FORMULATION AND EVALUATION OF COLCHICINE LOADED ETHOSOMAL GEL FOR TRANSDERMAL DELIVERY

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FORMULATION AND EVALUATION OF COLCHICINE LOADED ETHOSOMAL GEL FOR TRANSDERMAL DELIVERY

by

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
°C	Celsius
µl	Microliter
ANOVA	Analysis of Variance
BE	Binary ethosomes
CE	Classical ethosomes
cm	Centimetre
cm^2	Squared centimetre
FDA	Food and Drug Administration
HCL	Hydrochloric acid
HETP	Height equivalent of a theoretical plate
HLB	Hydrophilic Lipophilic Balance
HPLC	High Performance Liquid Chromatography
hr	Hour
LOD	Limit of Detection
LOQ	Limit of Quantification
M	Molar
m^2	Square metre
mg	Milligram
mg/ml	Milligram per millilitre
min	Minute
ml	Millilitre
ml/min	Millilitre per minute
mM	Millimole
mV	Millivolt
NaOH	Sodium hydroxide
NE gel	Non-ethosomal gel
ng	Nanogram
ng/ml	Nanogram per millilitre
nm	Nanometer

Pa	Pascal
Pa.s	Pascal second
PdI	Polydispersity index
PTFE	Polytetrafluoroethylene
RH	Relative Humidity
rpm	Revolutions per minute
RSD	Relative Standard Deviation
SD	Standard deviation
Sec	Second
TEL	Transethosomes containing Labrafil®
TENa	Transethosomes containing Sodium taurocholate
TEM	Transmission Electron Microscopy
TET	Transethosomes containing Tween 20®
UK	United Kingdom
USA	United States of America
USP	United State Pharmacopoeia
UV	Ultraviolet
w/v	Weight per volume
w/w	Weight per weight
η	Viscosity
ZP	Zeta potential

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ABSTRAK

Colchicin digunakan untuk rawatan gout, pseudo-gout dan demam Mediterranean. Ia diketahui mempunyai terapetik indeks rendah dan biokeperolehan oral rendah yang disebabkan oleh kesan laluan pertama yang teruk, dan ia juga dikaitkan dengan kesan sampingan gastrousus teruk berikutan pemberian oral. Untuk mengatasi kelemahan pengambilan oral, gel etosom bermuatan colchicin disediakan dalam usaha untuk penghantaran drug secara transdermal. Tiga jenis etosom pembawa nano telah disediakan dengan menggunakan kaedah sejuk. Lima set reka bentuk faktorial eksperimen telah dijalankan, satu factorial 2^3 untuk etosom klasik, satu factorial 2^4 untuk etosom binari, dan tiga factorial 2^4 untuk transetosom. Sistem etosom dengan purata saiz vesikel $\leq$ 150 nm, polydispersity indeks (PdI) $\leq$ 0.3, dan zeta berpotensial (ZP) $\geq$ -20 mV dikaji lebih lanjut untuk penggabungan drug dalam etosom. Sistem etosom optimum dengan kecekapan pemerangkapan > 75 %, kemudian digabungkan ke dalam gel asas (Carbopol 940$^\circledR$). Gel etosom itu dicirikan mengikut purata saiz vesikel, PdI, ZP, pH, kelikatan dan nilai alah. Semua formulasi gel etosom menujukkan aliran plastik bukan Newtonian tanpa thixotropy. Dua gel etosom optimum dipilih iaitu TET 2 dan TENa 4 gel. Gel TET 2 memiliki purata saiz vesikel, PdI, ZP, pH, kelikatan dan nilai alah, masing-masing 111.4 $\pm$ 1.6 nm, 0.157 $\pm$ 0.01, -40.56 $\pm$ 5.32 mV, 6.74 $\pm$ 0.05, 66.25 $\pm$ 2.75 Pa.s, 131.91 $\pm$ 1.94 Pa. Manakala TENa 4 gel memiliki purata saiz vesikel, PdI, ZP, pH, kelikatan dan nilai alah masing-masing 110.5 $\pm$ 1.1 nm, 0.162 $\pm$ 0.014, -43.54 $\pm$ 2.56 mV, 6.81 $\pm$ 0.04, 55.68 $\pm$ 0.76

Pa.s, 122.71 ± 0.99 Pa. Kaedah HPLC dibangunkan dan divalidasi bagi kuantifikasi colchicin dalam gel etosom dan dalam cecair media. Kajian *ex-vivo* ketelapan kulit dijalankan untuk menilai dan membandingkan parameter ketelapan kulit antara formulasi gel etosom optimum dan gel bukan etosom. Formulasi gel TET 2 mempunyai penelapan kulit lebih tinggi (188.90 kali ganda) berbanding gel colchicin bukan ethosom. Gel TET 2 dipilih sebagai formulasi akhir gel etosom optimum. Kajian kestabilan gel TET 2 menunjukkan bahawa formulasi stabil pada keadaan penyimpanan 4 ± 2 °C. Anggaran jangka hayat formulasi gel TET 2 adalah 14.44 bulan. Kesimpulannya, formulasi gel colchicin etosom untuk penghantaran transdermal telah berjaya disediakan, dan ia mungkin berguna sebagai laluan alternatif kepada pemberian colchicin secara oral.

FORMULATION AND EVALUATION OF COLCHICINE LOADED ETHOSOMAL GEL FOR TRANSDERMAL DELIVERY

ABSTRACT

Colchicine is used for the treatment of gout, pseudo-gout and familial Mediterranean fever. It is known to have low therapeutic index and low oral bioavailability due to extensive first pass effect, and also associated with sever gastrointestinal side effects following oral administration. To overcome its oral intake drawbacks, colchicine loaded ethosomal gels were prepared in an attempt to deliver the drug transdermally. The three types of ethosomal nanocarriers were prepared using the cold method. Five sets of factorial designs experiments were conducted, one of 2^3 for classical ethosomes, one of 2^4 for binary ethosomes, and three of 2^4 for transethosomes. Ethosomal systems with vesicular size $\leq$ 150 nm, polydispersity index (PdI) $\leq$ 0.3, and zeta potential (ZP) $\geq$ -20 mV were further investigated for drug incorporation studies. The optimized ethosomal systems with entrapment efficiency > 75 % were then incorporated into gel base (Carbopol 940®). The prepared ethosomal gels were characterized for vesicular size, PdI, ZP, pH, viscosity and yield values. All the formulated ethosomal gels exhibited non-Newtonian plastic flow with no thixotropy. Two optimized ethosomal gels were selected TET 2 and TENa 4 gels. TET 2 gel possessed mean vesicular size, PdI, ZP, pH, viscosity and yield values of 111.4 $\pm$ 1.6 nm, 0.157 $\pm$ 0.01, -40.56 $\pm$ 5.32 mV, 6.74 $\pm$ 0.05, 66.25 $\pm$ 2.75 Pa.s, and 131.91 $\pm$ 1.94 Pa, respectively. While TENa 4 gel possessed mean vesicular size, PdI, ZP, PH, viscosity and yield value of 110.5 $\pm$ 1.1 nm, 0.162 $\pm$ 0.014, -43.54 $\pm$ 2.56 mV, 6.81 $\pm$ 0.04, 55.68 $\pm$ 0.76 Pa.s, 122.71 $\pm$ 0.99 Pa, respectively. HPLC method was developed

and validated for the quantification of colchicine in ethosomal gel and dissolution media. *Ex-vivo* skin permeation studies were conducted to evaluate and compare the skin permeation parameters between the optimized ethosomal formulations and the non-ethosomal gel. The TET 2 gel formulation had higher skin permeation (188.90 folds) compared to non-ethosomal colchicine gel. The TET 2 gel was selected as the final optimized ethosomal gel formulation. The stability studies of TET 2 gel formulation revealed that the formula was stable at the storage condition of (4 ± 2 °C). The estimated shelf life for the TET 2 gel was estimated and was found to be 14.44 months. In conclusion, colchicine ethosomal gel formulation for transdermal delivery was successfully prepared, and could be useful alternative route to oral administration of colchicine.

CHAPTER 1

INTRODUCTION

1.1 Gout

Gout is a form of inflammatory arthritis where swelling and severe pain develop in joints, and negatively interfering with patient's basic daily activities. It was first recognized in 2640 BC by the Egyptians (Omole et al., 2009). The worldwide reported prevalence of gout ranges from 0.1 % to approximately 10 %, while its incidence vary from 0.3 to 6 cases per 1,000 person-years. Over the past 50 years, the global burden of gout management was substantial, and seems to be increasing in many parts of the world (Kuo et al., 2015). Gout can manifest as acute or chronic arthritis.

Acute gout is an intense acute inflammatory reaction that occurs due to articular deposits of monosodium urate (MSU) crystals. It is one of the most painful conditions experienced by humans (Martillo et al., 2014). Acute gout typical symptoms include sever red, hot, and swollen joint with an excruciating pain. The acute form of the disease may advance to chronic gout if not properly treated. Acute gout treatment relies on various therapeutic approaches such as anti-inflammatory agents including nonsteroidal anti-inflammatory drugs (NSAIDs), colchicine, uricosuric agents and corticosteroids (Choi et al., 2005), as well as systemic and intraarticular glucocorticoids, and biological agents that inhibit the action of interleukin (IL)-1 beta (Terkeltaub, 2010). In 2009, the United States food and drug administration (FDA) approved colchicine for treatment of acute gout. A scheduled oral dose for the first 24 hours of the attack was set with an initial dose of 1.2 mg, one hour later another 0.6

mg is administrated, making a sum of 1.8 mg as a total dose on the first day of therapy (Burns & Wortmann, 2012)

Chronic gout is a progressive disorder of urate metabolism characterized by symptomatic hyperuricemia, and monosodium urate crystals deposition in joints as well as soft tissues (Novak et al., 2007), which leads to a series of repeated pain episodes, as well as inflammation and degenerative ramification, in addition to joint damage and motion loss. The treatment of chronic gout goal is to completely dissolve MSU crystals and prevent new crystals formation. To achieve such status, the serum urate, and uric acid levels within the tissues and joints should decrease below MSU saturation point (Perez, 2009), by using uricosuric agents such as allopurinol (> 300 mg), and prophylactic colchicine doses (0.5 – 1 mg daily), and/or a non-steroidal anti-inflammatory drug (NSAID) (Roddy et al., 2007).

1.2 Pseudo-gout

It is known as acute attacks of synovitis due to calcium pyrophosphate dihydrate (CPPD) crystals. Clinically, the disease looks like gouty arthritis. CPPD crystals deposition can lead to symptoms like septic arthritis, as well as poly-articular inflammatory arthritis which usually are misdiagnosed as rheumatoid arthritis or degenerative osteoarthritis (McCarthy, 2008). The diagnosis of pseudo-gout is conducted by the demonstration of CPPD crystals in synovial fluid. Both gout and pseudo-gout can also coexist in a single inflammatory effusion, as 20 % of patients with CPPD will have hyperuricemia, and later, a quarter of these patients will develop gout (Rosenthal., 2005). The current available treatments of pseudo-gout are limited to fixing the ongoing metabolic abnormalities, and treating their associated symptoms,

these treatments include NSAIDs, colchicine, corticosteroids and adrenocorticotropic hormone (Choy, 2005).

1.3 Familial Mediterranean fever (FMF)

Familial Mediterranean fever (FMF) is a hereditary inflammatory disease characterized by recurrent febrile episodes accompanied by peritonitis, pericarditis, pleuritis and/or arthritis, it is sometimes associated with an erysipelas-like skin rash. Serious complications can happen if the disease is not treated such as end-stage renal disease and malabsorption secondary to amyloid deposition in the kidneys and digestive tract, male and female infertility, and growth retardation in children (Zadeh et al., 2011). FMF treatment relies mainly on preventing the development of painful attacks and amyloidosis. Colchicine is the only available drug for treating FMF and is considered as the gold standard in FMF therapy. It is thought to inhibit the increased chemotactic activity of neutrophils during FMF attacks (Ben-Chetrit & Levy, 1998). Colchicine can also improve height development and weight parameters in children with FMF (Ozcakar et al., 2010). A daily lifelong administration of colchicine is required to prevent both the fever/pain attacks, and the silent amyloid deposition. NSAIDs are used to mask the FMF symptoms of fever and pain. (Zadeh et al., 2011).

1.4 Colchicine

1.4.1 Physicochemical properties

Colchicine ($C_{22}H_{25}NO_6$) is a yellowish-white amorphous or crystalline powder with a structure described in figure 1.1. It is a potent alkaloid obtained from the dried corns and seeds of plants of the genus colchicum, which belongs to *Liliaceae* family. Among

this family, the commonly used plants are *colchicum autumnale* "meadow saffron" and *colchicum autumn* crocus. The drug has a neutral chemical nature, with a partition coefficient (log P) and pKa values of 1.3 and 1.85 respectively. It has a molecular weight of 399.437 Da, water solubility of 45 mg/ml and a melting point of 156 °C (Singh et al., 2015; Bombuwala et al., 2006).
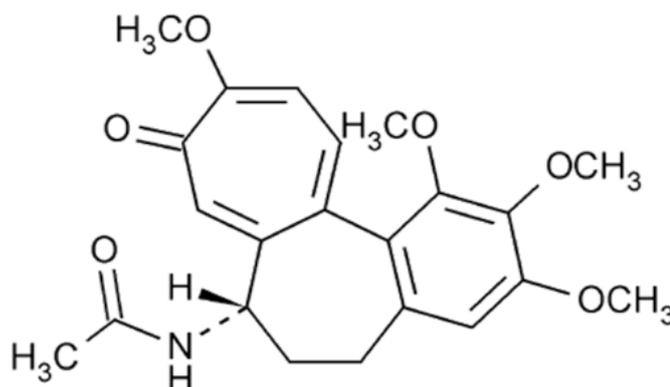
Fig. 1.1: The chemical structure of colchicine.

1.4.2 Pharmacodynamics

Colchicine has been used for the treatment of acute gout since more than 2000 years ago. It was also used to treat pseudo-gout and familial Mediterranean fever (FMF) for several decades. It is highly effective in the treatment of acute gout especially when given in the first 12 – 36 hr of the gouty attack (Terkeltaub, 2009). Colchicine relieves pain and inflammation of gouty arthritis without altering the metabolism or excretion of urates and without other analgesic effects. It produces its anti-inflammatory effects by binding to the intracellular protein tubulin, thereby preventing its polymerization into microtubules, leading to the inhibition of leukocyte migration and phagocytosis. It also inhibits the formation of leukotriene B4 (Katzung et al., 2009).

Colchicine oral administration linked to several mild to severe gastrointestinal side effects including nausea, vomiting, diarrhea, stomach cramps or pain. (Ahern et al., 1987). Even at therapeutic doses, around 80 percent of patients will develop these side effects (Harris et al., 1999; Putterman et al., 1991) While the intravenous administration is associated with potential serious adverse effects including tissue necrosis cytopenias, disseminated intravascular coagulation, and death (Evans et al., 1996), accordingly the intravenous route of administration was banned by the FDA (Burns & Wortmann, 2012).

1.4.3 Pharmacokinetics

Colchicine has a low therapeutic index (Terkeltaub, 2009). After oral administration, colchicine is absorbed in the jejunum and ileum with a single zero-order rate process, showing a low bioavailability of 25 % to 50 % due to the extensive first pass effect (Kesselheim & Solomon 2010). Within the first 24 hours of colchicine intake, 15 % to 30 % of it excreted in the urine, and its excretion still can be measured for day 10 of the intake. Up to two thirds of the drug eliminated via feces. After around one hour of oral intake the maximum serum concentration is reached, and it varies between 2.2 and 6.7 ng/ml (Rochdi et al.,1994; Girre et al.,1989). Serum half-life time of colchicine is speculated to be ~10 to 20 hours (Girre et al., 1989; Poffenbarger & Brinkley, 1974). In FMF patients with renal amyloidosis and are on hemodialysis, it was found that the drug elimination half-life was increased significantly (400 %), and total clearance was reduced compared to healthy patients (Ben-Chetrit et al.,1994).

1.5 Skin and the transdermal drug delivery

1.5.1 Skin structure

The skin covers a total body surface area of approximately 1.8 m^2, it is providing the contact between the human body and the outer environment. It is the largest and most readily accessible organ of the body, serving as a potential route of drug administration for systemic effects. Skin has three main layers namely: the epidermis which is the outermost layer of skin, beneath the epidermis layer there is the dermis layer which contains tough connective tissue, hair follicles, and sweat glands, the third deeper layer is the subcutaneous tissue (hypodermis) layer which is made of fat and connective tissue. The epidermis is itself divided into at least four separate parts, a fifth part is present in some areas of our body. The order from the most superficial layer of the epidermis to the deepest, these layers (strata) are: the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. One of the main challenges for percutaneous drug delivery is the nearly insuperable nature of stratum corneum layer of the epidermis (Barry, 2001), its permeability is approximately 1000 times less than other biological membranes and this is due to the (brick and mortar) arrangement of corneocytes, flattened mononucleated keratinocytes, with interspersed lipids and proteins (Hadgraft, 2001a; Hadgraft, 2001b).

1.5.2 Transdermal drug delivery

Transdermal drug delivery is currently one of the most appealing alternatives to oral drug delivery as well as hypodermic injection route. Transdermal drug delivery systems offer many advantages, such as avoidance of first-pass metabolism by the liver, controlled delivery of drugs, reduced dosing frequency, and improved patient

compliance, as they are noninvasive and can be self-administered (Prausnitz & Langer, 2008; Schoellhammer et al., 2014).

The first transdermal patch containing scopolamine for the treatment of motion sickness was approved in the US in 1979. A decade later, nicotine patches became the first transdermal blockbuster. Till 2008 a variety of 19 transdermal delivery systems for single or multiple drug delivery were available in the market such as (fentanyl HCl, methylphenidate, selegiline and rotigotine) with an estimation of more than one billion transdermal patches that are manufactured each year (Prausnitz & Langer, 2008).

1.6 Ethosomal nanocarriers

A new era of research in the field of transdermal drug delivery was opened with the use of liposomes for topical delivery of triamcinolone (Mezei & Gulasekharam, 1980), and since then a wide range of novel lipid-based vesicular systems were developed. The deformable or elastic liposomes which are currently known as transfersomes® were introduced by Cevc and Blume in 1992 (Cevc & Blume, 1992), and followed by the innovative work of Elka Touitou and her team (Touitou et al., 1996), that led to the discovery of a novel lipid vesicular system called ethosomes. Ethosomal systems differ from liposomes because they contain relatively high concentrations of ethanol in addition to phospholipids and water (Touitou et al., 2000). New generations of ethosomal systems have been introduced since then by adding other compounds to the basic ethosomal formula in an attempt to enhance the vesicular characteristics and the skin permeation.

1.6.1 Ethosomal systems classification

Ethosomal systems are classified into three classes on the basis of their compositions namely: classical ethosomes, binary ethosomes, and transethosomes (Figure 1.2) (Abdulbaqi et al., 2016).
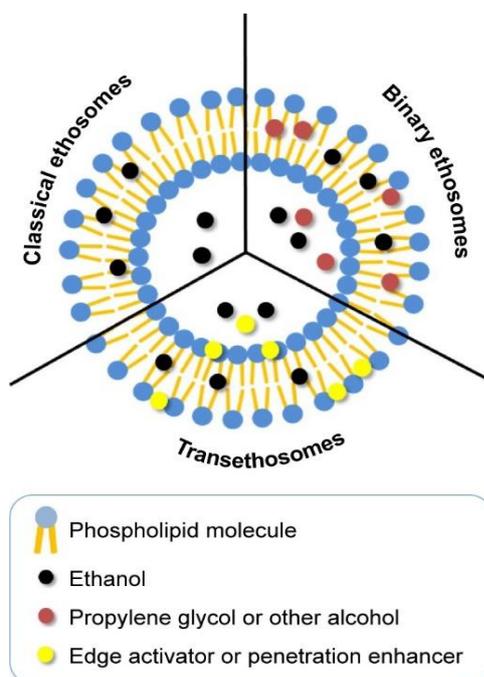
Fig. 1.2: Schematic representations of the different types of ethosomal systems.

1.6.1(a)Classical ethosomes

Classical ethosomes are a modification of classical liposomes and are composed of phospholipids, high concentration of ethanol up to 45 %w/w and water. The classical ethosomes were reported to be superior over the classical liposomes for transdermal drug delivery because they have smaller size, negative zeta potential, higher entrapment efficiency. Moreover, classical ethosomes showed better skin permeation and stability profile compared to classical liposomes (Touitou et al., 2000; Sarwa et al., 2014; Jain et al.,2014). The molecular weights of drugs entrapped in classical

ethosomes were ranging from 130.077 Daltons to 24 K Daltons (Zhang et al., 2012; Mishra et al., 2010).

1.6.1(b) Binary Ethosomes

Binary ethosomes were first introduced by Zhou et al in 2010 (Zhou et al., 2010). Basically, they were developed by adding another type of alcohol to the classical ethosomes. The most commonly used alcohols in binary ethosomes are propylene glycol (PG) and isopropyl alcohol (IPA) (Li et al., 2012; Zhang et al., 2012; Akhtar & Pathak, 2012; Dave et al., 2010; Shen et al., 2014).

1.6.1(c) Transethosomes

Transethosomes are the new generation of ethosomal systems which were first reported by Song et al in 2012 (Song et al., 2012). This ethosomal system contains the basic components of classical ethosomes and additional compound such as a penetration enhancer or an edge activator (surfactant) in their formula. These novel vesicles were developed in an attempt to combine the advantages of classical ethosomes and deformable liposomes (Transfersomes®) in one formula to produce transethosomes. Many researchers have reported superior properties of transethosomes over classical ethosomes (Ainbinder & Touitou, 2011; Bragagni et al., 2012; Meng et al., 2013; Chen et al., 2014; Chenet al., 2014; Fang et al., 2008; Ascenso et al., 2015). Different types of edge activators and penetration enhancers have been investigated to produce ethosomal systems with better characteristics. Transethosomes were reported to entrap drugs with molecular weights ranging from 130.077 Daltons to 200–325 K Daltons (Ainbinder & Touitou, 2011; Chen et al., 2014). Table 1.1 shows the

comparison of classical ethosomes, binary ethosomes and transethosomes properties in their initial suspension form.

Table 1.1: Comparison of Classical Ethosomes, Binary Ethosomes and Transethosomes

Parameter	Classical ethosomes	Binary ethosomes	Transethosomes
Composition	1. Phospholipids 2. Ethanol 3. Stabilizer 5. Charge inducer 6. Water 7. Drug/agent	1. Phospholipids 2. Ethanol 3. Propylene glycol (PG) or other alcohol 4. Charge inducer 5. Water 6. Drug/Agent	1. Phospholipids 2. Ethanol 3. Edge activator (surfactant) or penetration enhancer 4. Charge inducer 5. Water 6. Drug/agent
Morphology	Spherical	Spherical	Regular or irregular spherical shapes
Size	Smaller than the classical liposomes	Equal to or smaller than classical ethosomes	Size based on type and concentration of penetration enhancer or edge activator used
Zeta Potential	Negatively charged	Negatively charged	Positively or negatively charged
Entrapment efficiency	Higher than classical liposomes	Typically higher than classical ethosomes	Typically higher than classical ethosomes
Skin permeation	Typically higher than classical liposomes	Typically equal to or higher than classical ethosomes	Typically higher than classical ethosomes
Stability	More stable than classical liposomes	More stable than classical ethosomes	No particular trend determined

1.6.2 Mechanisms of ethosomal system skin permeation

Ethanol and phospholipids are reported to act synergistically to enhance the skin permeation of drugs in ethosomal formulations. Ethanol fluidizes the lipid bilayers of the ethosomal vesicles and the stratum corneum simultaneously, changing the arrangement and decreasing the density of skin lipids. Therefore, the highly malleable and soft vesicles of an ethosomal system will penetrate the altered structure of the stratum corneum and create a pathway through the skin. The release of the therapeutic agent occurs by the fusion of these vesicles into cell membranes in the deeper layers of the skin (Touitou et al., 2000; Ainbinder et al., 2010; Singh et al., 2014). It is suggested that transethosomes have superior skin-permeation properties over classical ethosomes. This is because transethosomes contain both ethanol and the edge activator or the penetration enhancer, which both act together to increase vesicular malleability and skin-lipid perturbation (Bragagni et al., 2012; Ascenso et al., 2015). A proposed mechanism is illustrated in figure 1.3.
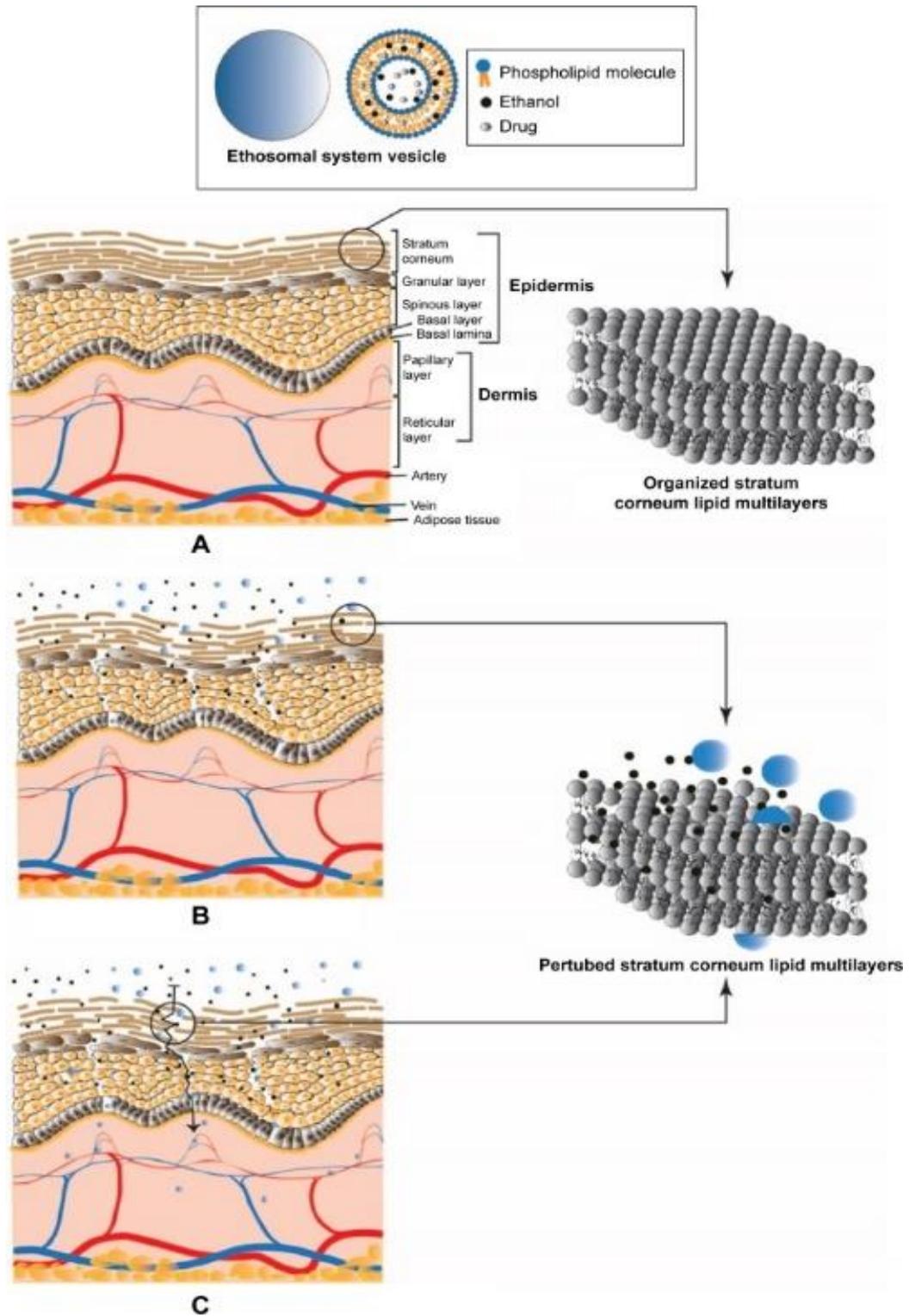
Fig 1.3: Proposed mechanism for permeation of molecules from ethosomal system through the skin. Notes: (A) Normal skin; (B) Skin-lipid perturbation by ethanol effects; (C) Penetration of the soft malleable ethosomal system vesicles.

1.6.3 Preparation methods

1.6.3(a) The classical cold method

This is the simplest and most widely used method for the preparation of ethosomal systems. The organic phase is obtained by dissolving the phospholipids, (in addition to the surfactant or penetration enhancer for the preparation of transethosomes) in ethanol, or in mixture of solvents (ethanol/PG for binary ethosomes) (Zhang et al., 2012), at room temperature (Cortesi et al., 2010), or at 30 °C (Touitou et al., 2000). The used aqueous phase is either water (Dubey et al., 2007; De la Presa et al., 2009; Chourasia et al.,2011), buffer solution (Dubey et al., 2010; Cortesi et al., 2010) or normal saline solution (Raza et al., 2013). The aqueous phase is added to the organic phase in a fine stream (Touitou et al., 2000) dropwise (Zhou et al., 2010) or using a syringe pump at a constant rate of 175 or 200 µl/min (Paolino et al., 2005). The mixture is stirred at a speed of 700 – 2,000 rpm (Touitou et al., 2000; Chourasia et al., 2011; Kaur & Saraf, 2011; Chen et al., 2010) using an overhead (Touitou et al., 2001) or magnetic (Elsayed et al., 2006) stirrer. The intended drug for incorporation within the ethosomal system will be dissolved in either the aqueous or the organic phase, depending on its physicochemical properties.

1.6.3(b) The ethanol injection–sonication method

In this method, the organic phase containing the dissolved phospholipid in ethanol is injected to the aqueous phase, using a syringe system (Zhaowu et al., 2009) at a flow rate of 200 µl/min, then homogenized with an ultrasonic probe for 5 min (Liu et al., 2011).

1.6.3(c) The hot method

In one vessel, phospholipid is dispersed in water and placed in a water bath at 40 °C until a colloidal suspension is obtained. In another vessel, ethanol is heated to 40 °C and then added dropwise to the phospholipid dispersion, under continuous mixing using a mechanical or magnetic stirrer (Ashoniya & Meenakshi, 2011). The drug is dissolved in either the organic or the aqueous phase, based on its hydrophilic/hydrophobic properties.

1.6.3(d) The thin-film hydration method

In this method the phospholipid is first dissolved in chloroform only (Park et al., 2014) or a chloroform–methanol mixture at ratios of 3:1 or 2:1 in a clean, dry, round-bottom flask, later, organic solvents are removed by a rotary vacuum evaporator at a temperature above the lipid-phase transition temperature. The traces of the solvents are removed from the deposited lipid film under vacuum overnight, then the lipid film is hydrated with a water–ethanol solution (Lopez-Pinto et al.,2005) or phosphate buffered saline–ethanol solution (Mishra et al., 2008).

1.6.3(e) The reverse-phase evaporation method

This is the least used method and specially designed to produce large unilamellar vesicles. The organic phase is prepared by dissolving the phospholipid in diethyl ether and then mixing it with the aqueous phase at a ratio of 3:1 v/v in an ultrasonic bath at 0 °C for 5 min to form a water-in-oil emulsion. The organic solvent is removed under

reduced pressure to produce a gel, which turns into a colloidal dispersion upon vigorous mechanical agitation (Maestrelli et al., 2009).

1.6.3(f) Transmembrane pH-gradient method

In this method, the drug is loaded "actively", based on the pH-gradient difference between the acidic interior of the internal phase and the basic exterior of the external phase of the ethosomal system. At first, the empty ethosomal suspension is prepared using any of the aforementioned methods, but the aqueous phase or the hydration process uses an acidic buffer (usually citrate buffer, pH 3). Secondly, the active drug is loaded into the empty ethosomal suspension, followed by continuous stirring. In order to make the external phase more alkaline and to establish the pH gradient between the acidic internal (pH 3) and basic external phases of the ethosomal system, an alkali, usually a sodium hydroxide solution of 0.5 M is added to make the external pH 7.4. In the third stage, the ethosomal system is incubated at a specified time and temperature (30 – 60 °C) to give the opportunity for the unionized drug to actively pass the bilayer of the ethosomal vesicles and get entrapped (Zhou et al., 2010).

1.7 Literature review

The ability of ethosomal systems to penetrate intact skin, delivering molecules with diverse physicochemical properties in therapeutic amounts to the blood circulation, has been reported by many researchers (Abdulbaqi et al., 2016). For instance, the pharmacokinetics and pharmacodynamics effects of a valsartan-loaded ethosomal system were studied in albino Wistar rats. The bioavailability of transdermal

ethosomal valsartan was significantly higher (3.03 times) than the oral valsartan suspension; the area under the concentration–time profile curve from time 0 to infinity for the transdermal ethosomal formulation was 177,298.82±665.01 ng/ml/hr., while for the oral suspension it was 55,554.54±774.01 ng/ml/hr. The dose of valsartan used in both routes was 3.6 mg/kg. The reported maximum drug concentration (C_{max}) and the time taken to reach maximum concentration (T_{max}) values for the oral administration were 13,100±101.12 ng/ml and 1±0.01 hr, respectively. The C_{max} and T_{max} values for the transdermal route were 7,944±134.32 ng/ml and 5.0±0.83 hr., respectively (Ahad et al., 2013). Ethosomal valsartan was shown to effectively reduce blood pressure by 34.11 % for 48 hr in hypertensive rats (Ahad et al., 2015).

The incorporation of ethosomal dispersions in suitable vehicles like gels, patches, and creams represents an important step to get better skin-permeation and therapeutic results. Several researchers have studied the skin permeation and disposition properties of various drugs from ethosomal gels, and reported the high efficiency and superiority of these formulations in dermal or transdermal drug delivery over the traditional or marketed gels or creams. (Puri & Jain, 2012) compared the *in vitro* skin permeation properties of 5-fluorouracil from ethosomal gel and marketed cream using Franz diffusion cell and albino rat skin, and found that the transdermal flux of 5-fluorouracil from ethosomal gels was 4.9 folds higher than the marketed cream. Moreover, the skin disposition of the drug from the ethosomal gel was 9.4-fold higher than the marketed cream (Puri & Jain, 2012). Other researchers found that *in vitro* transdermal flux of aceclofenac from ethosomal gel was higher (226.1 $\mu g/cm^2/hr$) than Zynac gel (131.1 $\mu g/cm^2/hr$) (Dave et al., 2010). The development of colchicine loaded ethosomal gel for transdermal delivery may offer a new route for administration of this venerable

drug to solve the several dose dependent side effects associated with its oral administration and enhance its bioavailability.

1.8 Problem statement

Colchicine is a highly effective drug in the treatment of gout, pseudo-gout, FMF and many other illnesses, but it is known to have a low therapeutic index and oral bioavailability of 25 % to 50 % due to the extensive first-pass metabolism, its oral administration is associated with mild to severe gastrointestinal side effects including: abdominal cramps, pain, nausea, vomiting and diarrhea that will develop in around 80 percent of patients. Colchicine was used to be administered intravenously until it was banned by the United States food and drug administration (FDA) in 2008 due to its potential and serious adverse effects such as tissue necrosis cytopenias, disseminated intravascular coagulation, and even death.

Therefore, colchicine transdermal administration by ethosomal gels may offer an alternative route of administration of this drug to overcome its adverse reactions, and low oral bioavailability problems associated with the oral route.

1.9 Study Objectives

The objectives of the proposed study are:

I. To optimize colchicine ethosomal systems using 2^3 and 2^4 factorial designs.

II. To develop and validate an HPLC method for the quantification of colchicine in ethosomal gel and in the release media.

III. To formulate and optimize colchicine loaded ethosomal gel.

IV. To evaluate the stability of the optimized gel formulation.

V. To study the skin permeation behavior of the drug from the optimized gel formulations.

CHAPTER 2

DEVELOPMENT AND CHARACTERIZATION OF COLCHICINE ETHOSOMAL SYSTEMS

2.1 Introduction

Colchicine is used for the treatment of acute gout, familial Mediterranean fever (FMF) and pseudo-gout (Martinon et al., 2006). However, colchicine is known to have low oral bioavailability (25 – 50 %) due to the extensive first-pass metabolism (Ben-Chetrit & Levy, 1998) and low therapeutic index (Terkeltaub, 2009).

Oral administration of colchicine is causing severe gastrointestinal side effects, including abdominal cramps and pain, nausea, vomiting and diarrhea (Niel & Scherrmann, 2006).

To overcome these drawbacks, and to improve colchicine bioavailability, some researchers studied and prepared microemulsions (Ganju et al., 2012) nanoemulsions (Shen et al., 2011), transdermal patches (Kumar et al., 2012), iontophoresis (Kulkarni et al., 1996) and elastic liposomes (Singh et al., 2009).

Ethosomal systems are novel nanocarriers especially designed for the delivery of drugs dermally or transdermally, and may represent useful tools for the delivery of colchicine. Therefore, the aim of present study was to prepare colchicine ethosomes for transdermal delivery. The three types of ethosomal systems were prepared namely: classical ethosomes (CE), binary ethosomes (BE) and transethosomes (TE). The 2^3 and 2^4 factorial designs were used in the optimization of ethosomal formulations.

2.2 Materials

Colchicine (purity > 97 %) was purchased from (Acros organics, New Jersey, USA). Phospholipon 90G® (PL90G) (contains > 90 % Phosphatidylcholine from soybean) generously donated by (Lipoid LLC, New Jersey, USA). Absolute ethanol (purity ~ 99.7 %) was bought from (Fine Chemicals Inc., USA). Tween 20® (purity ≥ 99 %) and sodium stearate were purchased from (R & M Chemicals, Essex, UK). Sodium taurocholate, cholesterol and Sephadex® G-25 were bought from (Sigma-Aldrich, St. Louis, MO, USA). Propylene glycol (PG) acid was obtained from (QRëC, Selangor, Malaysia). Labrafil® M 1944 CS (Oleoyl macrogol-6 glycerides EP) was purchased from (Gattefossé, Lyon, France). Ultrapure water was prepared by Elga PURELAB Classic UVF water purification system, France.

2.3 Methods

2.3.1 Preparation of the ethosomal systems

The three types of ethosomal systems namely classical ethosomes (CE), binary ethosomes (BE) and transethosomes (TE) were prepared using the cold method (Abdulbaqi et al., 2016; Touitou et al., 2000). In this method the organic phase was obtained by dissolving the PL90G at 30 °C in ethanol for preparation of CE or mixture of solvents (ethanol/polyethylene glycol) for the preparation of BE. Surfactants (either Tween 20®, Sodium taurocholate or Labrafil®) were added into organic phase for the preparation of TE. The aqueous phase (Ultrapure water) was heated to 30ºC as well and then added to organic phase dropwise with continuous stirring at 1200 rpm, using magnetic stirrer. The mixing continued for 45 min to get the ethosomal dispersions which are further subjected to size-reduction process by multiple extrusions through