Alshikhey completed under the supervision of Prof. Raimar Löbenberg and the Co supervisor Prof. Michael Doschak 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.

Dedication

To my loving father Mohammad Alshaikhi, To the kindest heart my mother Rihana Alshaikhi, for their endless love, support, and belief in me. This accomplishment would not have been possible without both of you.

To the stars in my sky, my sisters and brothers, I will always cherish the support you have given me, your encouraging words, your surprising gifts, and your love. You are my lights and guidance in the dark nights.

To Serghei Vascov, who has been there throughout this roller-coaster ride. I do not think you realize how much your support has meant to me.

Acknowledgment

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Chapter 1

1. Literature Review

1.1. Veterinary Compounding

Veterinary compounding has always been and will continue as a vital aspect to deliver safe and effective medications to veterinary patients. The history of veterinary compounding was initiated parallel to that of human compounding, in which compounded products dominated since the 1930s and 1940s of the twentieth century [88]. Nowadays, compounding drugs to fulfill the animal therapeutic needs is in increasing as the availability of animal approved medications for all species and illnesses are limited. In particular, as it estimated by Food and Drug Administration (FDA), 75,000 pharmacies annually fill 6,350,000 compounded animal prescriptions in the United States [89]. According to United State Pharmacopeia USP, compounding is defined as preparation, mixing, packaging, and labeling of a drug based on the prescription ordered by the practitioner [2]. Also, as is stated elsewhere [3], compounding can be described as a manipulation of the original dosage form to produce an easily administered drug or to meet the therapeutic needs for veterinary patients when the original dosage form is not in the ideal form for the species being treated.

Although veterinarians might prepare compounding medications for animals, pharmacists are the primary compounders. Moreover, the attention that is gained by pharmacists toward veterinary medicine is an indication of the improved care standard for veterinary patients associated with the lack of compounding training in veterinary practice [4]. However, pharmacists and veterinarians should be aware of

Regulations and Compliance Policy Guidelines (CPGs)[90] for veterinary compounding. In particular, compounding of animal drugs is legalized explicitly by the Animal Medicinal Drug Use Clarification Act (AMDUCA) [91]; however, the compounding must be employed under the relevant provisions of extra-label drug use (ELDU). Specifically, the latter refers to the use or intended use of drugs approved by Health Canada in the veterinary patients [92].

The growth in veterinary compounding practice has been a beneficial, and vital adjunct to the veterinary profession and the patients in need. In other words, the importance of compounding including but not limited to providing therapy when there is no appropriate government-approved (USP/FDA) drugs are available, or approved medications in an unsuitable dosage form for certain species. Similarly, approved medicines in an unacceptable flavor for some animals (e.g., bubblegum or citrus flavor are not accepted by cats). In these instances, compounding is of an essential need to improve the adherence in an individual animal patient [5].

Due to the high prevalence of hyperthyroidism in cats, MET oral liquid is considered to be one of the top 10 drugs that are compounded for veterinary patients [3].

1.2. Methimazole

MET is a pharmacological agent that is used to treat hyperthyroidism in cats [6] . It is a thioureylene antithyroid drug that is actively concentrated in the thyroid gland (Figure 1.1). The primary action of MET is to inhibit the formation of thyroid hormones; by impeding the iodination of the thyroid peroxidase of tyrosine residues in thyroglobulin, and, thus preventing the synthesis of thyroxin (T4) and triiodothyronine (T3), which are the primary hormones produced by the thyroid gland [7]. Therefore, MET can effectively inhibit the production of new thyroid hormones as it does not affect the existing or stored thyroid hormones [8] .

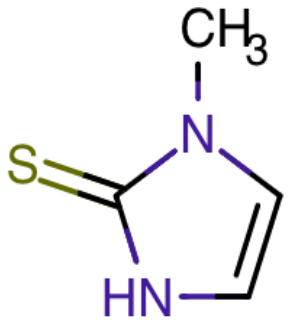


Figure1.1 The structure of MET (Molecular Weight 114).
Structure retrieved from https://chemicalize.com/#/calculation

The initial recommended oral dose to treat cat's hyperthyroidism is 10-15 mg as once or twice daily [9] . However, 2.5 to 5 mg once or twice daily will be useful in cats being treated at the earlier stage of the disease or with less severe clinical manifestations [88]. Although, up to a dose of 10 mg (0.1 mL) can be applied to the ear pinnae of a cat [6] . a topical dose of transdermal PLO gel as 2.5-5 (0.1 mL) mg has been demonstrated to be used in cats every twelve hours even though the safety and efficacy have not

been established [10] . Vomiting was observed as one of the adverse effects of MET in cats as a result of the unpalatable taste of the oral product. Another less frequent side effects such as anorexia, pruritus, anemia, neutropenia, hepatotoxicity, and thrombocytopenia can be observed in cats. In addition, hematologic changes can be detected in 15% of the treated cats [10,11] . Given the well-documented side effects coupled with conventional treatments, an alternative approach using transdermal MET would be an effective substitute. According to the study that has been conducted by Sartor et al., to evaluate the efficacy of transdermal MET compared to oral product. This study based on forty-seven cats diagnosed with hyperthyroidism, and it was concluded that transdermal MET route was associated with fewer gastrointestinal adverse effects compared to the oral application [12]. Furthermore, there is another documented study that was performed by Lécuyer et al., to evaluate the clinical safety and efficacy of transdermal MET in the treatment of feline hyperthyroidism. This trial based on thirteen cats diagnosed with hyperthyroidism and it was concluded that transdermal MET is an adequate and safe alternative to the conventional oral formulations [10].

Monitoring of MET therapy is an essential tool to ensure providing well-managed symptoms as well as effective treatment. According to a survey that was performed by Higgs et al. of 603 veterinarians, to assess the monitoring parameters for medically treated cats, represents that body weight, serum total thyroxin (TT4)

concentration, and renal biochemistry were the most common parameters to monitor [13]. Furthermore, an excellent guideline of the recommended baseline monitoring parameters has been published by Daminet et al., the baseline monitoring parameters include (modified from ref. [14]);

- Thorough case history and physical examination (including cervical palpation and emphasis on cardiac assessment)
- Bodyweight and body condition score
- Blood pressure measurement to establish the baseline and to familiarize the cat with the procedure
- Ophthalmologic examination
- Circulating TT4 concentration
- Complete blood cell count (CBC)
- Blood biochemistry, including liver enzymes and
- Urinalysis: urine-specific gravity (USG), dipstick analysis and sediment examination as a minimum. Urine culture is ideal.

As long as the pharmacological treatment of hyperthyroid patients established, it is critical to assess the cat's condition and observe the progress. The cat needs to be reassessed at 2 to 3 weeks after the start of the treatment by measuring the total serum T4 concentration. Furthermore, the cat needs to be medicated and closely monitored until the euthyroid status has reached. Once reached, the dose needs to be reduced to the lowest amount possible and monitored every 3 to 6 months [115].

Methimazole Physiochemical Properties

Physicochemical properties are the primary factors that influence the transdermal absorption of MET. It has been believed that very hydrophobic drugs will be retained in the stratum corneum as it is considered as a lipophilic layer while medications with solely hydrophilic properties will be unable to penetrate the stratum corneum (an upper layer of the skin). Therefore, drugs possess water and lipid solubility are considered to have better skin permeation compared to those with monophasic solubility [15].

MET is a hydrophilic drug, with an intrinsic solubility of 4.18 mg/ml (Figure 1.2) while solubility of MET in water is estimated to be 275g/L [93]. Moreover, drug molecules need to partition into the membrane as this partitioning is a crucial step in the diffusion through the skin membrane [94]. In which hydrophilic compounds have lower log P values in comparison to higher log P value in lipophilic compounds, however, compounds with log P value ranges between 1-3 are considered suitable candidates for transdermal drug penetration as they possess both hydrophilic and lipophilic properties due to their ability to pass the stratum corneum (lipophilic) and epidermis (hydrophilic) layers of the skin [95]. The distribution coefficient can be described as the ratio of the total concentrations of the ionized and non-ionized forms of the compound in both oil and water phase. Similarly, log D is used as an indication of how hydrophobic or hydrophilic the compound is [96].

Furthermore, It was found that this compound represents the same value of log P and log D as 0.75 at the pH ranged between 4.2-7.4 (Figure 1.3); Thus, incorporating of MET in a transdermal vehicle with a sufficient amount of surfactant would enhance its permeation through the skin [16] while its higher hydrophilicity would increase its ability to diffuse through deeper hydrophilic skin layers.

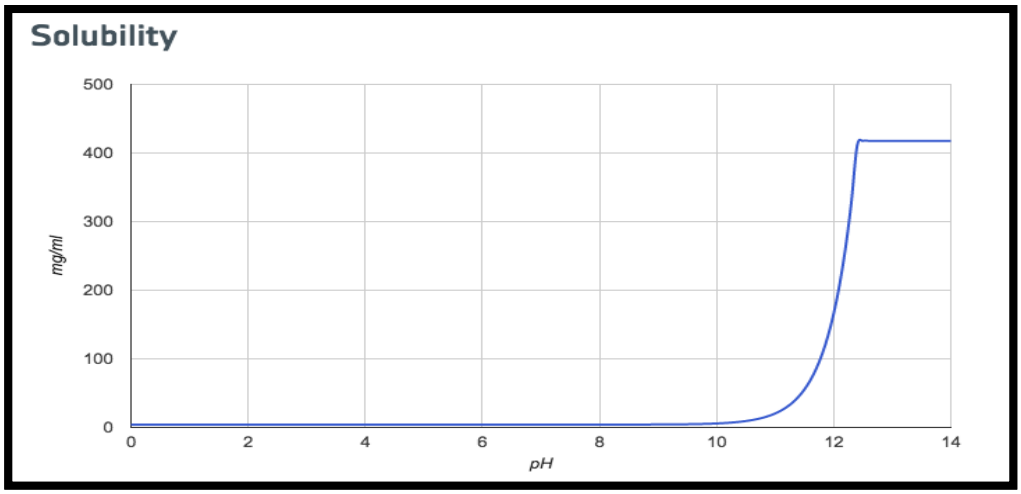


Figure 1.2 The representative MET Solubility as a function of pH. The drug solubility is shown in logarithmic form where logS is the solubility 10-based logarithm measured in mol/l.
The Figure retrieved from https://chemicalize.com/#/calculation

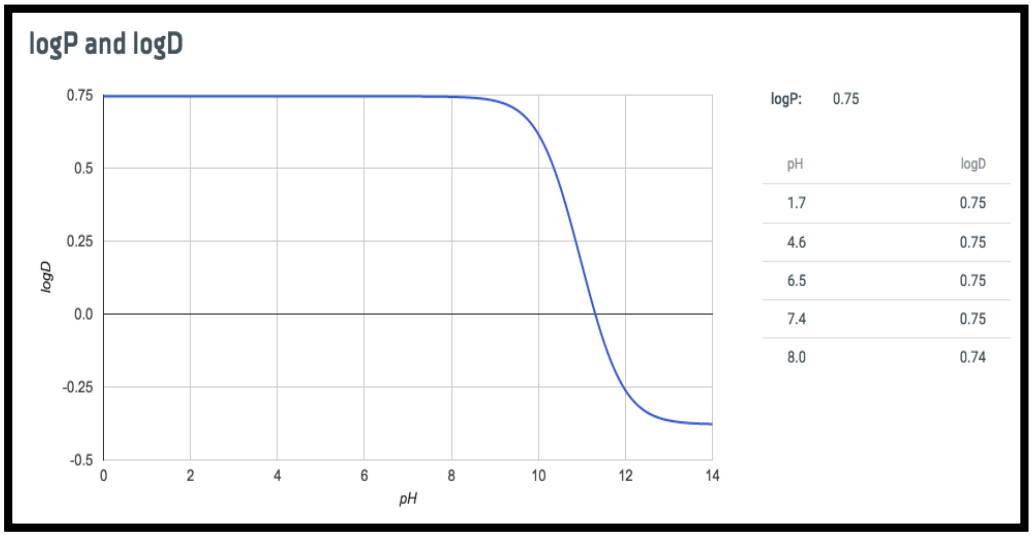


Figure 1.3 The representative hydrophilicity of MET as a function of pH
The Figure retrieved from https://chemicalize.com/#/calculation

1.3. Feline Skin Structure and Function

As the skin is considered the largest organ of a cat's body, it plays a dramatic role in the functioning of an animal's body. It comprises numerous components that support an animal's body existence and activity. The skin structure of cats consists of three main layers, such as epidermis, dermis, and subcutaneous layer (Figure 1.4).

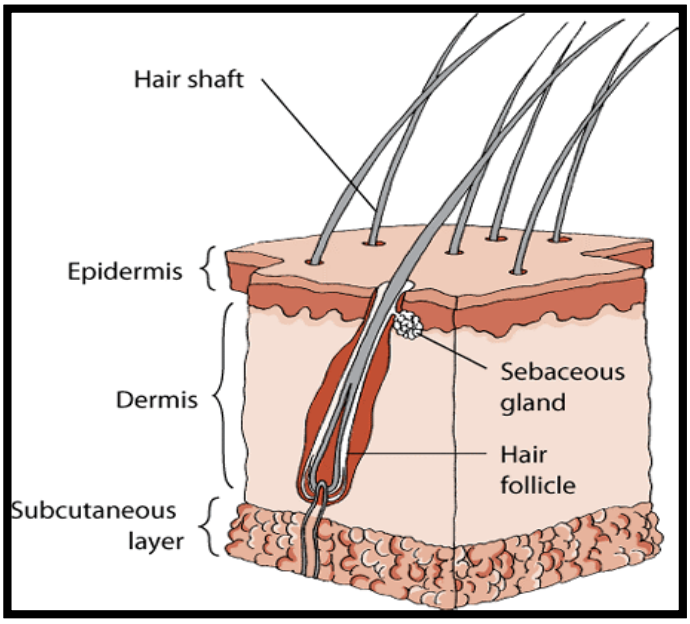


Figure 1.4 Anatomy of feline's skin including three major layers
Adapted from https://www.msdvetmanual.com/cat-owners/skin-disorders-of-cats/structure-of-the-skin-in-cats#v6493316

Firstly, as the outer layer, the epidermis performs a protective function against the environment as well as regulate the temperature. The epidermis consists of three layers; for instance; stratum corneum, stratum granulosum, and stratum germinativum [97]. In particular, stratum corneum is the outer layer, closest to the epidermis. The stratum corneum is shaped by corneocytes "bricks" connected by corneodesmosomes, in which the corneocytes are separated by a lipid matrix known

as “mortar”. The stratum corneum is a lipid membrane; thus, it is considered as a significant permeation barrier to the transdermal drug application. As a brick and mortar structure, the corneocytes consist of a highly insoluble keratinized cells that hinder the penetration of hydrophilic compounds. In general, small, non -polar lipophilic compounds are the most readily absorbed compounds [98].

Keratinocytes, melanocytes, and Langerhans cells are the mainly regenerative cells that compose the epidermis. Dead cells are located on the surface of a cat’s skin in which such surface contains vital elements for cats such as fluids, salts, nutrients, and water [99]. Nevertheless, new cells from the inferior part of the epidermis permanently replace the useless dead cells. In particular, numerous factors determine the speed of this transformation, including a cat’s nutrition, hormones, tissue characteristics, and immune cells in the skin [17]. In general, the epidermal structure in cats is very thin, and its thickness in haired skin ranges between 0.1 – 0.5 mm while in footpad is found to be up to 1.5 mm thick [99]. Basement membrane zone is located in the middle between the epidermis and the dermis; this layer performs the connective function. Moreover, the basement membrane zone provides a protective role as well [17].

Secondly, the dermis is the middle layer in a cat’s skin. It has a significant role in supporting, nourishing and elasticity, as the dermis contains collagen and elastin

proteins as well as blood vessels, which plays an essential role in supplying the cat's skin with nutritious elements and providing tensile strength [100].

Thirdly, the hypodermis known as (Subcutis layer) is the most in-depth and thickest layer of the skin. The subcutis rich in adipose cells, and contains a network of fibrous tissues that are connected to the dermis and the underlying fascial areas [99].

The thickness of the three main layers varies among different breeds and from parts to another in the cat's body. Specifically, skin in cats found to be the thickest on the forehead, neck, thorax, and the tail's base while the thinnest area of skin is located in the ears. In general, the skin thickness in cats ranges from 0.4 to 3.6 mm. Moreover, adult male cats have thicker skin than female cats. However, skin pH is higher in older and female cats when compared to young and male cats [101].

The pinna is a part of the outer ear, which consists of cartilage overlaid with the skin. The amount of hair on pinnae is extremely minimal in comparison to the other parts of a cat's body. The convex surface is covered with more hair than the concave surface. The structure of the skin of ear pinnae consists of three main layers as well. As is stated by Monteiro-Riviero et al., the epidermal thickness of the cat's ear is 10.01±1.53μm while the stratum corneum thickness at the ear is 8.90±0.91μm [102].

The epidermis on the skin of the pinnae contains many small blood vessels like the one in the skin on other parts of a cat's body such as nick, thorax, and forehead [103].

1.4. Overview of Feline Hyperthyroidism

Hyperthyroidism or thyrotoxicosis is the most common glandular disorder in cats resulted from over secretion of thyroid hormones from the thyroid gland that leads to an increase of the circulating thyroid hormones (Thyroxin T4, and the active form Triiodothyronine T3) [18]. Feline hyperthyroidism is of increasing prevalence of disease as its annual incidence varies geographically, which reaches up to 11.92 % and is specifically high in older feline patients over nine years of age [19].

The thyroid gland, where approximately 80% of such disorder might occur [20], is formed of two lobes present in the mid-cervical region next to the lateral surfaces of the trachea. The anatomy of the thyroid gland is considered similar to that in humans; however, such bilobed gland in cats is connected by an isthmus of thyroid tissue [1]. The functional unit of thyroid gland structure is called follicle which is surrounded by a type of cells termed as parafollicular cells or C cells. Moreover, such follicular cells are responsible for producing both thyroid hormones T4 and T3, while parafollicular cells produce calcitonin. As a result, the thyroid hormones play a crucial role in the feline body in which bone formation and fetal development are in effect [104].

The thyroid gland immensely depends on iodide which plays a significant role in producing its hormones (T4 and T3). Besides, thyroglobulin is the most abundant protein in the thyroid gland within the follicular lumen. Its primary function is to provide the polypeptide backbone for synthesis and storage of thyroid hormones which in response the thyroid hormones diffuse into the blood circulation for healthy development and regulating metabolism [105]. In particular, thyroid hormone release is controlled by thyroid-stimulating hormone (TSH) produced by the anterior pituitary. Furthermore, TSH binds to the TSH receptor on the thyroid follicular cells and boost the synthesis and secretion of T4 and T. The secretion of TSH from the pituitary gland is regulated by the thyroid releasing hormone (TRH) which is produced in hypothalamus [21](Figure 1.5).

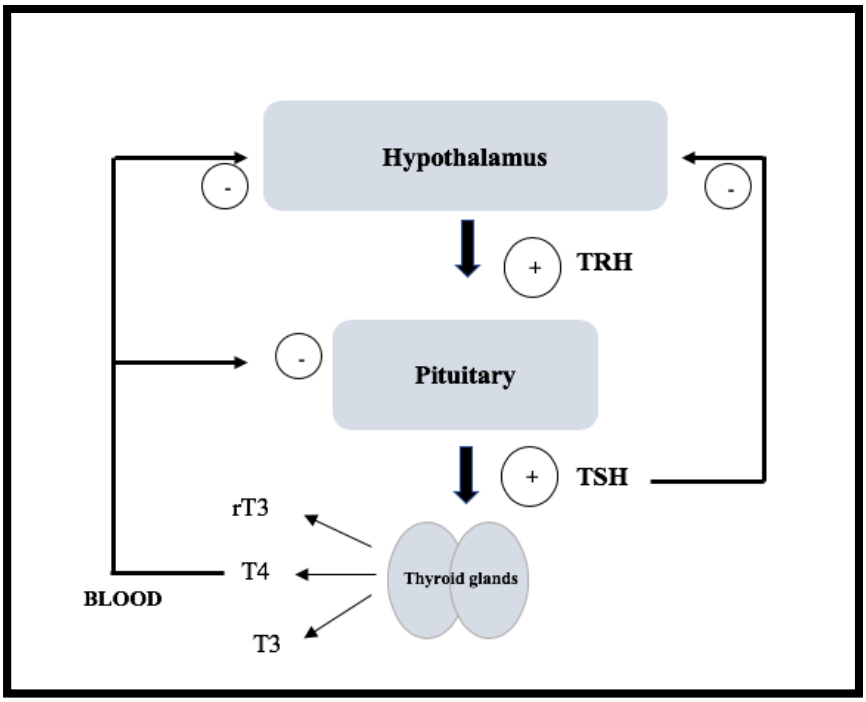


Figure 1.5 The hypothalamic-pituitary-thyroid axis, a classical negative feedback. Reproduce from ref [1].

In addition, hyperthyroidism can be caused by some reasons such as mutations or autonomous replication of follicular cells or TSH receptor [106]. However, the exact underlying cause behind the hyperthyroidism in feline patients has not been determined thus far [1].

The clinical manifestations of hyperthyroidism vary from subtle, barely noticeable signs to severe ones. Examples of mild symptoms are weight loss, tachycardia, and hyperactivity of the cat. More visible and more severe features can be observed as polyphagia, polydipsia or polyuria, vomiting, and diarrhea [106]. Nevertheless, these clinical features are not sufficient for a confirmed diagnosis of hyperthyroidism. The primary determinant finding of hyperthyroidism is the high serum concentration level of total T4 (TT4) which is considered as the profound initial diagnostic test for hyperthyroidism [107]. Besides, the free T4 and the T3 suppression tests can also be measured for confirmation purposes [108]. As it indicated elsewhere [22], over than 90% of hyperthyroid cats might experience an elevation in serum liver enzymes; thus, alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) would be a helpful tool in the diagnosis as well.

In accordance to the guidelines for the management of feline hyperthyroidism (2016), there are four different approaches to manage the hyperthyroidism in cats [23]: surgical thyroidectomy, radioactive iodine, dietary therapy, and medical therapy.

Though, medication administration is the most familiar option for feline hyperthyroidism in which MET is considered the standard treatment since the discovery of the disorder in the 1980s [24].

1.5. The Principle of Transdermal Drug Delivery

Drug delivery methodologies are continuously advancing in the attempts to find the most suitable route that can overcome dosage form's limitations in a particular species. For instance, oral administration in felines particularly for chronically administered medications is of concern; thus, one of the most promising approaches is the transdermal route, in which the transdermal drugs are applied onto intact skin to be subjected for penetration, and systemic absorption accordingly [25].

The mechanism of transdermal drug delivery can be achieved by one of two possible routes, transepidermal (e.g., intercellularly or intracellularly) or trans appendageal (Figure 1.6).

1. *Transepidermal routes*

This route is divided into the intercellular and intracellular routes. In the intercellular pathway the drug molecules crosses in between the cells of the stratum corneum, thus, allows diffusion of non-polar and lipophilic solutes through the lipid matrix. Instead, in intracellular which is known as transcellular route, the drug molecules

pass through the stratum corneum cells as it is distinct for the polar and hydrophilic solutes transport [26, 27]. The stratum corneum is a complex consisted of proteins and lipids which is structurally organized as " bricks and mortar. The very hydrophobic lipids in the SC is uniformly dispersed where this high lipid composite is classified into lamellar membranes that encircle the corneocytes [28].

2. *Transappendageal route*

This pathway involves the passage of the drug through skin appendages such as sweat gland and hair follicles. It then reaches the dermal microcirculation where it travels to systemic organs through the bloodstream [27].

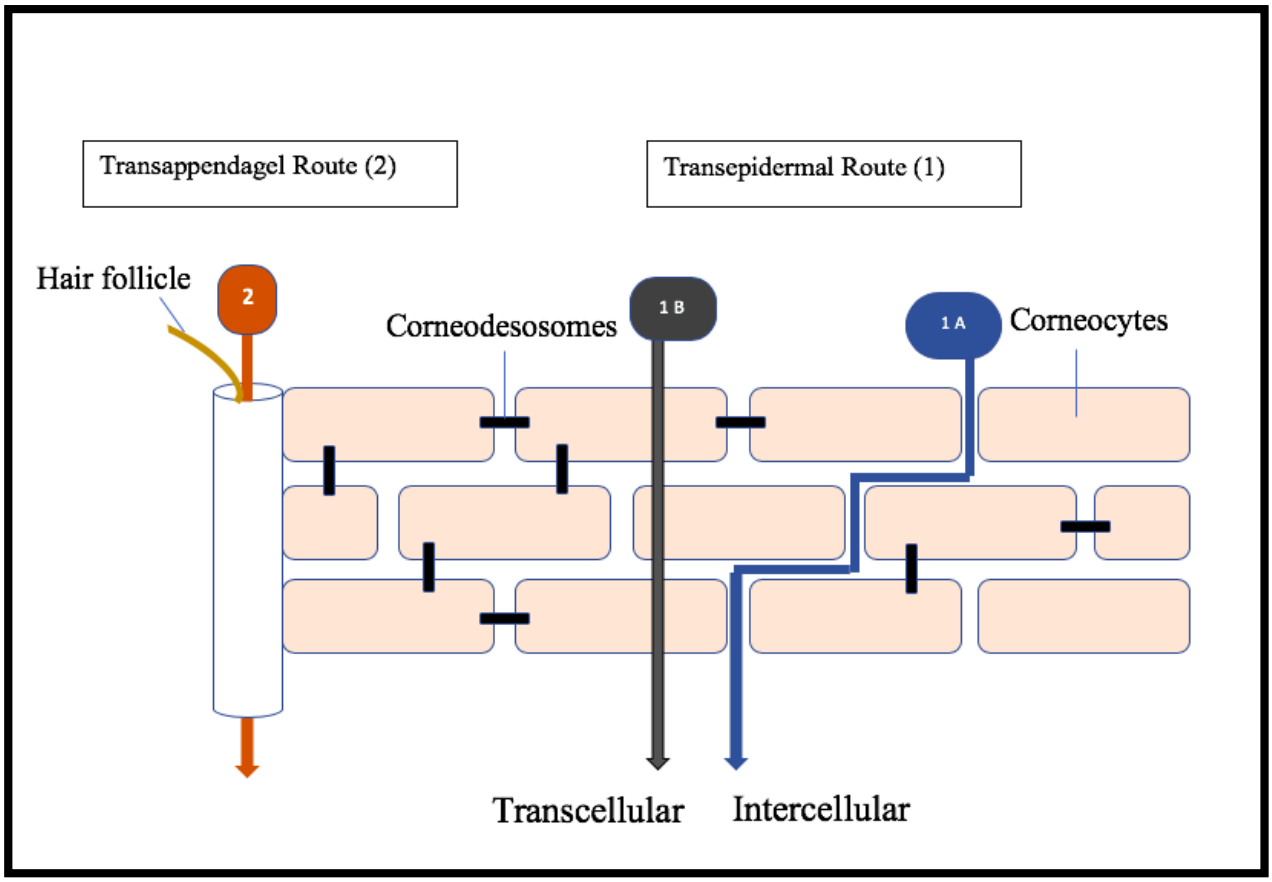


Figure 1.6 Brick and mortar model and routes of transdermal permeation. Reproduce from ref [28].

Indeed, several factors influence the transdermal drug absorption such as skin pH, skin thickness, and hair follicle density. In particular, skin pH plays a significant role in transdermal permeation; and since the stratum corneum is acidic, the acidification of the drug is required [29] . Also, it has been demonstrated by Hill et al., that the drug absorption can be significantly affected by the skin region in which the administration of drugs in the ear pinna of cats had greater absorption than the skin of the groin, thorax, and neck of the cats [30].

The pharmacological drugs must retain specific properties to be delivered successfully through the transdermal system. For instance, drugs with a molecular weight range between 100 - 500 Daltons (1 Dalton = 1gram/mole) were found to be ideal for transdermal transport. Moreover, a drug possesses features of having both hydrophilicity and hydrophobicity in nature, low melting point, potent in small concentration, non-irritant or allergic to the skin, as well as not undergoing heavy metabolism in the skin layers before reaching circulation, are considered as a suitable candidate for transdermal applications [31].

There are several advantages of transdermal drug delivery, some of which are avoiding of hepatic first-pass effect, reducing plasma concentrations and thus decreasing the adverse effects, decreasing the dosing frequency and accordingly, improving patient and owner satisfaction [32]. A very successful example of such drugs is MET, which is currently a registered long-term treatment for hyperthyroidism in cats.

1.6. Microemulsions

Microemulsions are an optically isotropous mixture of water, surfactant, and oil, which usually contains a mixture of hydrocarbons and olefins as constituent elements of the co-surfactant and the oil [33]. The surfactant and the co-surfactant in the mix serve as stabilizers for the microemulsion droplets. Furthermore, microemulsions are predominantly clear and are also highly stable thermodynamically. This thermodynamic stability differentiates microemulsions from conventional emulsions as regular emulsions are kinetically stable, but unstable thermodynamically . Such stability has an essential role in the relative ease in the formation of microemulsions as high energy and shear conditions are not required for their formulation [34]. Likewise, such lower energy requirement allows microemulsions to be more commercially viable than regular emulsions.

The concept of microemulsions was introduced in the 19th century by Professor Jack Shulman, and it was initially defined as a mixture produced by mixing hexanol with a milky emulsion [35]. The term microemulsion was not used until 1959 to describe a multiphase system consisting of water, oil, surfactant, and cosurfactant, which is in effect form a transparent solution [35]. The microemulsion particle size was discovered to be ranging approximately from 1 to 100 nm, usually 10 to 50 nm [36].

Microemulsions were found to be effectively used in the transdermal delivery of certain medications as a result of their numerous advantages. Some of these

advantageous properties are the thermodynamic stability, the unnecessity for handling special equipment, the possibility of utilizing both hydrophilic and lipophilic drugs, and the low cost of preparation [37]. Since microemulsions are possessing a remarkable penetrating enhancing ability, they have become more preferable than other dermatological preparations to permeate the external barrier provided by the skin.

The combination of the microemulsion and the therapeutic agent is mainly dependent on the internal structure of the microemulsion used and the quantities of its components. Indeed, the main components of microemulsions are water, oil, surfactant, and co-surfactants. Several studies have indicated the drug permeation across the skin is primarily affected by the type of oil as well as the combination of the surfactant and co-surfactant used in the microemulsion [37].

Based on the microemulsions structure, they can be divided into three distinct categories; for example Oil-in-Water, Water-in-Oil, and Bi-continuous structures. Oil in water microemulsions, which is the most prevalent type used, primarily comprised of droplets of oil that are surrounded by droplets of water in the dispersed phase [38]. Conversely, Water in Oil microemulsions possesses a reverse structure by constituting droplets of water surrounded by droplets of oil. Moreover, they could also exist as bi-continuous structures or sponge structures, where oil and water exist

in bi-continuous phases and are separated by the surfactant in the mixture as the sponge (Figure 1.7). In each of these structures, the surfactant and the co-surfactant both stabilize the droplets in the mixtures by reducing the internal surface tension that exists between the two continuous phases to almost zero [38].

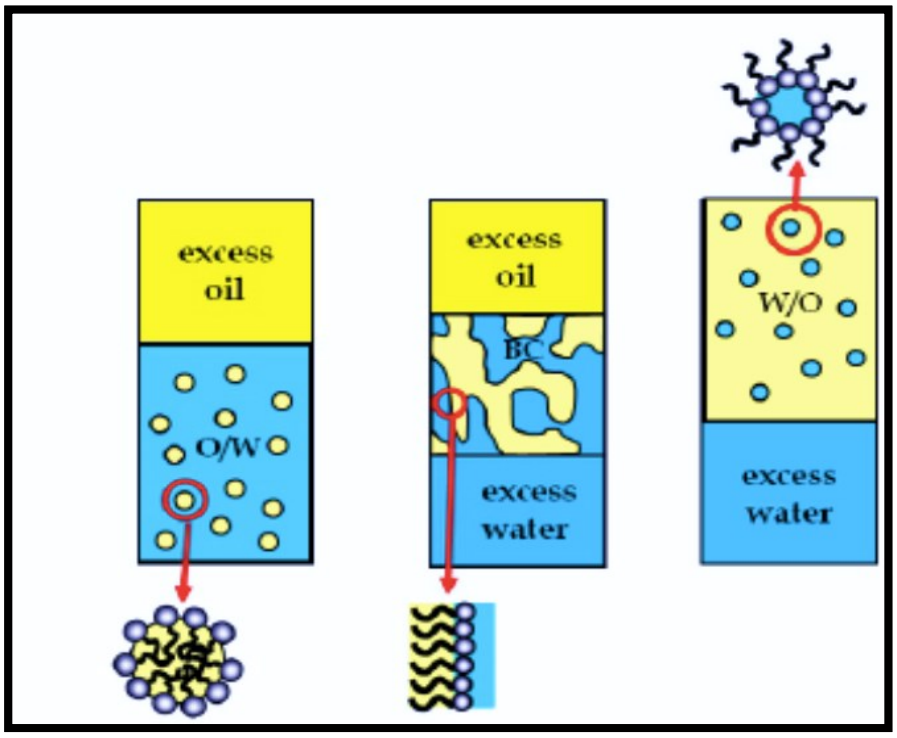


Figure 1.7 Schematic illustration of the microemulsion structures. Adapted from https://www.researchgate.net/figure/Winsor-classification-of-microemulsion-equilibria-Microemulsion-phase-sequence-as-a_fig2_224830304

The surfactant usually contains a charged hydrophilic head group alongside the hydrophobic carbon tail. The surfactant usually stabilizes the mixture by keeping its head group resides in one phase while the tail is retained in the corresponding continuous phase. Besides the cosurfactant is providing stability to the mix, it is reducing the intermolecular forces in the charged head of the surfactant (Figure 1.8) [38] . Furthermore, Hydrophilic-Lipophilic Balance (HLB) is considered as an

empirical expression for the hydrophilic and hydrophobic groups of a surfactant. In particular, higher HLB value is referred to more water-soluble the surfactant [109].

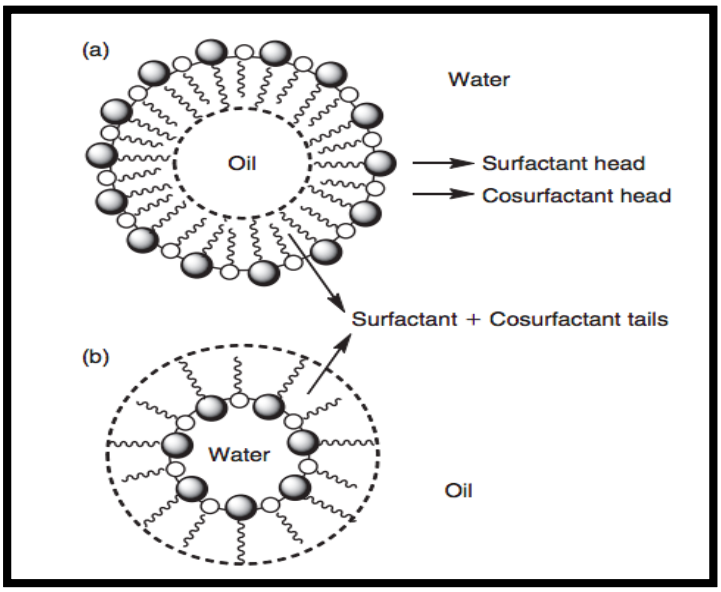


Figure 1-8 Microemulsion like-droplet: (a) O/W Microemulsion, (b) W/O Microemulsion. Retrieved from ref [39].

1.7. *In vitro* Release Testing (IVRT)

One of the most efficient techniques to evaluate the transdermal permeation of MET in cats is the Franz cell apparatus. In accordance to the FDA's guidance for industry on Scale-Up and Post Approval Changes for Semisolid Dosage Forms (SUPAC-SS), *In vitro* release tests using vertical diffusion cell procedure to study the pre-change and post-change by SUPAC 's related changes approval. In particular, VDC consists of two chambers; the donor compartment and the receptor compartment between which the membrane is placed, in which the donor chamber serve as a holder for the formulation, and the receptor chamber contain the receiver medium as well as serve as a sampling point. As it stated in Mills et al., Hill et al., bath temperature was

maintained at approximately 32°C to mimic the *in vivo* skin condition of dogs for the former and the cats for the latte [110, 40].

Indeed, diffusion cells can be categorized into two types: static type or flow-through type, in which static model can be sub-categorized based on the membrane positioning into Horizontal or vertical apparatus [41]. Nevertheless, vertical diffusion cell (VDC) is the most frequently used apparatus to assess and validate the IVRT where the membrane positioned toward the air [42] (Figure 1.9).

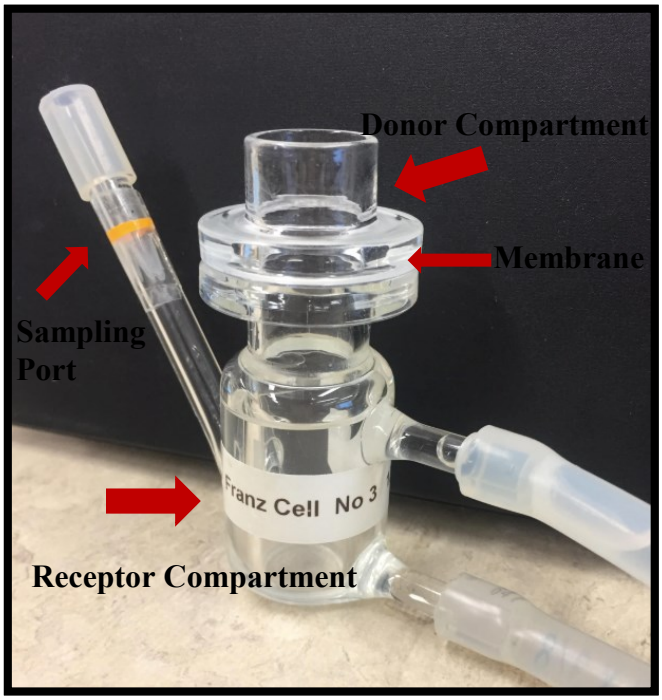


Figure 1.9 Typical diagram of vertical diffusion apparatus.

In general, as is described in USP chapter <1724>, there are three essential dimensions of Franz cell apparatus that should be considered; firstly, the size of the donor chamber as it is necessary for maintaining the infinite dose theory throughout the experiment. Secondly, the orifice diameter plays a role in choosing the suitable

concentration applied to the donor compartment. Thirdly, the capacity of the receptor chamber should be considered to maintain the sink condition at each sampling time[42] .

Indeed, it is essential to maintain the temperature in the system constant as it should be measured in each cell at the beginning and the end of the experiment. Also, it should be taken into consideration that the synthetic membrane used is compatible with the active ingredient and with the chosen receiver medium[42] .

The principle of IVRT is to determine the diffusion of the drug from the semisolid dosage form, through a membrane, into a suitable receiver. The data can be mathematically calculated based on Fick's first law of diffusion **(Equation 1)**:

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

(Equation 1)

where J is the rate of transfer per unit area (flux) (g cm^2 h^{-1}), C is the concentration gradient (gcm $^{-3}$), x is the linear distance travelled (cm), and D is the diffusion coefficient (cm^2 h^{-1}). The equation adapted from ref[41] .

1.8. Rationale, Hypothesis, Study Objectives

1.8.1. Rationale

Numerous studies have been conducted to evaluate the MET Efficacy. One of which is a study titled as the safety of transdermal MET in the treatment of cats with hyperthyroidism and it was concluded that cats treated with oral MET had experienced a higher incidence of gastrointestinal side effects compared to those treated with transdermal MET [113].

Another case study has been conducted in this regard which is titled as; "Carbimazole associated hypersensitivity vasculitis in a cat". And it was concluded that a cat experienced a hypersensitivity vasculitis which resulted in tail necrosis [112].

Based on previous studies as the oral Carbimazole (as antithyroid drug) and oral MET might not be the most favorable dosage form to treat hyperthyroidism in cats, so the Microemulsion-based System for transdermal drug delivery of MET is tended to be used in this veterinary project.

1.8.2. Hypothesis

Microemulsion system can improve the *In vitro* permeation of MET. Hence, the formulated drug-loaded microemulsion is capable of enhancing the penetration of transdermal applied MET.

1.8.3. Objectives

1- To formulate ME-based foam that acts as an appropriate drug vehicle for MET.

2- To characterize the properties of the prepared ME-based foam.

3- To evaluate the stability of the formulated ME-based foam through performing physiochemical tests.

4- To assess the *In vitro* drug release performance of MET from the formulated ME-based foam as compared to different compounded bases formulations.

Chapter 2

2.Evaluation and Characterization of a Microemulsion-Based Foam as a Transdermal Drug Delivery of Methimazole

2.1. Introduction

Topicals are pharmaceutical dosage forms that tend to be formulated for effective drug delivery concerning maximizing patient compliance and safety [43] . Although a few drugs have received Food and Drug Administration (FDA) approval for the use in cats and their usability are limited due to either palatability or lack of suitable tablet size and strength. There is a growing number of topical pharmaceutical products being registered and marketed in veterinary medicine [44'45]. MET (Felimazole®) is an FDA approved medication as an orally administered drug for the treatment of hyperthyroid cats [46] . At the same time, compliance with chronic oral medications can be a problematic in feline patients due to having difficulty administration to some cats or containing unpleasant tasting substance [44]. Besides, drug absorption in a feline with intestinal malabsorption may exhibit poor availability after oral administration [12]. In response, veterinary compounding pharmacies have started using transdermal medications as an alternative route of drug administrations for cats and formulating transdermal gels that can be applied to the cats' ears [46]. Transdermal drug delivery, in particular, was brought to attention as this technique of drug delivery possess numerous advantages over traditional routes of administration. The efficacy of transdermal application could be and not limited to reduction of first-pass metabolism in liver, improvement of the therapeutic dose efficiency and delivering the drug to the systemic circulation at a fixed rate [47]. As the application of topical and transdermal drug therapy in veterinary medicine have

gained considerable attention, several studies have been conducted to study the efficacy of transdermal MET application in feline patients. One of which is a study that was performed by Hill et al., to characterize the percutaneous absorption of MET in a lipophilic vehicle versus PLO vehicle. This trial based on six cats and it was concluded that MET was significantly better absorbed when administered in the lipophilic vehicle than PLO gel [40]. For transdermal delivery, the drug must be formulated in the appropriate vehicle that can effectively transport the active ingredient throughout skin layers to systemic circulation [45]. The use of microemulsions is increasingly popular as a drug delivery vehicle due to their numerous advantages such as thermodynamic stability, ease of preparation and scale up, enhancement of drug solubilization as well as improvement of skin permeation [48'49]. Therefore, this study aimed to evaluate the *In vitro* performance of MET through preparing and examining a MET-loaded foamable microemulsion formulation. A direct comparison was performed with marketed and compounded products.

2.2. Materials and Methods

2.2.1. Materials

MET was purchased from Sigma-Aldrich. A compounded topical MET-formulation of a Lipoderm base purchased from a local pharmacy Exp. Date: 09/2018. Labrasol (Caprylocaproyl polyoxyl-8 glycerides NF), Plurol Oleique (polyglyceryl-3 dioleate NF), and Labrafac (Medium-Chain Triglycerides NF) were received as a kind gift

from GatteFosse, (Montreal QC). Carbopol 934P NF was from L.V. Lomas Limited (Brampton ON). Oleabase and Versapro base for topical formulation was received from Medisca. 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. MET Assay

The quantitative determination of MET was performed by reversed-phase high-performance liquid chromatography method (HPLC) at λmax = 252 nm. A calibration curve was then obtained (Y = 1E+08x - 109394), in which Y was concentration [mg/mL], X was peak area, and r^2 was 0.9999. The standard plot of MET has performed over the concentration range of 0.002 to 0.2 mg/mL.

2.2.2.2. HPLC Method for Quantification of MET

The high-performance liquid chromatography (HPLC) analysis of MET in the microemulsion formulation was carried out using a Shimadzu system. The HPLC system was equipped with CBM-20A controller, SIL-10A auto-injector, LC 10AS pump, CTO-10A column oven, SPD-M10A VP diode array detector. Chromatographic separation was achieved using a LiChrospher RP-18 column (5 µm packing, 4.6 mm × 12.5 mm) and maintained at 40°C. The isocratic mobile phase consisted of 10% Methanol, pumped at a flow rate of 1 ml/min. The assays were

acquired by injecting 20 µl of sample and fixing the UV detector wavelength at 252 nm. The retention time of MET was determined in 3 minutes after the start of each run. And then, the data was quantified by using EZStart 7.4 SP1 software.

2.2.2.3. Preparation of Drug-loaded Microemulsion (ME)

The ME formulation was prepared experimentally based on ref [50] , by incorporating the following components: Labrasol (caprylocaproyl polyoxyl-8 glycerides NF) as a surfactant (with an HLB value of 12) [111] and Plurol Oleique (polyglyceryl-3 dioleate NF) as a cosurfactant at 6:1 ratio into the Labrafac as oil phase (caprylic capric triglycerides NF). At room temperature, water was added to the above mixture and mixed gently. Different concentrations of 0.25%, 1%, and 2.5% (w/w) MET were compounded with the Labrafac-based microemulsions. The mixtures were finally mixed with the aid of a magnetic stirrer at 600 rpm room temperature for 5 minutes, and transparent drug-loaded O/W MEs were obtained (Table 2.1). In this study, (2.5% w/w) 25mg/mL MET was the highest concentration used which is within the therapeutic range for the feline transdermal application. Generally, MET concentration of 2.5 mg/mL up to 100 mg/mL as a transdermal compound can be used topically on the cats' ears [51'6].

Table 2.1 Components Composition (% w/w) of the drug-loaded foamable ME formulations.

Excipients	ME	Drug-loaded ME
Labrasol (Caprylocaproyl polyoxyl-8 glycerides NF)	18	18
Plurol Oleique (Polyglyceryl-3 dioleate NF)	3	3
Labrafac (Medium-Chain Triglycerides NF)	0.5	0.5
MET	-	0.25, 1, 2.5
Purified Water	q.s.	q.s.

2.2.2.4. Preparation of Plain Carbopol Gel Base

Carbopol 934P gel base was prepared by gradually dispersing 1% (w/w) Carbopol into distilled water and mixing it using a magnetic stirrer at 1200 rpm for at least 30 min [52]. The mixture was allowed to hydrate and swell for 24 hours. Next, Carbopol was then neutralized with 10 % sodium hydroxide (10% NaOH) solution that was added dropwise until the desired pH value for topical application was approximately reached between 4-7 [53'54].

2.2.2.5. Preparation of Plain Pluronic Lecithin Organogel (PLO) Base

The plain PLO base was prepared as described elsewhere[55] : 30% Pluronic gel (F127) was first cooled at 4 °C until a clear solution was obtained, and then, PLO was prepared by mixing 30% Pluronic gel solution and Lecithin/Isopropyl palmitate in a ratio of 4:1.

2.2.2.6. MET in Different Compounded Bases

The compounded MET based gel formulations were prepared by adding the desired amount of MET into the selected base (PLO, Versapro, or Oleabase) and blended via geometric dilution [56]. The final concentration of MET based gel formulations were 2.5% (w/w).

2.2.2.7. Drug Solubility Determination

For measuring the drug solubility in the surfactant, cosurfactant, oil phase and the prepared foamable ME, an excess amount of MET was added to 2 g of each of the vehicles. Mixtures were shaken and kept in a shaker at 25°C for 72 hours. Afterward, the sample was withdrawn at 24 hours, 48 hours, and 72 hours and at each time interval the sample was subjected to centrifugation using a centrifuge (Heraeus Biofuge Pico) at 10,000 rpm for 10 min. The concentration of MET in the supernatant was diluted with an HPLC grade methanol and analyzed by HPLC (Shimadzu) at a wavelength of 252 nm [57] [54] .

2.2.2.8. Physicochemical Evaluation of the Prepared Foamable ME

The following thermodynamic stability tests were conducted to evaluate the physical stability of foamable ME formulation.

2.2.2.8.1. Physical Appearance

The prepared ME and ME loaded with MET were visually inspected for precipitation, homogeneity, or any changes in their colors [58].

2.2.2.8.2. pH Measurements

The pH values of the formulated ME with and without the drug was obtained using a digital pH meter (Accumet XL20, pH meter). The pH meter was calibrated by applying a 3-point calibration with standard pH solutions of 4, 7, and 10. Afterward, the electrode was rinsed with doubled distilled water and blot was dried with a clean tissue paper. The electrode was then inserted into the test solution, and the pH was recorded when reading was stable. All measurements were performed for three times at room temperature [59].

2.2.2.8.3. Drug Content Estimation

For the determination of drug content, 10 mg of MET was dissolved in the previously prepared ME. Then, 1mL of the mixture was diluted in 100 mL of distilled water and

filtered. The drug content of the resultant solution was quantified by HPLC method at a wavelength of 252 nm. All measurements were performed in triplicate [60] (Equation 2).

$$\text{Drug loading efficiency} = \frac{\text{Amount of drug in known amount of formulation}}{\text{Initial drug load}} \times 100$$

Equation 2

2.2.2.8.4. Centrifugation Test

The foamable ME-based formulation and MET-loaded foamable ME were evaluated for phase separation and homogeneity alteration by subjecting the formulations to centrifugation using Centrifuge (Heraeus Biofuge Pico) at 11,000 rpm for 30 min, and they were visually inspected at 25℃. The samples were then taken to heating and cooling cycles [61].

2.2.2.8.5. Heating-Cooling Cycles

Heating and cooling cycles are one of the physiochemical stability tests that were performed to evaluate the stability of the formulations under thermal condition. Six cycles between the refrigerator (4°C) and oven temperature (45°C) of both the foamable ME and drug-loaded foamable ME were conducted for not less than 48 h for each cycle [59]. At the end of the experiment, both formulations were evaluated for physical characteristics such as pH, homogeneity, and consistency.

2.2.2.8.6. Percentage Transmittance

The transparency of the foamable ME and drug-loaded foamable ME was detected by measuring the percentage transmittance of the formulations using UV-Visible spectrophotometer (Genesys 10 Bio UV). Percentage transmittance of the formulations was examined at 650 nm keeping the purified water as a blank, and three replicates were measured for each sample [60].

2.2.2.8.7. Particle Size Measurement

By the principle of dynamic light scattering, the Zetasizer Nano-DTS 1060 (Malvern Instruments Ltd, UK) was used to determine the particle size at 25°C. The samples of the foamable ME and drug-loaded foamable ME were diluted in degassed purified water and were kept in disposable cuvettes. All the measurements were performed in triplicate. The polydispersity index (PDI) was used as a parameter for droplet-size distribution by indicating the aggregation in the particles [62].

2.2.2.8.8. Transmission Electron Microscopy (TEM) Analysis

The Particles' shape and surface structure of the drug-free ME and drug-loaded ME was examined using Philips / FEI (Morgagni) transmission electron microscopy (TEM) operated with Gatan Digital Camera for taking the images. Samples were prepared for staining as follows: diluted formulations (1 in 10 dilutions) were dropped gently onto a copper grid. A filter paper was used to remove the excess

amount. A drop of 2% aqueous solution of phosphotungstic acid was then placed into the copper grid and left for 30–60 seconds to stain and the excess was removed using a filter paper. The dried grid was held on a slide and covered with a coverslip, before performing the observation of the sample under TEM with different magnification [62].

2.2.2.9. Qualitative Studies Analysis

2.2.2.9.1. Electric Conductivity Measurement

The electroconductivity of the formulated foamable ME with and without the drug was measured using (Accumet XL20 conductivity meter) that armed with 1.0 accumet probe. The conductivity meter was standardized by using a 3-point standardization of 23, 447 and 1500 Microsiemens per centimeter (μS/cm) standard solutions. The probe was then inserted into the test solution, and the conductivity value was recorded when reading was stable. All measurements were performed for three times at room temperature.

2.2.2.9.2. Dye Solubility Test

Since the staining test is considered as a parameter to determine the type of ME, a water-soluble dye was used to evaluate the type of the drug-free and drug-loaded ME. The staining test was performed by dispersing a water-soluble dye in the ME systems to inspect the dispersion visually. A uniformly dissolved dye in the system

is an indication of an oil in water (O/W) ME while observing clump on a surface is associated with the W/O ME type [63].

2.2.2.10. *In vitro* Drug Release Studies

In vitro release studies were performed using static Franz glass diffusion cells (minimum of 3 replicates) to determine the cumulative percentage drug release and the flux rate of MET from the foamable ME and other vehicles. The area for diffusion was 1.79 cm^2 (15.1 mm diameter orifice). The Franz diffusion cells were set up and allowed to equilibrate for 30 minutes before the samples were applied. In the meanwhile, synthetic 0.45 μm pore diameter hydrophobic Polyvinylidene Fluoride (PVDF) membranes soaked in double-distilled water for 30 minutes. The membranes were then carefully positioned between the donor and receptor compartments. The receptor compartments were thermoregulated using a circulating water bath (Haakel D2, Germany) and maintained at 32.0 ± 0.5 °C to mimic the skin temperature of cats on the surface of the membrane [7]. The receptor fluid consisted of double-distilled water because of the sufficient solubility of MET in the chosen receptor medium. The receptor chamber volume varied from 12 to 13 ml. Each diffusion cell contained a magnetic bar and was magnetically stirred at 600 rpm (IKA, USA) during the experiment to keep homogenous concentrations within the acceptor medium and to minimize stagnant layers. 0.5 g of the formulations (containing 2.5% w/w of MET) were accurately weighed and placed in the donor compartments. 100 μL samples were withdrawn through the sampling port at five points time intervals (0.5, 1, 2, 4

and 6 h) using a syringe needle, and diluted with 900 µL fresh acceptor medium. The same volumes were replaced with fresh double-distilled water to maintain a constant volume. The samples were analyzed by HPLC method at 252 nm. The cumulative percentage release of MET and the Flux were calculated.

Formula for Determination of Percentage of Release of Drug MET from *In vitro* Release Testing, adapted from reference [64] (Equations 3)

Equations 3.1. Concentration of drug (µg/ml)= (slope × absorbance) ± intercept.

Equations 3.2. Amount of drug released mg/ ml = (Concentration × Dissolution bath volume × dilution factor)/1000.

Equations 3.3. Cumulative percentage = Volume of sample withdrawn (ml) × P (t – 1) + Pt release (%) Bath volume (v) Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to 't'.

Formula for Determination the Flux (J) of Drug MET, adapted from reference (Equation 4) [65]

Equation 4 J= Q/(At)

Where Q is the total quantity of drug travelling across the membrane in time t, and A is the area of exposed membrane in cm^2. For this experiment the diffusion area was 1.79 cm^2.

The release profiles of MET from the foamable ME formulation was compared with five different compounded preparations consisting of commercial and compounding

bases (LipodermA, Versapro, PLO, and Oleabase) each contains 2.5% (w/w) MET, which is a commonly available strength in the market. A strength of 0.25% w/w, which is the lowest strength available in the market, was also evaluated and compared with 2.5% (w/w) MET-loaded ME and 2.5% (w/w) MET free ME as a control. Indeed, different drug strength mimics the variability of an individual's need.

2.2.2.11. Foam Quality

Abram and Hunt ranking of 0–5 is used to evaluate the foam quality. As shown in (Figure 2.1), the rank "0" is demonstrating full, fine and stable bubble foams where the rank "5" is demonstrating large bubble foams or foams that immediately break to large bubbles. Overall, the higher foam stability, the lower value on the scale [66].

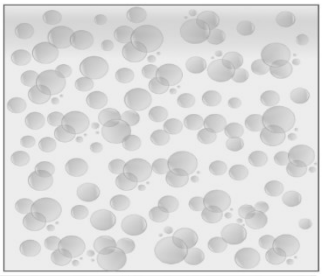
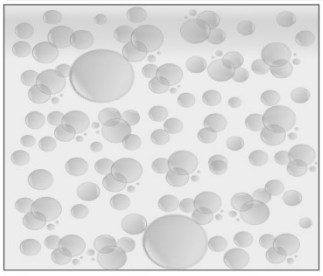
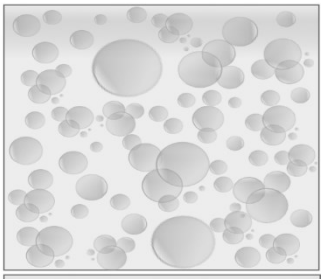
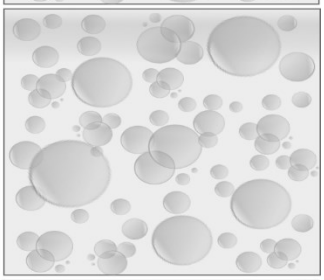
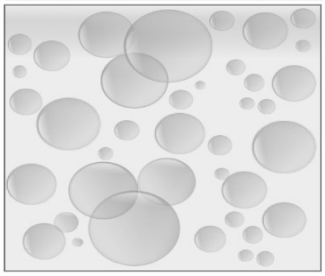
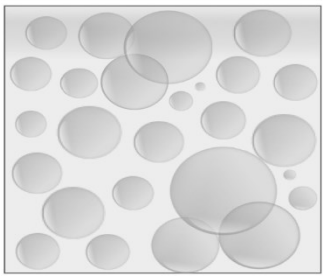
Scale	Structure	Description
0		Full, fine, stable (holds structure or only a very slow, small collapse over 30-60 sec).
1		Mostly fine with a couple of coarser bubbles on surface than stable, or fine then slightly coarser over time.
2		Slightly coarse initially but reasonably stable, or fine (possibly 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 2.1 A visual assessment of Abram and Hunt's scale for evaluating foam structure. Retrieved from ref [66].

2.2.2.11.1 Production of Foam from Foamable ME

One of the techniques to produce foam from the ME system is bubbling method. Bubbling method can be performed by injecting the foamable ME formulation and gas through the narrow opening of the syringe. Two of 10 ml syringes with a Luer-Lok™ tip was used. In particular, 2 ml of the foamable formulation and 4ml of ambient air was injected through the narrow opening in one syringe where the second one enclosed 8ml of ambient air. Then, Baxter sterile Rapid-Fill™ connector Luer lock-to-Luer lock was placed in between the two syringes to connect them. The foam was generated by pushing the solution and ambient air from one syringe to the other (Figure 2.2) [66] .

Figure 2.2 Generating of foam via bubbling method

2.2.2.12 Statistical Analysis

All the measurements were performed in triplicate, and data were expressed as mean ± SD. The statistically significant differences between formulations were determined by using one-way analysis of variance (ANOVA) and student t-test at the probability level of 0.05. A non-parametric post hoc test (Tukey's test) was used for comparing differences between individual means. A p-value of p=0.05 was considered to be statistically significant. The Statistical analysis was done using by SPSS software (version 24), and Microsoft Office Excel (version 16.15). Additionally, DDSolver software was used to compare drug release profiles by using one-way ANOVA and similarity factor f2. Calculations of f2 values, which is a measurement of the similarity in the (%) release between two curves, was done according to the Equation (5).

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

Equation (5).

Where n indicates the number of time points, Rt is the dissolution value of the reference product at time t, and Tt is the dissolution value of the test product at time t. f2 values must be higher than 50 (50-100) to ensure closeness or equivalence of two dissolution curves as well as the performance of the test and reference products [67].

2.3. Results and Discussion

2.3.1. Determination of Drug Solubility

Solubility is an essential criterion in choosing the appropriate microemulsion as a vehicle for transdermal drug delivery. The suitable solubility of the drug in the oil phase, surfactant, and cosurfactant would help the microemulsion to maintain the drug in dissolved form. Moreover, the surfactant as a primary component of microemulsion stated to act as a penetration enhancer. Additionally, cosurfactant plays a role in boosting the fluidity of the interface by penetrating into the surfactant layer [68,16,69]. In particular, caprylic capric triglycerides was chosen as an oil phase, caprylocaproyl polyoxyl-8 glycerides as a surfactant and Polyglyceryl-3 dioleate NF as a cosurfactant. In this study, MET was exhibited reasonably good solubility profile in the tested ME components. (Table 2.2).

Table 2.2 MET solubility in oil phase, surfactant and cosurfactant. **(mean ± SD, n=3).**

Phase type	Excipients	Drug solubility mg/g
Oil	Caprylic capric triglycerides	3.35 ± 0.06
Surfactant	Caprylocaproyl polyoxyl-8 glycerides	159.53 ±1.49
Cosurfactant	Polyglyceryl-3 dioleate NF	41.34 ± 2.11
Water	Double distilled water	275 g/L

2.3.2. Physical Appearance

The physical observation of the prepared foamable ME and MET-loaded ME were liquids that translucent in color for the former and yellow-colored for the later. Both formulations were found to be transparent, clear, and homogenous texture. The generated foam from the dispenser was white-colored with a fine surface of bubbles structure.

2.3.3. pH Measurement Analysis

The plain ME formulation had suitably observed pH value of 4.07 ± 0.14. Significant changes in pH were observed for ME drug-loaded (5.12 ± 0.11) as compared to ME drug-free (P = 0.00048) (Table 2.4). Accordingly, The results of pH measurements of ME drug-free and ME drug-loaded have lied in the range of appropriate pH value of 4.0-7.0 for dermal applications [54].

2.3.4. Drug Content %

The drug content of the formulated foamable ME was observed to be 98.03 ± 1.63% (Table 2.4). In other words, the higher values of drug content, the better estimation of minimal drug loss during the formulation process.

2.3.5. Phase Separation

Microemulsions are generally considered as a type of emulsions. In which emulsions were believed to be thermodynamically unstable and eventually lead to phase separation while microemulsion systems do not [70]. The microemulsion of pure and drug-loaded formulations was found to be optically monophasic even after being subjected to stress stability testing like centrifugation. Subsequently, no signs of drug precipitation nor phase separation were detected, which is a confirmation of the physical stability of the ME system.

2.3.6. Heating-Cooling Cycle Analysis

Regarding the physical transparency, the foamable ME with and without the drug showed no signs of breaking or drug precipitation when subjected to six heating-cooling cycles. To confirm its physical stability, the pH measurement after each cycle was considered, as the changes in the pH of both formulations were not significant (P= 2.85 and P= 2.27) for plain ME and drug-loaded ME, respectively. Accordingly, The physical appearances of the prepared plain ME and MET-loaded foamable ME were unchanged as well as no significant changes in pH were detected, indicating that both formulations were physically stable (Figure 2. 3).

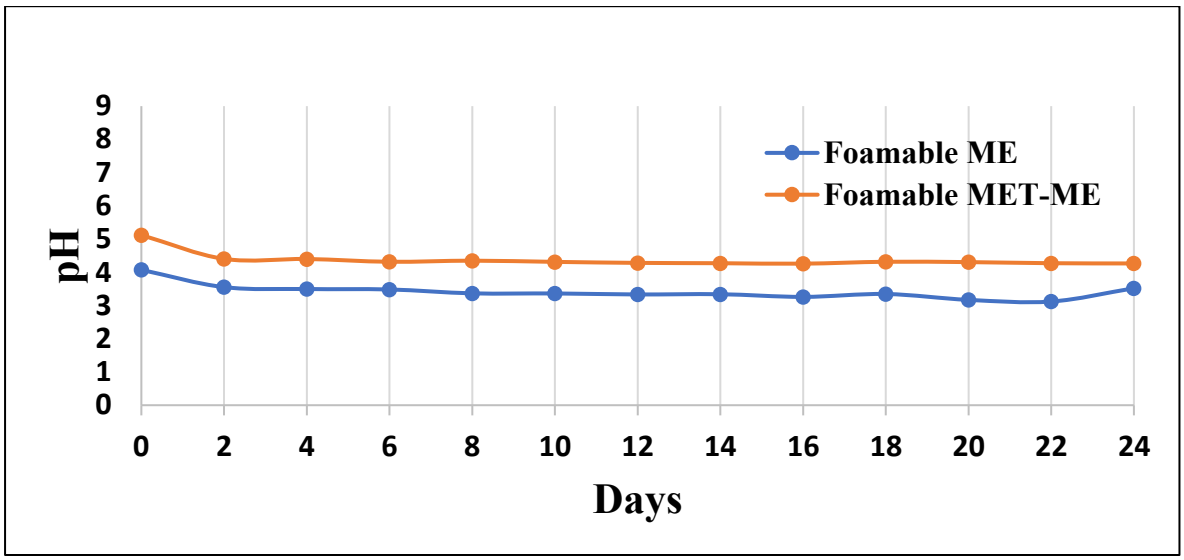


Figure 2.3 Changes in the pH values of the drug-free and drug-loaded ME throughout the heating-cooling cycles.

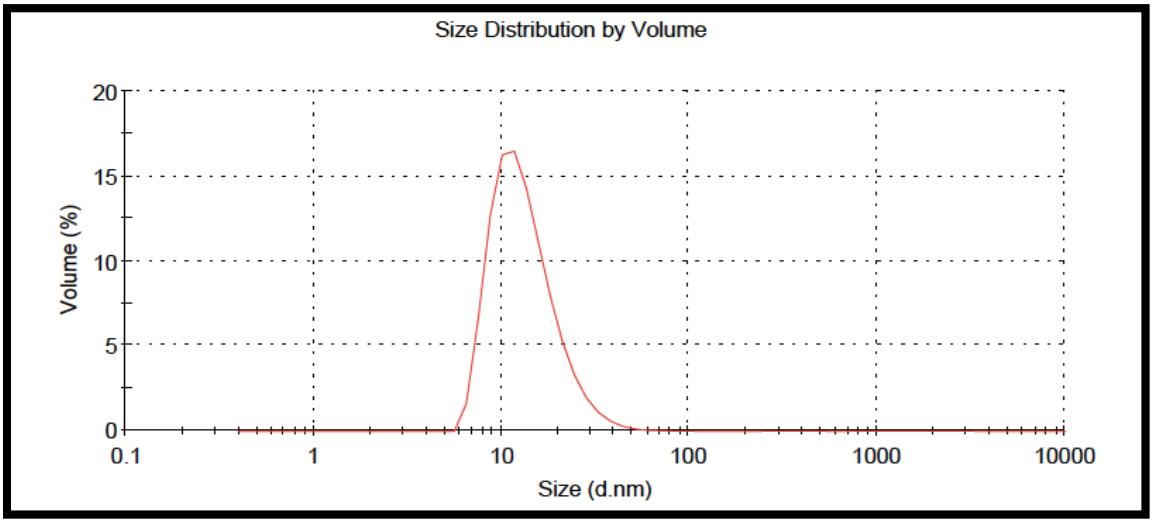
2.3.7. Percentage Transmittance

The clarity of the formulated microemulsion system was evaluated based on the optical transparency, measured as percentage transmission. The higher value of the percentage transmittance is indicating the smaller the amount of light absorbed by the sample. Likewise, a value of percentage transmittance (%T) closer to 100%, this shows that the selected formulation is clear, and transparent [71]. It was found that the ME free drug and the ME loaded with MET have transmittance values at 650 nm of (98.53± 0.39) and (98.60± 0.27), respectively. There was no significant difference between the plain ME and drug-loaded ME, p-value = 0.8251. Percentage transmittance values of the measured formulations were indicating high clarity and transparency of the systems (Table 2.3).

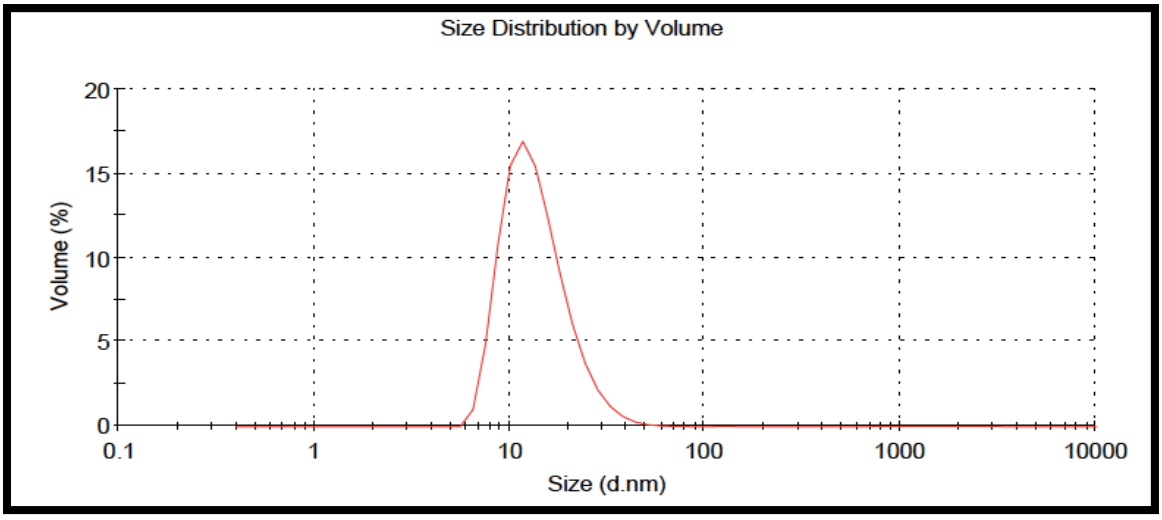
2.3.8. Particle Size Analysis

Particle size measurement is one of the essential criteria to evaluate the physical stability of the microemulsion system for effective transdermal permeation. In the present study, the particle size measurements were carried out using Zetasizer for the blank ME and ME drug-loaded system. It was revealed that the mean particle size of the MET in the prepared ME were (25.98 ± 2.34) in comparison to the plain foamable ME formulation (25.45 ± 1.05) (p= 0.7383). Accordingly, no significant reduction in particle size was observed upon incorporating the MET into ME formulation; the results are shown graphically in (Figure 2.4). As the examined mean globule size was found to be in the microemulsion range, this plays a significant role in skin permeation and thus enhancing *in vivo* efficacy of the formulation [72]. The average particle size was determined to be within the size of the microemulsion in which the size range of the dispersed phase of microemulsion ranging approximately from 1 to 100 nm, usually 10 to 50 nm [36]. It is believed that the smaller the particle size, the larger surface area are explicitly obtained, hence better skin permeability is delivered. Likewise, the polydispersity index (PDI) is a dimensionless number gives information about the uniformity of the particle size distribution in a microemulsion system having a value between 0 and 1. When polydispersity value is closer to zero, this indicated the more uniform and homogenous the formulations [54]. PDI of ME loaded with MET decreased slightly in comparison to the mean droplet size of the drug-free ME; however, no significant reduction was observed ($p > 0.05$). As is

represented in (Table 2.3), the observations of particle size measurements having droplet size in the nano-range and a very low PDI of the measuring systems (< 0.4), these results justified the homogenous and uniform nature of the prepared microemulsion systems.



(a)



(b)

Figure 2.4 Particle size distribution of (a) drug-free foamable ME (25.45 ± 1.05 nm), and (b) MET- loaded foamable ME (25.98 ± 2.34 nm).

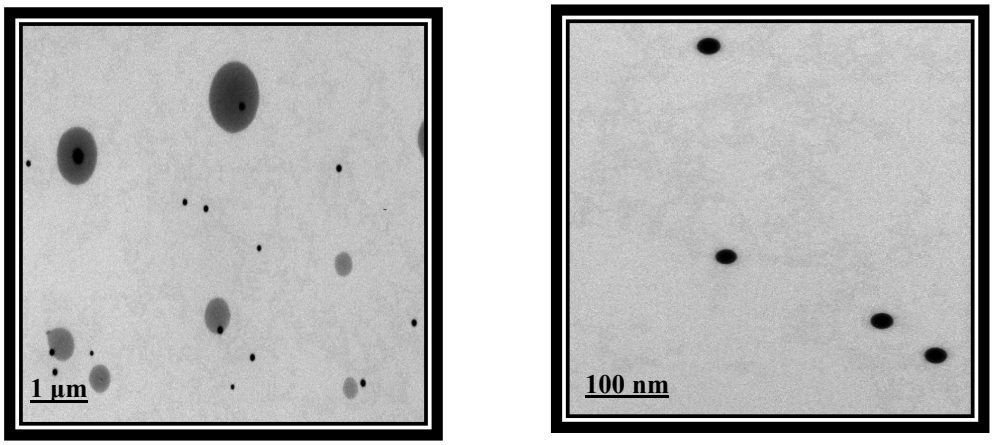
Table 2.3 Physicochemical characteristics of the prepared formulations (mean ± SD, n=3).

Formulation	Transmittance (%)	Particle size (nm)	PDI
ME	98.53± 0.39	25.45 ± 1.05	0.38 ± 0.12
MET ME	98.60± 0.27	25.98 ± 2.34	0.25 ± 0.01

2.3.9. Transmission Electron Microscopy (TEM) Analysis

TEM is one of the fundamental technique to investigate the morphology and the structure of microemulsion droplets [71]. As depicted in (Figure 2.5), the TEM images revealed that the droplets were approximately spherical for the prepared ME and drug-loaded ME. Moreover, the morphological features of prepared microemulsion systems were observed to be in the nanometer size range which was confirmed by Zetasizer.

(a) (b)



(c) (d)

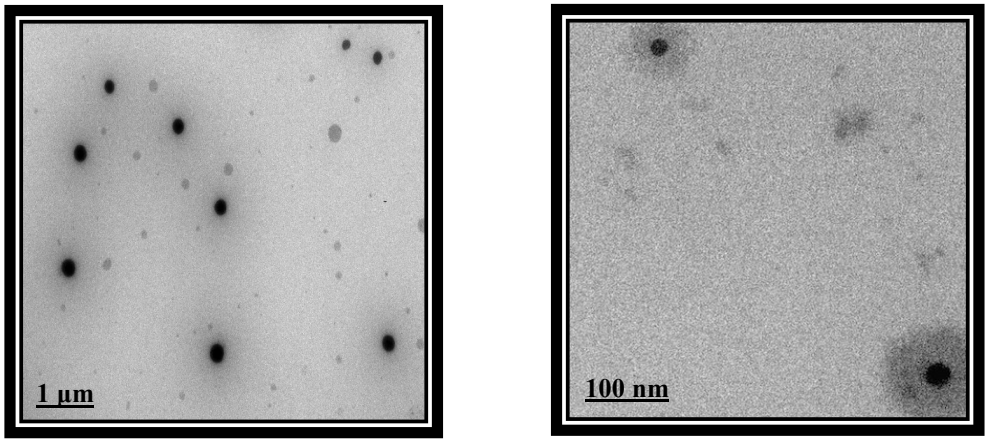


Figure 2.5 TEM images of (a) and (b) of o/w foamable drug-free ME droplets (Magnification 14,000X and 44,000X, respectively), (c) and (d) foamable MET-loaded ME (Magnification 14,000X and 56,000X, respectively).

2.3.10. Qualitative Studies Analysis

Electrical conductometry is a useful tool to evaluate the conductivity behavior of microemulsion samples. Correspondingly, O/W microemulsions exhibit higher conductivity values than the W/O microemulsions [73]. It was found that the conductivity of MET sample in ME was 24.61±1.44 while the plain ME sample 26.82 ±1.05 µS/cm which represent o/w ME structure (Table 2.3). Given that, the added drug did not disrupt the conductivity behavior of the system, the stability nor the visual consistency of the formulation ($p > 0.05$). Similarly, o/w structure of MEs was confirmed after conducting the staining test.

Table 2.4 Drug solubility, pH, drug content, and conductivity measurements of the prepared formulations (mean ± SD, n=3).

Formulation	Drug Solubility mg/g	pH	Drug content %	Conductivity µS/cm
ME	—	4.07 ± 0.14	—	26.82 ±1.05
MET ME	237.21± 2.06	5.12 ± 0.11	98.03 ± 1.63	24.61±1.44

2.3.11. *In vitro* Drug Release Studies

Based on the conducted release tests through the hydrophobic membranes, the cumulative release percentage of MET from Microemulsion (ME) was calculated and compared with four different compounded base formulations as it is shown in (Figure 2.6). The data revealed that after 6 hours, the foamable MET-loaded ME formulation exhibited the highest dug release among all formulations. Foamable MET loaded ME formulation had (84.65 ± 6.44 %) of drug release. A Microemulsion-free formulation with 2.5 % MET was used as a control and only (1.52 ± 2.68 %) of the drug was out of the formulation after six hours (Figure 2.7). The data represents a 55-fold increase in permeability of MET-loaded ME formulation relative to control. Less than 50% of the drug was released from the commercial Lipoderm-based formulation (47.86 ± 7.35 %). While Oleabase formulation showed no release of the drug, the release of MET from Versapro gel and PLO gel were about (33.54 ± 3.40 %) and (33.08 ± 4.93%) respectively. Thus,

the release is considered less than half of the release of the drug-loaded ME after six hours. Despite the low drug strength, the 0.25% w/w foamable MET-loaded ME revealed a higher drug release (67.74 ± 3.76 %) relative to the commercial and compounded formulations. As the Flux (J max) is considered an additional permeation parameter, the observed data demonstrated that the diffusion rate increased in the order of MET-ME > MET-Lipoderm > MET-Versapro > MET-PLO > MET-Oleabase as presented in (Table 2.5). The flux in 6 hours of MET-ME was significantly higher compared to the other tested formulations.

From the current *In vitro* release study, it was seen that the MET-loaded ME, had significantly higher drug releases as compared to compounded formulations, and the control. *f2* comparison of release profiles of two different strength of MET-loaded ME to compounded formulations indicated no similarity at ($p= 0.0001$) as shown in (Table 2.6). The observed high drug release from the ME may be explained based on three reasons. Firstly, the high solubility profile of MET in the ME based formulations could be a dominant reason for increasing the drug release rate. Secondly, a microemulsion of a nano range particle size improved the permeability of the drug by enhancing the adherence to the membrane during dug transportation, and the surfactant, reducing the surface tension between the water and oil interface [74] . Thirdly, higher content of water in O/W MEs leads to higher level of membrane hydration that confirmed by high MET permeation [75].

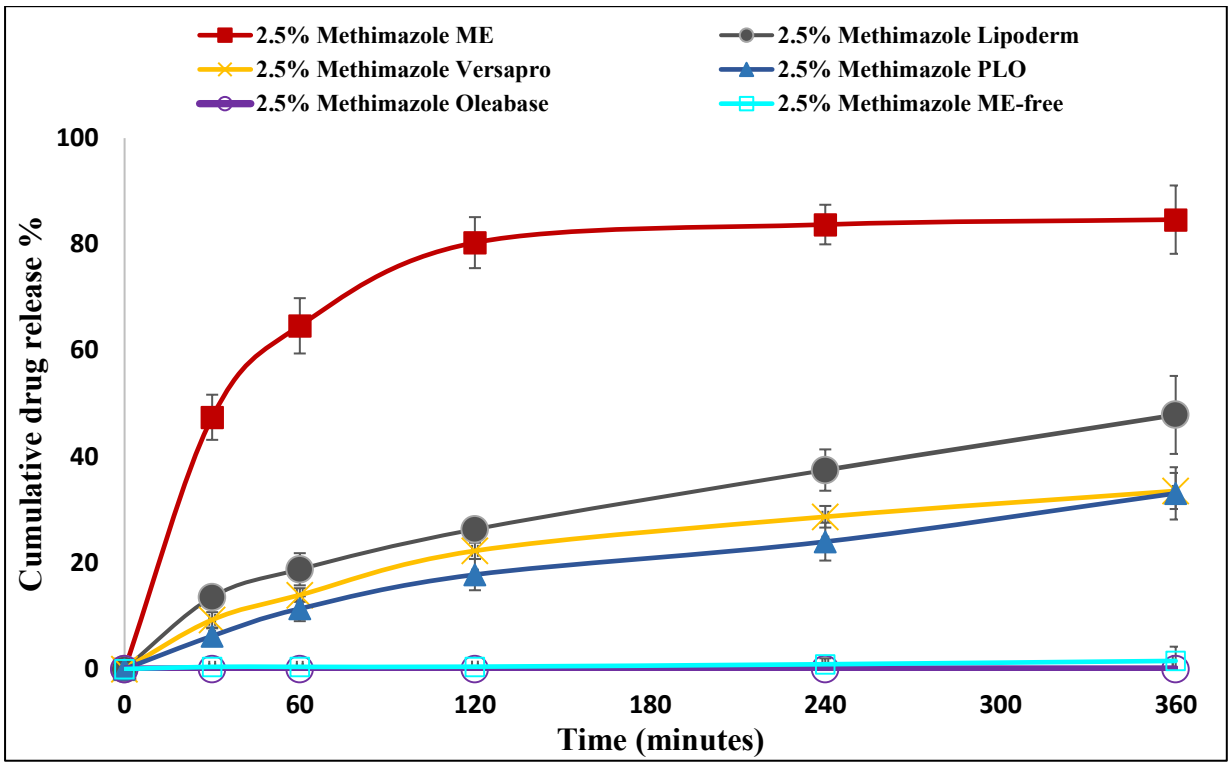


Figure 2.6 *In vitro* release profiles of MET through Hydrophobic PVDF 0.45 µm membranes from the ME, different compounded formulations, and control (mean± SD).

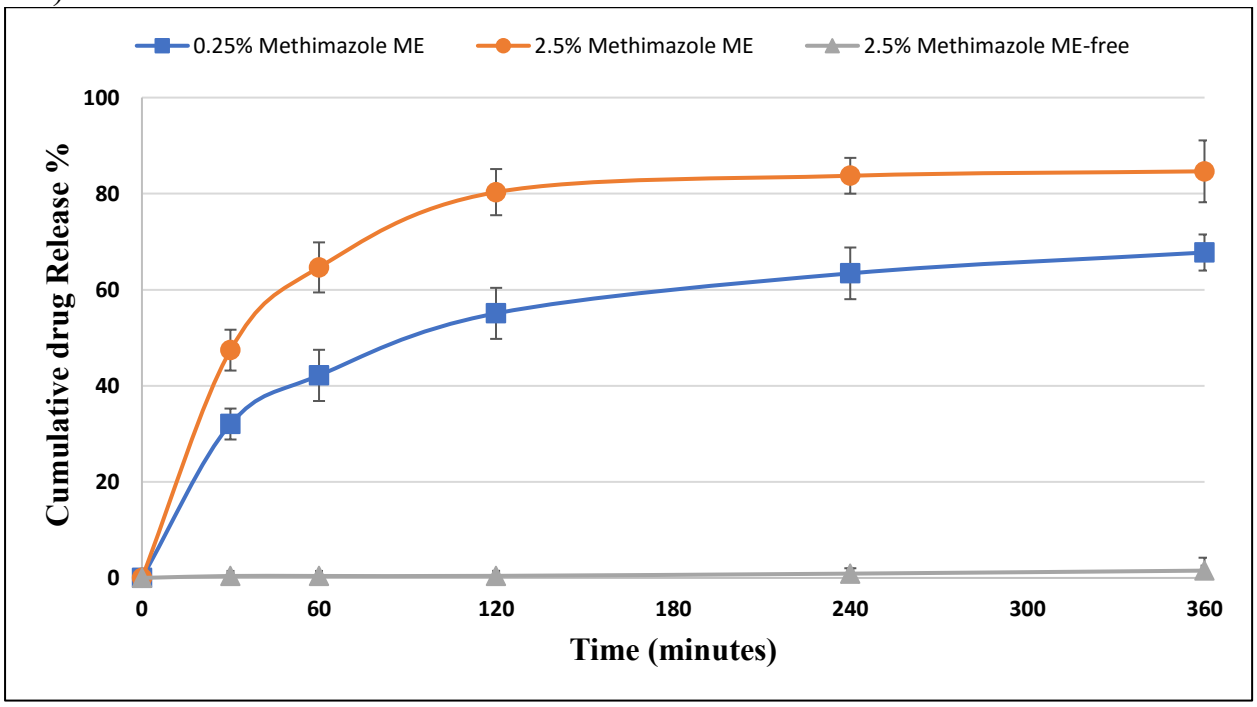


Figure 2.7 Comparison of *In vitro* release profiles of two different MET's strengths and control through Hydrophobic PVDF 0.45 µm membranes (mean ±SD).

Table 2.5 Summary of average MET flux from microemulsion based system, other compounded bases and control after 6 h for individual Hydrophobic PVDF 0.45 µm synthetic membrane.

Formulations	**Flux (mg/ cm^2/hr) ± SD**
MET ME	1.54 ± 0.12
MET Lipoderm	1.11 ± 0.17
MET PLO	0.77 ± 0.11
MET Versapro	0.78 ± 0.08
MET ME-free	0.03 ± 0.05
MET Oleabase	0

Table 2.6 Results of similarity factor (*f2*) for the release profile of two strengths of MET-ME in comparison to different compounded formulations and the control.

Formulation	Similarity factor (*f2*)			
	Lipoderm gel	Versapro gel	PLO gel	MET ME-free
2.5% (w/w) MET-ME	19.83	16.57	15.28	8.87
0.25% (w/w) MET-ME	33.32	27.48	25.43	15.78

2.3.12. Foam Quality

Foam quality is one of the principles for an acceptable foaming structure in foams process, good manufacturing practice (GMP) and quality control (QC) [76]. Different foams are likely accountable for their different quality. Foam quality can be

evaluated by visually inspecting the physical appearance of the foam [77]. Hence, the foam scale of Abram and Hunt was used for comparative purposes of various foam bubble structure and constancy over time [76]. As represented in (Figure 2.8), the foam generated from the drug-free ME was fine (possibly some slight dimples) with a couple of flat bubbles appearing on the surface which categorizes the foam as "2". Whereas the drug-loaded ME produced a stable, mostly fine foam with a couple of coarser bubbles on the surface, that classify the foam as "1".

(a) (b)

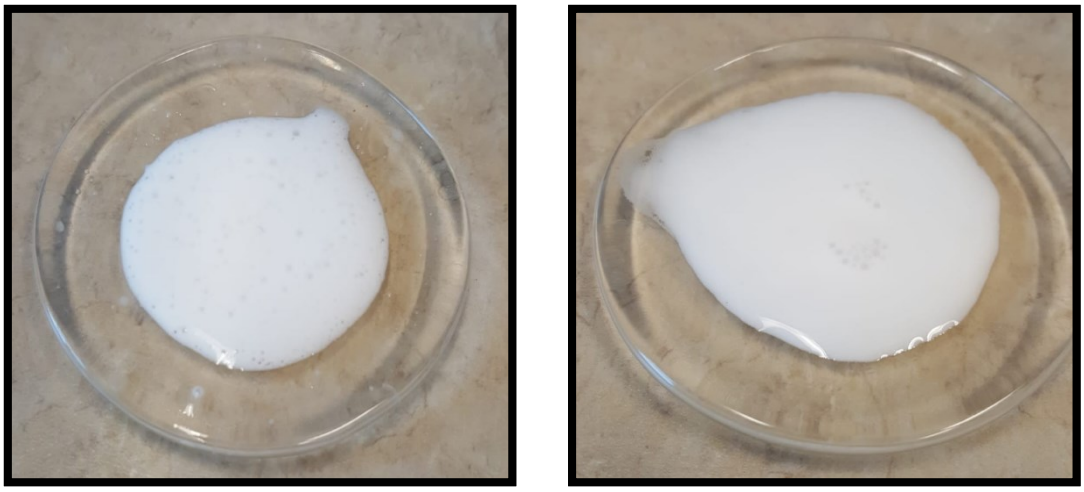


Figure 2.8 Macroscopic images of (a) foam generated from drug-free ME, and (b) foam generated from MET-loaded ME.

2.4. Conclusion

The present study proved the potential applicability of the foamable microemulsion based formulations as an alternative dosage form for enhancing the *In vitro* permeation of MET. The successfully prepared foamable ME contained caprylocaproyl polyoxyl-8 glycerides / polyglyceryl-3 dioleate at a ratio of (6:1), caprylic capric triglycerides, and water. The foamable formulations proved their ability for yielding a physicochemical stable nano-foam, and after a series of *In vitro* release tests, the foamable drug-loaded ME formulation has demonstrated its ability to deliver MET at a higher rate in comparison to other carriers. Therefore, it can be concluded that the prepared MET-loaded ME-based foam has a great potential for the delivery of MET via transdermal route of administration for hyperthyroidism in cats, as this formulation proved its physiochemical stability during the tested period in the laboratory.

Chapter 3

3. Quantitative relationship between the octanol/water partition coefficient and the membrane diffusion

3.1. Introduction

Octanol is an organic compound, and it belongs to one of the organic compounds classes known as fatty alcohols. This compound has low water solubility stated as 0.532 mg/ml [14]. Octanol and water are immiscible solvents, in which the partition coefficient of a compound is determined by calculating the distribution of a molecule between octanol and water [78]. Log P value of octanol, accordingly, is thought to be log Kow=3 [79]. In general, drug molecules need to partition into the membrane as this partitioning is a crucial step in the diffusion through the membrane [80]. Hydrophilic compounds have lower log P values in comparison to higher log P value in lipophilic compounds, however, compounds with log P value ranges between 1-3 are considered suitable candidates for transdermal drug penetration as they possess both hydrophilic and lipophilic properties due to their ability to pass the stratum corneum (lipophilic) and epidermis (hydrophilic) layers of the skin [81].

Diclofenac Sodium is one of the nonsteroidal anti-inflammatory drugs (NSAID) of the phenylacetic acid class that possesses anti-inflammatory, analgesic, as well as antipyretic properties [82]. Diclofenac is a hydrophobic molecule was found to exhibit a high value of log P of 4.26-4.51, while the log D value was found to be 3.7-1.1 at the pH ranged between 4.2-7.4 (Figure 3.1) [66].

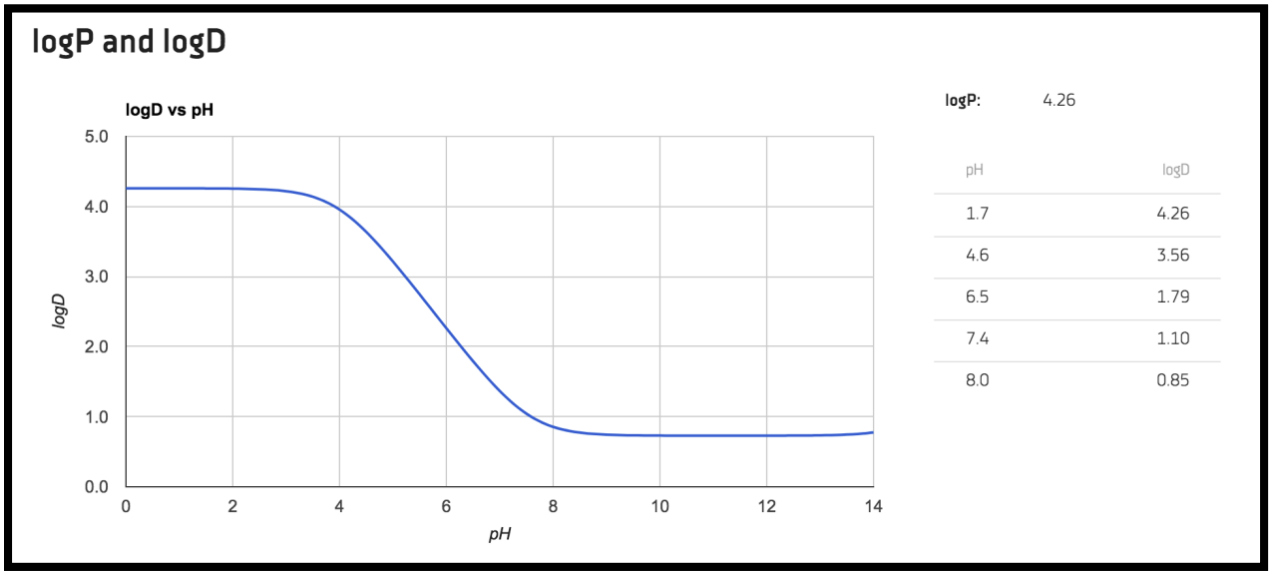


Figure 3.1 The hydrophobicity of Diclofenac as a function of pH
The Figure retrieved from https://chemicalize.com/#/calculation

MET is one of the antithyroid drugs that has been used to medically manage hyperthyroidism as it inhibits the formation of thyroid hormones [40]. It was found that this compound represents the same value of log P and log D as 0.75 at the pH ranged between 4.2-7.4 (Figure 3.2); accordingly, MET is considered as a hydrophilic drug [83,19].

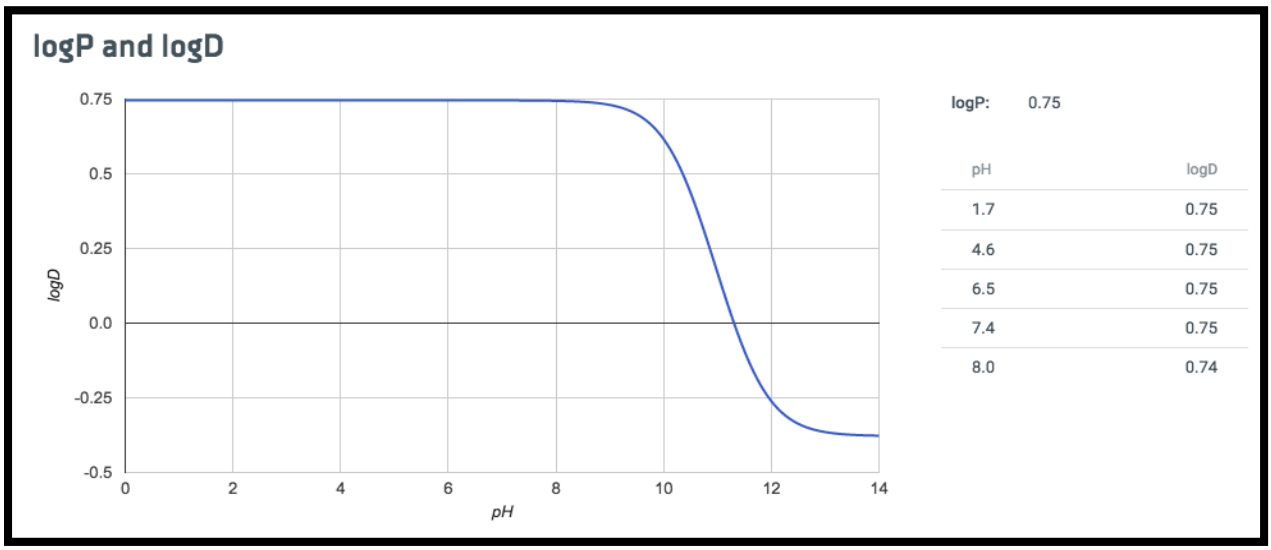


Figure 3.2 The hydrophilicity of MET as a function of pH
The Figure retrieved from https://chemicalize.com/#/calculation

It has been stated that wettability, in addition to porosity, has a significant role in the process of permeation through a membrane [84]. Also, the maximum favorable performance of membranes can be reached by establishing air bubble-free system as well as the thoroughly wetted surface. Henceforth, this project aimed to study the influence of octanol as a new model in the partitioning of transdermal application drugs through the *In vitro* release tests [85] .

3.2. Materials and Methods

3.2.1. Materials

Diclofenac Sodium (DS) USP was obtained from PCCA (London, ON). Labrasol (Caprylocaproyl polyoxyl-8 glycerides NF), Plurol Oleique (polyglyceryl-3 dioleate NF), and Labrafac (Medium-Chain Triglycerides NF) were received as a generous gift from GatteFosse, (Montreal QC). MET was purchased from Sigma-Aldrich. Carbopol 934P NF was from L.V. Lomas Limited (Brampton ON). Double distilled water was used for the MEs preparation. All other solvents and materials used were of analytical grade.

3.2.2. Methods

3.2.2.1. Diclofenac Sodium Assay

The quantitative determination of DS was carried out using UV spectrophotometry (Genesys 10 Bio) at λmax = 277 nm. A calibration curve was afterward obtained (Y = 42.936x + 0.0011), in which Y was concentration [mg/mL], X was absorbance, and r^2 was 1. The standard plot of DS was performed over the concentration range of 0.0003 to 0.025 mg/mL.

3.2.2.2. MET Assay

The quantitative determination of MET was performed by reversed-phase high-performance liquid chromatography method (HPLC) at λmax = 252 nm. A calibration curve was then obtained (Y = 1E+08x - 109394), in which Y was concentration [mg/mL], X was peak area, and r^2 was 0.9999. The standard plot of MET has performed over the concentration range of 0.002 to 0.2 mg/mL.

3.2.2.3. HPLC Method for Quantification of MET

The high-performance liquid chromatography (HPLC) analysis of MET in the microemulsion formulation was performed using a Shimadzu system. The HPLC system was equipped with CBM-20A controller, SIL-10A auto-injector, LC-10AS pump, CTO-10A column oven, SPD-M10A VP diode array detector. Chromatographic separation was achieved using a LiChrospher RP-18 column (5 µm packing, 4.6 mm × 12.5 mm) and maintained at 40°C. The isocratic mobile phase

consisted of 10% Methanol, pumped at a flow rate of 1 ml/min. The assays were attained by injecting 20 µl of sample and fixing the UV detector wavelength at 252 nm. The retention time of MET was determined in 3 minutes after the start of each run. And then, the date was quantified by using EZStart 7.4 SP1

3.2.2.4. Preparation of Drug-loaded Microemulsion (ME)

3.2.2.4.1. DS-loaded ME

The ME formulation was prepared experimentally based on reference [50], by incorporating the following components: Labrasol (caprylocaproyl polyoxyl-8 glycerides NF) as a surfactant and Plurol Oleique (polyglyceryl-3 dioleate NF) as a cosurfactant at 6:1 ratio into the Labrafac as oil phase (Medium-Chain Triglycerides NF). At room temperature, water was added to the above mixture and mixed gently. 0.5% (w/w) DS was compounded with the Labrafac-based microemulsions. The mixtures were finally mixed with the aid of a magnetic stirrer at 600 rpm room temperature for 5 minutes, and transparent drug-loaded O/W MEs were obtained (Table 3.1). In this study, (0.5% w/w) 5mg/mL DS was the concentration used which is lower than the therapeutic range for the topical application. Indeed, 0.5% concentration was intended to be used in this study to study the effect of octanol-based membrane diffusion in the low therapeutic range of DS. According to the FDA-highlights prescribing information by the inventor, a 2-4 g of DS is the

recommended dose as a topical dosage form; and the total daily dose should not exceed 32 g [86].

3.2.2.4.2 MET-loaded ME

The preparation method that was carried out on MET- loaded foamable ME was previously mentioned in chapter 2 (Table 3.1).

Table 3.1 Components Composition (% w/w) of the drug-loaded foamable ME formulations

Excipients	ME	DS-loaded ME	MET-loaded ME
Labrasol (Caprylocaproyl polyoxyl-8 glycerides NF)	18	18	18
Plurol Oleique (Polyglyceryl-3 dioleate NF)	3	3	3
Labrafac (Medium-Chain Triglycerides NF)	0.5	0.5	0.5
Diclofenac Sodium	-	0.5	-
MET	-	-	2.5
Purified Water	q.s.	q.s.	q.s.

3.2.2.5. Preparation of Plain Carbopol Gel Base

Carbopol 934P gel base was prepared by gradually dispersing 1% (w/w) Carbopol into distilled water and mixing it using a magnetic stirrer at 1200 rpm for at least 30 min [52]. The mixture was allowed to hydrate and swell for 24 hours. Next, Carbopol was then neutralized with 10 % sodium hydroxide (10% NaOH) solution that was added dropwise until the desired pH value for topical application was approximately reached between 5-7 [53].

3.2.2.6. Preparation of MET-loaded no Surfactant Gel

For the reason of evaluating the octanol as a membrane wetting agent on the diffusivity of the membrane, MET was gradually added, under continuous stirring, to the previously mentioned plain Carbopol gel. The final concentration of MET in the gel formulation was 1% w/w (Table 3.2).

Table 3.2 Percentage Composition (%w/w) of the MET based-gel formulation

Excipients	MET based-gel %
MET	1
Carbopol 943P	1
10 % NaOH	Drops
Purified Water	q.s.

3.2.2.7. Physicochemical Evaluation of the Prepared MET-loaded ME

All the physiochemical experiments that were carried out on the MET-loaded foamable ME were previously mentioned in chapter 2.

3.2.2.8. *In vitro* Drug Release Studies

Static Franz glass diffusion cells (minimum of 3 replicates) were used to assess the effect of octanol-soaked membranes on the cumulative percentage drug release and the flux rate of two different drug compounds DS and MET. The area for diffusion was 1.79 cm^2 (15.1 mm diameter orifice). The Franz diffusion cells were set up and allowed to equilibrate for 30 minutes before the samples were applied. The tested synthetic membranes with the needed pore diameter were immersed in octanol. The membranes were then carefully positioned between the donor and receptor compartments. The receptor compartments were thermoregulated using a circulating water bath (Haakel D2, Germany) and maintained at 32.0 ± 0.5 °C. The receptor chambers volume varied from 12 to 13 ml and were filled with double-distilled water in both experimental studies of DS and MET. Each diffusion cell contained a magnetic bar and was magnetically stirred at 600 rpm (IKA, USA) during the experiment to keep homogenous concentrations within the acceptor medium and to minimize stagnant layers. 0.5 g of the formulations were accurately weighed and placed in the donor compartments. 100 µL samples were withdrawn through the sampling port at five points time intervals (0.5, 1, 2, 4 and 6 h) using a syringe needle,

and diluted with 900 μL fresh acceptor medium. The same volumes were replaced with fresh double-distilled water to maintain a constant volume. The cumulative percentage release and the Flux of both compounds were calculated.

The data of the release rate of DS is mainly performed on the basis of the linear regression of the cumulative release of the active ingredient per unit area articulated as a function of the square root of time known as Higuchi diffusion model [42] .

3.2.2.8.1. Octanol-Based Surfactant Formulations for Evaluation of Diclofenac Sodium Drug Delivery

Diclofenac Sodium DS (0.5% w/w) was used as a drug-loaded ME formulation (pH adjusted to 4.18). In regards to the porosity and the membrane properties, artificial hydrophobic and hydrophilic octanol- soaked membranes with 0.22 and 0.45-micron pore size were used to examine the DS release profile across the membranes. Comparatively, water-soaked hydrophobic and hydrophilic membranes were employed with 0.22 and 0.45-micron pore size to compare the permeability data through octanol and water- hydrated membranes. The DS samples were then analyzed by a spectrophotometric determination at a wavelength of 277 nm.

3.2.2.8.2. Octanol Surfactant-Free Formulations for Evaluation of MET Drug Delivery

MET (2.5% w/w) was compounded as a drug-loaded hydrogel formulation. Synthetic hydrophobic and hydrophilic membranes with 0.22 and 0.45-micron pore size were used to compare the MET release profile through the membranes. MET samples were analyzed by HPLC method at a wavelength of 252 nm.

3.2.2.8.3. Comparison of octanol-Based Surfactant versus octanol Surfactant-Free for Evaluation of MET Drug Delivery

MET (1% w/w) was prepared as a drug-loaded microemulsion to be experimentally compared to MET (1% w/w) drug-free microemulsion formulation. Synthetic hydrophobic and hydrophilic membranes with 0.22 and 0.45-micron pore size were used to analyze the MET release profiles through the membranes. MET samples were quantified by HPLC method at a wavelength of 252.

The Formula for Determination of the Percentage of Release of Drug from *In vitro* Release Testing, adapted from reference (Equations 3)[64]

Equations 3.1 Concentration of drug (µg/ml)= (slope × absorbance) ± intercept.

Equations 3.2 Amount of drug released mg/ ml = (Concentration × Dissolution bath volume × dilution factor)/1000.

Equations 3.3 Cumulative percentage = Volume of sample withdrawn (ml) × P (t – 1) + Pt release (%) Bath volume (v) Where Pt = Percentage release at time t Where P (t – 1) = Percentage release previous to 't'.

Formula for Determination the Flux (J_{max}) of Drug, adapted from reference (Equations 4) [65]

$J = Q/(At)$ **Equations 4**

Where Q is the total quantity of drug travelling across the membrane in time t, and A is the area of exposed membrane in cm^2. For this experiment the diffusion area was 1.79 cm^2.

The comparison between the release profiles of different pore sizes in hydrophilic and hydrophobic membranes was made for DS and MET. Series of *In vitro* release tests were carried out to compare octanol and water as wetting solvents of the membrane in DS based-formulations, to assess the impact of wetting agents on permeability performance of the membrane. In the experiments of MET-loaded hydrogel formulations, the octanol was used as a soaking agent of the synthetic membranes. Additional IVRT of 1% (w/w) MET-loaded ME and MET-free ME were tested and evaluated with the usage of octanol as a soaking agent.

3.2.2.9. Statistical Analysis

All the experiments were triplicated, and data were expressed as mean ± SD. The statistically significant differences between formulations were determined by using one-way analysis of variance (ANOVA) and student t-test at the probability level of p=0.05. A non-parametric post hoc test (Tukey's test) was used for comparing differences between individual means. A p-value of <0.05 was considered to be statistically significant. SPSS software (version 24), and Microsoft Office Excel (version 16.15) were used to perform the statistical analyses. The cumulative drug release profiles were fitted to Higuchi diffusion model by using DDsolver software.

3.3. Results and Discussion

All the performed *In vitro* drug release tests are illustrated in six figures as shown below. Based on the obtained release tests, the cumulative release percentage and the flux of DS-loaded ME formulation and MET-based formulations were calculated for both compounds through the hydrophobic and hydrophilic membranes after 6 hours.

As it presented in (Figure 3.3), on water-soaked hydrophilic membranes, the use of 0.45µm pore size membrane displayed a higher DS release (23.68 ± 1.10 %) compared to a 0.22µm hydrophilic membrane (4.08 ± 1.33 %). Water as a wetting agent was also tested on hydrophobic membranes as the release of DS through the 0.45µm membrane was also shown as an 8-fold higher (16.53 ± 3.63 %) relative to

DS release through a 0.22µm hydrophobic membrane (2.65 ± 0.75%), with a p-value of 0.0029.

On the other hand, evaluating the DS release from ME formulation through the octanol-soaked hydrophilic membranes, as is depicted in (Figure 3.4), showed that there was no statistically significant difference between the DS release across 0.45µm (2.27 ± 0.75 %) and 0.22µm (1.47 ± 0.07 %) hydrophilic membranes (p=0.1742). Similarly, octanol was used as a soaking solvent of hydrophobic membranes, and it revealed no statistically significant difference of the release of DS through the 0.45µm (13.36 ± 2.93 %) and 0.22µm (11.82 ± 5.28 %) hydrophobic membranes was found (p=0.6828).

In light of the above, as it represented in (Figure 3.5), the data showed that there was no statistical difference between the octanol and water as soaking solvents for DS drug release through 0.45µm hydrophobic membranes (p= 0.3037). In contrast, DS drug release was higher in 0.22µm octanol-soaked hydrophobic membrane compared to 0.22 µm water-soaked hydrophobic membrane (p=0.0004).

As it can be seen in (Figure 3.6), the superiority of the DS release was certainly observable across the 0.45µm water-wetting hydrophilic membrane in comparison to octanol and all other release profiles. The diffusion of DS through 0.22 µm

hydrophilic membrane exhibited no statistical difference between water and octanol membrane wettability (p= 0.8450).

As is shown in (Table 3.3) the average flux mg/cm^2/hr was the highest 0.09 ± 0.004 for water wetted 0.45µm hydrophilic membrane among all other profiles. The diffusion rate of DS drug for the other conditions was faster in the order of H_2O 0.45µm Hydrophobic > OCT 0.45µm Hydrophobic > OCT 0.22µm Hydrophobic > H_2O 0.22µm Hydrophilic > H_2O 0.22µm Hydrophobic > OCT 0.45µm Hydrophilic > OCT 0.22µm Hydrophilic membrane. For this reason, the difference in flux might be attributed to the difference in wetting agents, membrane' porosity, and membrane property. In general, the more lipophobic a membrane, the greater fluxes can be obtained [87] .

As it can be shown in (Figure 3.7), the octanol was used as a soaking agent to assess the MET release profiles from Carbopol formulations. There was no statistically significant difference of the MET release profiles between 0.45µm and 0.22µm hydrophilic membrane (p= 0.0653). As well as, it is shown that using 0.45µm and 0.22µm hydrophobic membrane to evaluate the MET release revealed no significant difference in their profiles (p=0.2167). The obtained results have agreed with the point that using water as a wetting agent can only hydrate the hydrophilic membrane, while alcohol can hydrate both hydrophilic and hydrophobic membranes [20].

Instead, the higher release profile as shown in (Figure 3.8) was observed with MET in a microemulsion vehicle compared to MET dissolved in H_2O solution, traveling through the octanol-saturated membrane while using 0.45µm pore size hydrophobic membrane. However, the data revealed that there was no significant difference between MET in a microemulsion vehicle and MET in a water solution across a 0.45µm hydrophobic membrane (p=0.5005).

From (Table 3.4), despite the differentiation in the pore diameter used, the average flux mg/cm^2/hr of MET in Carbopol gel formulation was shown no significant difference with the same membrane property for different pore size, as p-value was 0.0653 and 0.21689 for hydrophilic and hydrophobic membrane respectively.

By using the octanol as a wetting agent, the mathematical modeling of data revealed that the DS release profile through 0.45µm, 0.22µm hydrophilic and hydrophobic membranes followed Higuchi diffusion. On the other hand, the mathematical modeling of data using water as a wetting agent represented that the cumulative drug release of DS through 0.45µm hydrophobic membrane followed Higuchi diffusion, while DS release profile through 0.45µm, 0.22µm hydrophilic membrane as well as 0.22µm hydrophobic membrane do not follow Higuchi diffusion (Table 3.5).

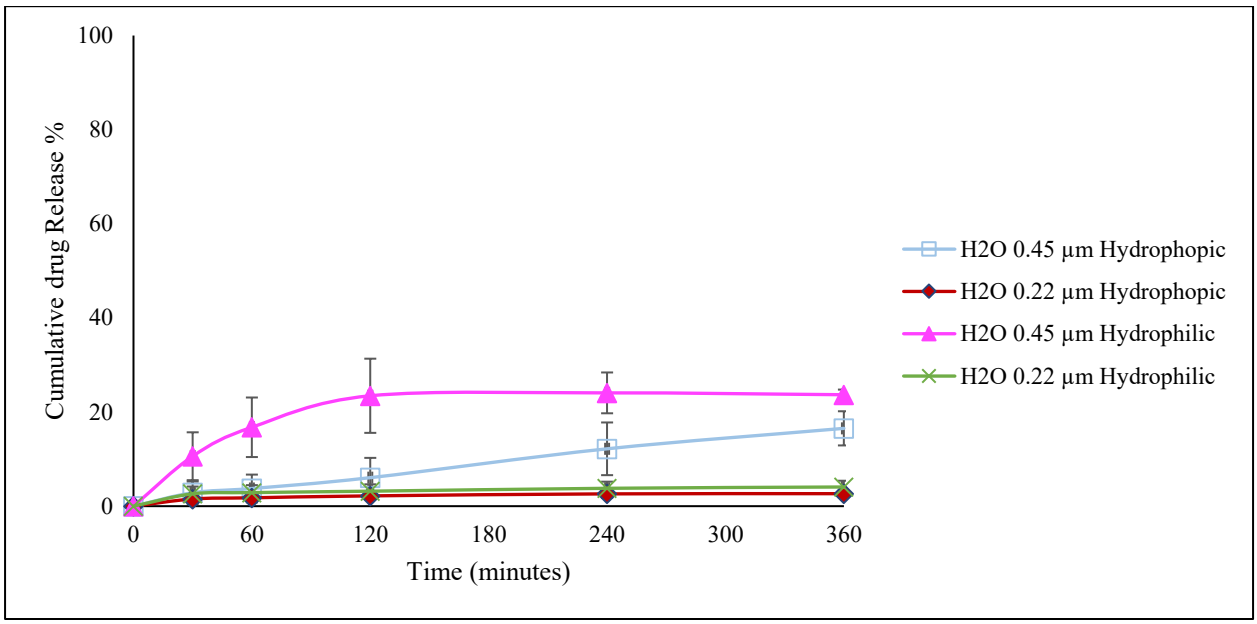


Figure 3.3 *In vitro* release profiles of Diclofenac Sodium through hydrophobic and hydrophilic membranes from the ME-based formulation using Water as a membrane wetting agent (mean± SD).

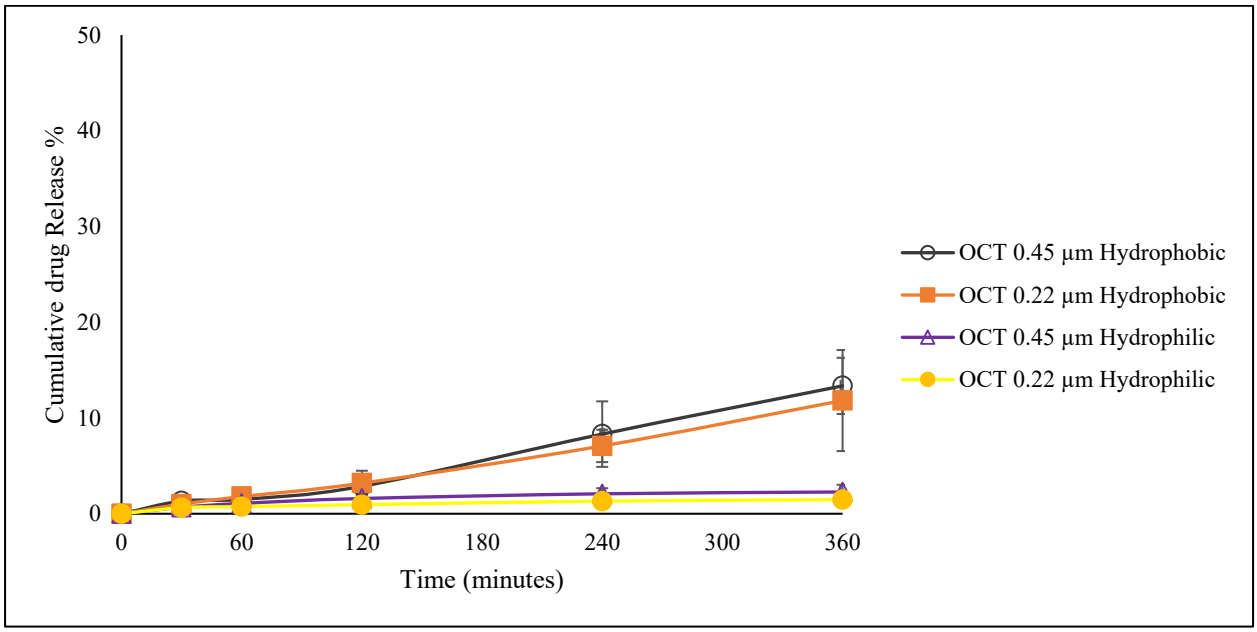


Figure 3. 4 *In vitro* release profiles of Diclofenac Sodium through hydrophobic and hydrophilic membranes from the ME-based formulation using octanol as a membrane wetting agent (mean± SD).

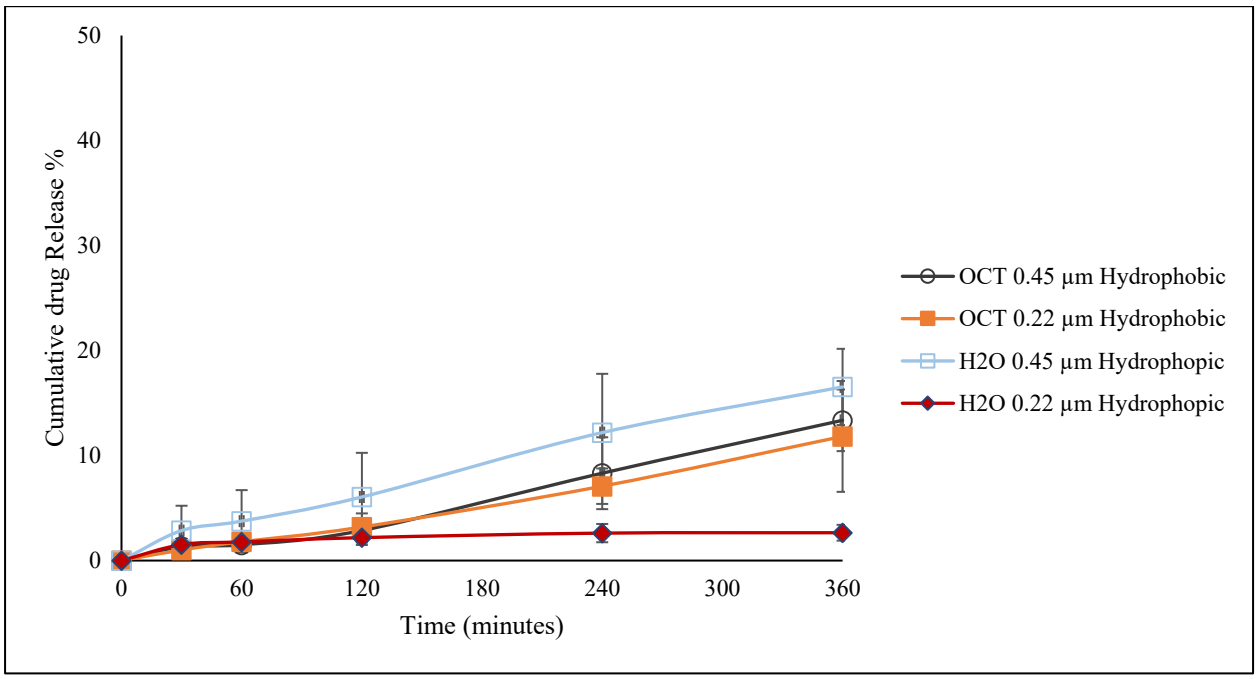


Figure 3.5 *In vitro* release profiles of Diclofenac Sodium through hydrophobic membranes from the ME-based formulation using Water and octanol as membrane wetting agents (mean± SD).

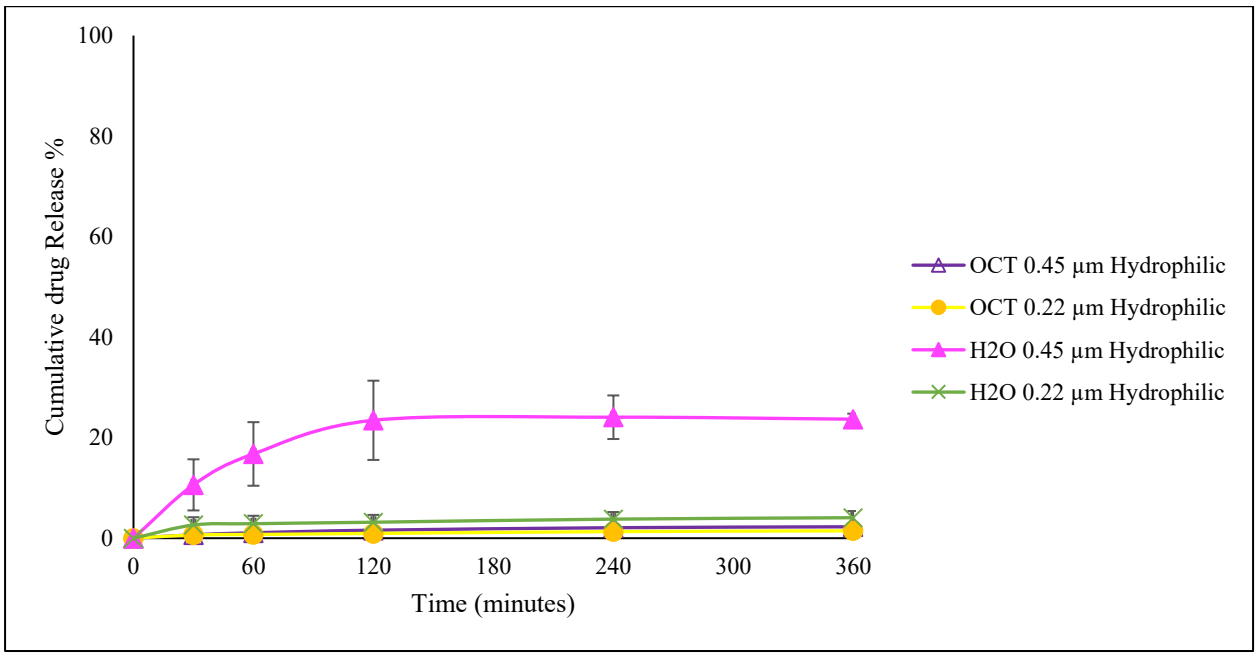


Figure 3.6 *In vitro* release profiles of Diclofenac Sodium through hydrophilic membranes from the ME-based formulation using Water and octanol as membrane wetting agents (mean± SD).

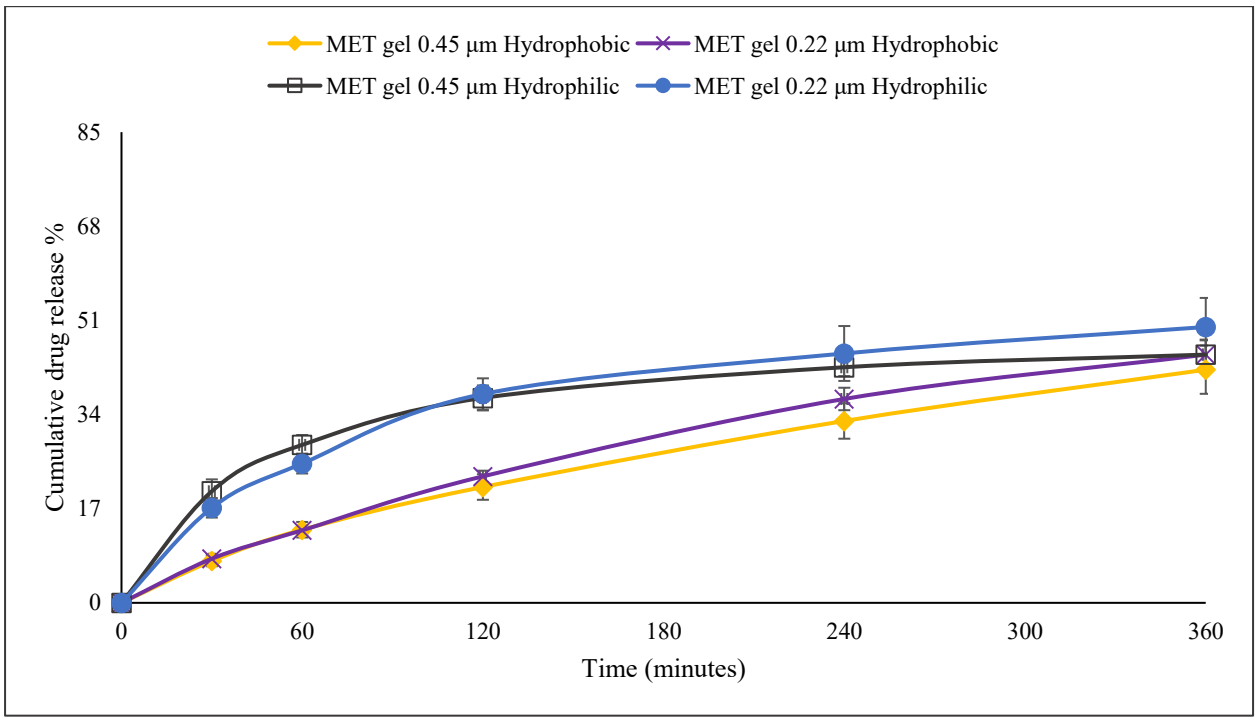


Figure 3.7 *In vitro* release profiles of MET through hydrophobic and hydrophilic membranes from the hydrogel-based formulations using octanol as a wetting agent (mean± SD).

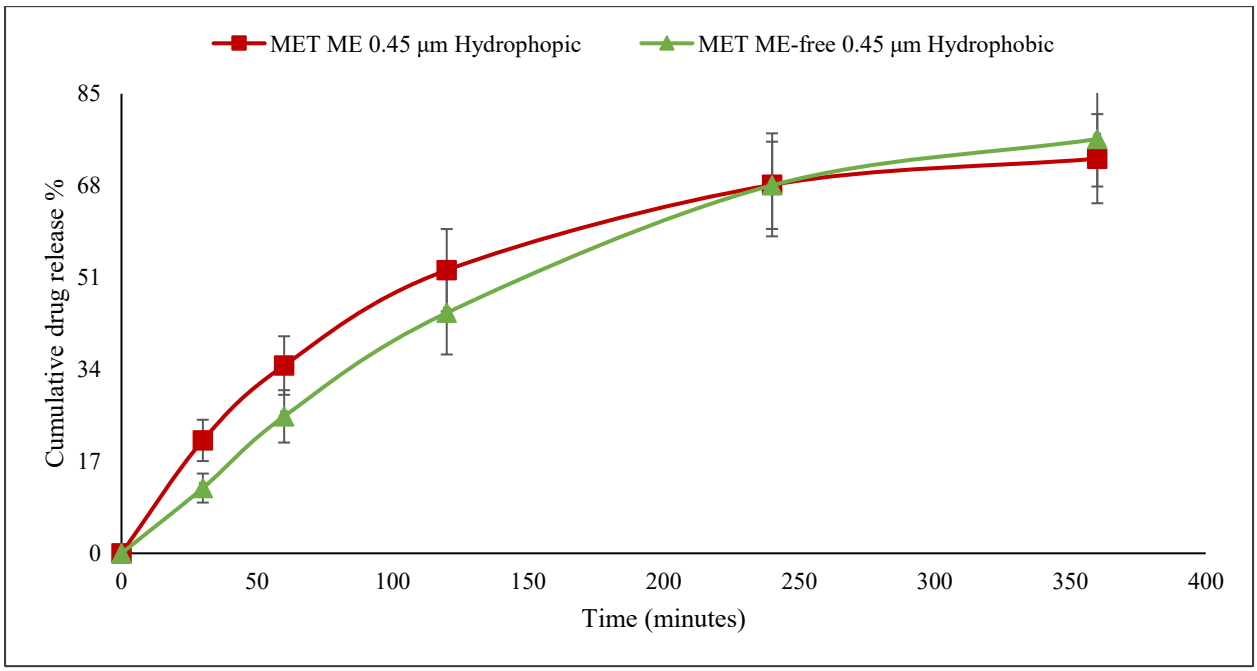


Figure 3.8 *In vitro* release profiles of MET through hydrophobic membranes from the ME formulation compared to MET ME-free formulation using octanol as a wetting agent (mean± SD).

Table 3.3 Summary of average DS flux (Jmax) from microemulsion based system across hydrophobic and hydrophilic membranes after 6 hours.

Experimental work	**Flux (mg/ cm^2/hr) ± SD**
OCT 0.45µm Hydrophobic	0.062 ± 0.014
OCT 0.22µm Hydrophobic	0.055 ± 0.024
OCT 0.45µm Hydrophilic	0.011 ± 0.003
OCT 0.22µm Hydrophilic	0.008 ± 0.003
H_2O 0.45µm Hydrophobic	0.077 ± 0.017
H_2O 0.22µm Hydrophobic	0.012 ± 0.003
H_2O 0.45µm Hydrophilic	0.110 ± 0.005
H_2O 0.22µm Hydrophilic	0.027 ± 0.004

Table 3.4 Summary of average MET flux (Jmax) from Carbopol gel , MET-ME and MET- H_2O across hydrophobic and hydrophilic membranes after 6 hours.

Experimental work	**Flux (mg/ cm^2/hr) ± SD**
MET-gel OCT 0.45µm Hydrophobic	3.13 ± 0.32
MET-gel OCT 0.22µm Hydrophobic	3.34 ± 0.19
MET-gel OCT 0.45µm Hydrophilic	3.34 ± 0.20
MET-gel OCT 0.22µm Hydrophilic	3.71 ± 0.39
MET-ME OCT 0.45µm Hydrophobic	2.18 ± 0.25
MET-H_2O OCT 0.45µm Hydrophobic	2.28 ± 0.26

Table 3.5 The correlation coefficient of Higuchi diffusion model.

Membrane	Octanol				Water			
	Hydrophobic		Hydrophilic		Hydrophobic		Hydrophilic	
	0.22µm	0.45µm	0.22µm	0.45µm	0.22µm	0.45µm	0.22µm	0.45µm
R^2	0.77	0.74	0.89	0.96	0.02	0.88	-1.03	0.28

3.4. Conclusion

According to obtained results it can be concluded that the drug release profile of octanol immersed membrane can be partitioned regardless of the membrane pore size, but due to the membrane type. In other words, octanol created channels that allow the drug molecules to pass through regardless of the membrane pore size. In contrary, the release performance of water submerged membranes was mainly influenced by the size of the pores in the membrane as water reduced the angle of contact between the liquid (semisolid formulation) and the solid (membrane) without changing the structure of the membrane. Furthermore, it can be also concluded that using octanol as a wetting agent might be used as a new model to investigate the release profiles of a compound.

Chapter 4

4.General Discussion and Conclusions

4.1. Conclusion

Since there have been issues raised with the use of MET in a PLO gel formulation and as oral formulations in domestic hyperthyroid cats, the purpose of this thesis was to formulate an efficient drug carrier and assess the release profiles through the in vitro release testing. When in fact, as it concluded by Hoffman et al. the bioavailability of transdermal MET in a Pluronic Lecithin Organogel (PLO) was shown to be poor and variable in a trial based on six adult cats [1]. In addition, a study performed by Hill et al., concluded that the MET absorption was significantly lower when administered into a PLO gel vehicle compared to a lipophilic vehicle [2].

Besides, it has been documented by Trepanier et al., that a single daily dose of oral MET is not an efficient approach to treat hyperthyroidism in cats, yet, increasing the dosing frequency is recommended [3]. Furthermore, an orally administered MET has been associated with unfavorable outcomes in domestic cats such as gastrointestinal (GI) problems [4]. Also, it has been concluded by Wu et al., that MET ointment exhibited fewer systemic adverse effects such as rash, liver dysfunction, and leucopenia (1.5%) compared to the administration of oral MET in hyperthyroid cats (12.3%; $p < 0.05$) [5].

Based on that, our rationale was to focus on evaluating the microemulsion-based foam as a vehicle for effective transdermal drug delivery of MET, and correspondingly, this thesis has hypothesized that transdermal penetration of MET might be enhanced. The first study in this dissertation proved that the developed drug loaded foamable microemulsion are physiochemically stable by taking a comprehensive evaluation of the pH, drug solubility, drug content, phase separation, particle size, and particle shape analysis, as well as qualitative study analysis of the microemulsion and MET-loaded microemulsion. Furthermore, the results presented in this thesis of the release tests through Franz cells diffusion provided evidence that foam-based microemulsion formulation was superior to cream-based formulations. In other words, besides the ease of preparation of microemulsion as no energy contribution required, the nanoparticle size foam gives larger surface area from which drug can be fast released. Moreover, the hydrophilic and hydrophobic domains of microemulsion enhanced the permeation of drug through the membrane.

For the purpose of investigating the relationship between the drug release and the membrane porosity as well as the membrane wetting agents, the second study was aimed to assess the influence of octanol as a new model in the partitioning of transdermal application through the In vitro release tests using Franz cells apparatus. The findings obtained from the release profiles across different pore size synthetic membranes, and using octanol as a wetting agent, proved that octanol could be used

as a new model in permeability tests as the findings showed no significant difference in the release profiles between 0.22 μm and 0.45μm pore size membranes.

In conclusion, the drug MET might be efficiently delivered using a novel foam-based microemulsion formulation, that would prove much more effective in the treatment of domestic pet cats than current formulations. Such finding is attributed to the evidenced thermodynamic, physiochemical stability, as well as improved permeability of drug-loaded microemulsion.

4.2. Future Directions

In light of the *in vitro* preliminary steps that have been performed:

- Evaluating the in vitro release profiles across excised ear of cat might be needed.
- Evaluating if a gender difference will affect the in vitro release profiles.
- An assessment of the amount of MET remains on cat's ear following the *in vitro* tests.
- In vivo evaluation of pharmacokinetics, bioavailability, and bioequivalence of MET
- The *in vivo* therapeutic assessment of the drug loaded foamable ME; TT4 level, hepatic enzyme levels such as ALT, and ALP.

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