A Thesis
entitled
Evaluation of the Percutaneous Absorption of Chlorpromazine Hydrochloride from PLO Gels Across Porcine Ear and Human Abdominal Skin

By
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Submitted to the Graduate Faculty as partial fulfillment of the requirements for
The Master of Science Degree in Pharmaceutical Sciences,
Industrial Pharmacy Option

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The University of Toledo
August 2015
An Abstract of
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The overall objective of this work is to determine the percutaneous absorption of chlorpromazine hydrochloride from PLO gels and verify the suitability of a topically applied chlorpromazine PLO gel for use in hospice patients to relieve symptoms such as vomiting and nausea at the end stages of life. The aims of the present study were to (a) prepare and characterize pluronic lecithin organogels (PLO gels) of chlorpromazine hydrochloride using isopropyl palmitate or ricinoleic acid as the oil phase, (b) assess the in vitro percutaneous absorption of chlorpromazine hydrochloride through porcine ear and human abdominal skin using isopropyl palmitate and ricinoleic acid PLO gels, and (c) assess the theoretical plasma concentrations of chlorpromazine from flux values. PLO gels of chlorpromazine hydrochloride were successfully prepared using isopropyl palmitate or ricinoleic acid as the oil phase and characterized for pH, morphology, stability, viscosity, thermal analysis using differential scanning calorimetry (DSC), in vitro drug release and stability. In vitro permeability studies were performed across pig ear and human abdominal skin using isopropyl palmitate PLO gel and compared with the ricinoleic acid PLO gel. The pH and viscosity of both PLO gels prepared with isopropyl palmitate and ricinoleic acid
were comparable. The thixotropic property of ricinoleic acid PLO gel was found to be better than the isopropyl palmitate PLO gel. Both formulations were found to be stable at 25°C, 35°C, and 40°C for up to 60 days. The permeation of chlorpromazine hydrochloride was higher from ricinoleic acid PLO gel than isopropyl palmitate PLO gel and pure drug solution. Theoretical steady state plasma concentrations (C_{ss}) of chlorpromazine from pure drug solution, isopropyl palmitate PLO gel and ricinoleic acid PLO gel were found to be 1.05, 1.20, and 1.50 ng/ml. PLO gels only marginally increased the flux and theoretical C_{ss} of chlorpromazine. However, theoretical C_{ss} values for chlorpromazine were much below the required therapeutic concentration for antiemetic activity in hospice patients. From this study it is clearly evident that PLO gels fail to deliver the required systemic levels of chlorpromazine following topical application. To achieve better chlorpromazine hydrochloride skin permeation and thus higher concentrations of chlorpromazine in plasma, following topical application on the skin, efficient permeation enhancers should be used.
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Chapter 1

1. Introduction

1.1. Overview

The concept of delivering medications through skin is not new since it was reported back in the sixteenth century. The husk of the castor oil plant in water was massaged on the head to get relief from pain. Today, transdermal drug delivery is decently acknowledged for delivering drug to the systemic circulation. Until now, the utilization of transdermal patches for pharmaceuticals has been restricted in light of the fact that just a couple of medications have shown to be effective in delivering drugs through the skin, for example cardiac drugs (e.g. nitroglycerin) and hormones (e.g. estrogen) [1, 2]. Transdermal delivery represents an attractive alternative to oral and parenteral delivery of drugs due to its accessibility and extensiveness [3-5]. The general difficulties that exist with the oral route in administering drugs to nauseous and/or unconscious patients led researchers to look for alternative methods such as the topical route. In transdermal delivery, active ingredients are delivered across the skin for local and/or systemic effects. The skin is the largest organ in the human body and it mainly consists of three layers: the epidermis, dermis, and hypodermis (Fig. 1.1). The skin protects the human body against penetration of foreign molecules and evaporation of water. The protective effects of the skin are mainly due to
the impermeable, multi-layered epidermis [6, 7].


**Figure 1-1:** Layers of the skin. Modified from http://www.freedigitalphotos.net/images/view_photog.php?photogid=2280

The epidermis is further divided into four distinct layers, namely, stratum corneum (outer most) stratum granulosum, stratum spinosum, and stratum germinativum (inner most) [6, 8]. The stratum corneum is composed of flattened cornified cells embedded in a lipid intercellular matrix. The barrier properties of stratum corneum prevents the absorption of approximately 90% of transdermal drugs [6, 7]. For several thousand years people have applied substances on the skin for local topical effects. Transdermal drug delivery offers several advantages compared to oral and parenteral delivery such as: avoids presystemic metabolism and hostile GI environment, is painless compared to parenteral administration, reduces dosing frequency, enhances patient compliance, and can be self-administered unlike parenteral administration. They have received
considerable attention in the area of pharmaceutical research over the last few decades. Transdermal systems have been used in treating several local and systemic diseases such as analgesia, angina, contraception, hormone replacement, hypogonadism, hypertension, motion sickness, and smoking cessation [9]. However, transdermal delivery is only amenable to a limited number of drugs with low molecular mass (< 400 Da) and log P values of 1-3. Transdermal delivery is also suitable for drugs that require doses of milligrams per day or less. Transdermal delivery of hydrophilic drugs and large molecular weight peptides has always been extremely challenging. Transdermal drug administration involves the delivery of drugs using: transdermal patches, gels, creams and ointments. The drug permeability from ointments and creams is often limited by the skin barriers. On the other hand, transdermal gels temporarily disrupt the lipid bilayers of the skin, specifically the stratum corneum. Pluronic lecithin organogel (PLO gel) is a commonly used transdermal gel. PLO gel allows the drug to permeate through the stratum corneum into the systemic circulation via dermal epidermal blood flow [5, 10-12].

1.2. Skin as Barrier to Transdermal Delivery

The skin is considered as the largest organs in the human body. It consists of two layers: the cellular outermost layer, which is called epidermis, and the inner connective tissue layer, dermis. Between these two layers is a microscopic structure, the basal lamina or basement membrane zone. The skin covers an area up to 1.73 square meters and weighs approximately 2 kilograms. The main function of the skin is to protect the body from its surrounding environment. The skin is the main permeation obstacle for exogenous molecules to permeate through the human skin. The skin has a pH range from 2.8 and 6.0 and extends in thickness from 0.05-2 mm. It is
extremely flexible. Contingent upon the body site, a normal square centimeter of skin contains 12 nerves, 100 sweat organs, 3 veins, 15 sebaceous glands and 10 hair follicles. The administered medication needs to cross various potential barriers before reaching the systemic circulation [7, 13, 14].

### 1.2.1 Skin structure:

The four principle barriers or hindrances that prevent chemical penetration into human skin can be categorized as follows [7, 15]:

1.2.1.1. **Stratum Corneum (SC)/horny layer** – It is the uppermost, non-viable epidermis. The stratum corneum is considered the primary barrier or the rate limiting step of xenobiotic diffusion besides acting as a reservoir for chemicals [16]. The stratum corneum is the deciding result of epidermal differentiation and is comprised of 15 to 25 cell layers covering the vast majority of the body surface. The corneocyte is the biggest cell in the stratum corneum, more or less 0.5 μm in thickness and 30 to 40 μm in width. It contains no organelles, but it is loaded with protein, 80% of which is high molecular weight keratin. The intercellular space is loaded with lipids sorted out into different bilayers and these lipids are of surprising organization. It is known that 14% of the stratum corneum (by weight) is lipids. Moreover, stratum corneum has a low water content [17]. Arrangement of the stratum corneum is also accompanied by the deposition of a 15 nm thick band of protein on the internal surface of the plasma membrane. The cornified cell envelope is a structure unique to keratinocytes and a sign of the terminal differentiation. Multiple cell differentiations show distinctive layers beneath the SC, specifically the stratum basale, stratum spinosum, stratum granulosum and stratum lucidum. As
the SC cells are sloughed off, the stratum basale is responsible for the replacement of these cells. It takes approximately 28 days for the aggregate turnover from the stratum basale to shedding [18]. The principle lipid segments of the stratum corneum are ceramides, cholesterol, and free unsaturated fats, which make up nearly 50, 25, 10 percent of the stratum corneum lipid mass, respectively. The minor lipid segments are glucosylceramides, cholesterol sulfate and cholesterol esters. Interestingly, with cell membranes, ceramides placed in the intercellular spaces cannot structure bilayers without additional input, so cholesterol, free unsaturated fats, and cholesterol sulfate are obliged to form ordered structures. The lipid bilayer containing these parts drastically diminishes skin penetration of exogenous chemicals [5, 18, 19].

1.2.1.2. Epidermis – It is the viable, cellular and avascular layer of the skin. The epidermis is composed of two sections: the living cells of the Malpighian layer and the dead cells of the stratum corneum usually referred to as the horny layer. The prime function of viable cells of the epidermis is to move continuously through a methodology of differentiation, inevitably lapsing to create the hindrance layer (the stratum corneum) (Fig. 1.2) [19, 20]. Over the greater part of the body the epidermis extends in thickness from 0.06 to 0.1 mm. The major cell of the epidermis is the keratinocyte and other critical cell components incorporate the melanocyte (the wellspring of melanin pigment). The skin layer arranged between the stratum corneum and the dermis, the viable cells of the epidermis, can be considered as fluid gel that doesn’t pose a striking hindrance to permeability, subsequently to improve percutaneous absorption, it is critical to the upgrade infiltration of a substance through the stratum corneum[21].
1.2.1.3. Dermis – It is the deepest layer that comes over acellular connective tissue. As illustrated in (Fig. 1.3), the dermis (0.1-0.5 cm thick) is the deepest layer of the skin that offers no imperviousness to passing particles. Comprised of collagen and elastin together with glycosaminoglycans, salts and water, the dermis gives an imperative segment of the body as it gives nutritive, resistant and other emotionally supportive networks. A system of lymphatics, veins and nerve endings is seen in the dermis which gives backing to the epidermis. Hair follicles and sweat channels derive from the dermis and at last give the appendageal course [18, 22].
1.2.1.4. Hypodermis – It is the internal subcutaneous fat layer. The hypodermis, overall known as the subcutis, is the deepest layer of the skin that backs the dermis. This greasy layer is comprised of fat cells, which are connected to the dermis by collagen and elastin filaments. It additionally contains different cells, chiefly fibroblasts and macrophages. The hypodermis joins the skin to the core muscles and supports the skin with neutral and vascular systems. It is evaluated that half of the body’s fat is comprised from the subcutis [17, 18].

1.3. Pluronic Lecithin Organogels (PLO gels):

Gel is defined as a soft, solid or solid-like materials which contains solid and liquid components.
The solid components (the gelator system) are introduced as mesh/network of aggregates, which immobilizes the fluid parts [23-25]. It is considered as a true two phase system since the inorganic particles are dispersed throughout the organic components and the gel is formed via physical or chemical interactions between the gelator system (e.g., lecithin) and appropriate solvents as illustrated in (Fig. 1.4). This prevents the flow of the solvent phase as a result of increasing surface tension [2]. As indicated by the United States Pharmacopeia (USP), gels are defined as a semisolid system comprising a dispersion made up of either small inorganic particles or vast organic molecules encasing and interpenetrated by fluid. The inorganic particles form a three-dimensional house of cards structure throughout the gel. There are many classification systems for gels. The first classification system according to USP classifies gels as a single phase or two phase gels. Based on the nature of solvents used, gels are classified as hydrogels (water based), organogels (organic or non-aqueous solvent), or xerogels [26]. Gels may be also classified depending on their rheological properties into plastic gels, pseudo-plastic gels, or thixotropic gels [26].

Figure 1-4: Different steps in the formation of a lecithin organogel. Modified from [48].
1.3.1. What Are Pluronic Lecithin Organogels (PLO gels)?

Pluronic lecithin organogels (PLO gels) are opaque, yellow-colored, and composed of isopropyl palmitate, soy lecithin, water, and Pluronic F127 (hydrophilic polymer). Chemical structures of these compounds are illustrated in Figures 1.5 and 1.6. The only difference between lecithin organogels (LO gels) and PLO gels is the presence of hydrophilic polymer (poloxamer 407), which gels in water and increases the quantity of aqueous phase in the formulation compared to the oil phase [25, 27, 28].

![Structure of lecithin](image1.png)

**Figure 1-5:** Structure of lecithin

![General structure of Poloxamers](image2.png)

**Figure 1-6:** General structure of Poloxamers
1.3.2. General characteristics of Pluronic Lecithin Organogels [28, 29]:

1.3.2.1. Viscoelasticity: PLO gels have both viscous and elastic properties. A PLO gel behaves like a solid with elastic properties at lower shear rates. The organogel structure will disrupt as the shear stress is increased resulting in flowability.

1.3.2.2. Non-birefringence: PLO gels will not allow polarized light to pass through its matrix; therefore, it appears as a dark matrix.

1.3.2.3. Thermo-reversibility: PLO gels lose their fiber structure and start to flow when the temperature of the organogel rises above the critical limit temperature. However, when cooled, PLO gels revert back to a more stable configuration.

1.3.2.4. Thermostability: PLO gels are thermostable due to the ability of the gelator system to undergo self-assembly, under appropriate conditions.

1.3.2.5. Optical clarity: Lecithin organogels are transparent while PLO gels are yellow-colored and opaque in nature.

1.4. Pluronic Lecithin Organogels history as vehicle for drug delivery

In 1991, two compounding pharmacists Marty Jones and Lawson Kloesel developed PLO gels from original lecithin organogels (LO gels) for topical and transdermal drug delivery[1]. LO gels were popularly used as vehicles for transdermal drug delivery before the development of PLO gels. LO gels are made by mixing a small amount of water with the organic solution of lecithin oil phase. Later, Pluronic F127 (poloxamer 407), a tri-block copolymer of polyoxyethylene and polyoxypropylene, was added to the system to stabilize the LO gels [30].
Multiple collaborations between physicians and compounding pharmacists led to the development of PLO gel formulations for a wide variety of drugs. Currently, PLO gels have been widely used in compounding pharmacies as a base for transdermal delivery of drugs including hormones, antiemetics, opioids, antipsychotic drugs, calcium channel blockers and local anesthetics [31, 32]. A PLO gel system facilitates the delivery of both hydrophilic and lipophilic drugs due to the presence of both oil and aqueous phases within the gel system. PLO gels enhance the transdermal permeability of drugs by temporarily altering the skin barrier functions [25].

**Figure 1-7:** Mechanism of gelation of poloxamer in presence of water. Modified from [52].

### 1.5. PLO gel preparation and characterization

PLO gels are usually prepared by mixing 1 part of oil phase containing lecithin soy in isopropyl myristate or isopropyl palmitate in a 1:1 ratio with 4 parts of aqueous phase containing 20-30%
Pluronic F127 to form a gel [33]. Dissolution of lecithin in isopropyl myristate or isopropyl palmitate at room temperature and dissolution of poloxamer in water at 4°C requires at least 12 h. The mechanism of gelation of poloxamer in the presence of water is illustrated in Fig. 1.7. About 0.1-0.2% w/w preservative is also added to enhance the stability of PLO gels [24]. Usually, hydrophobic drugs are added to the oil phase and hydrophilic drugs are added to the aqueous phase. Our group has recently suggested the use of ricinoleic acid as an alternative to isopropyl myristate or isopropyl palmitate, especially for delivering nonsteroidal anti-inflammatory drugs [34, 35]. Better thixotropic properties were observed for PLO gels prepared using ricinoleic acid compared to traditional PLO gels prepared using isopropyl palmitate [36]. PLO gels are generally characterized for the following:

1.5.1. Physical appearance: PLO gel mixtures are inspected visually for their color, homogeneity, and consistency.

1.5.2. pH: The pH of PLO gels is determined using a pH meter. The skin has a pH ranging between 2.8 and 6.0; therefore, adjusting the formulation pH close to the pH of skin at the application site is important.

1.5.3. Rheological and viscosity studies: Different shear stresses and rotational speeds (R.P.M.) are required to determine the rheological properties of PLO gels. It is crucial to study the rheological behavior of PLO gels used for transdermal and topical drug delivery. Rheological properties of PLO gels can also influence the following properties:

a. Spreadability

b. Bioadhesiveness on the skin site

c. Cohesiveness: It gives information about the structural reformation of the gel after
application of shear stress. Different shear stresses and rotational speeds (R.P.M.) are required to determine the rheological properties of the PLO gel.

d. Gel consistency: Consistency of the gel is a critical parameter and it should be modified in a favorable manner. PLO gel systems, before the addition of a polar phase, exhibit Newtonian behavior, but follow Maxwell’s rheological or viscoelastic behavior after the addition of the polar phase.

1.5.4. Stability studies: It is important for the drug formulation to have good stability over time. There are many signs of drugs instability which could be harmful to patients such as degradation of drug products into toxic or inactive products. Because of this harm, it is a crucial requirement to provide stability study profiles for each formulation in order to get approval for using the formulation in patients. In addition, it is possible to understand compatibility between drug and excipients during formulation development to obtain stable final products. Therefore, the expiration date could be estimated from the shelf-life calculation. There are many methods for performing stability studies such as: real-time stability testing, accelerated stability testing, retained sample stability testing, and cyclic temperature stress testing. Detailed information for all criteria and protocols for conducting stability studies and validation could be found in literature [37-41].

1.5.5. Drug content determination: Drug content determination has a significant role in developing any new formulation. Analytical method validation of the drug should be done prior to drug content determination [42, 43].
1.5.6. In vitro drug release and in vitro drug permeation using Franz-diffusion study

1.5.6.1. Description and uses of Franz-diffusion cells

Generally Franz diffusion cells are used to evaluate the permeation of drugs through the membrane of choice. The permeability of drugs could be used to calculate the flux of drugs across the membrane and amounts of the drug permeated and penetrated through the membrane [44, 45]. Figures 1.8 and 1.9 show the important components of a vertical Franz diffusion cell and the complete set-up for a permeability study.

1.5.6.2. Calculating Flux and Permeability Coefficient (Kₚ)

Flux (J) is the amount of compound permeated through the membrane per unit time. The unit of flux is mass/(area.time). When a permeant is applied in a finite dose, then flux is calculated by the following equation:

\[ J = \frac{Q}{A \cdot t} \]  
Eq. 1

where \(Q\) is the quantity of compound crossing the membrane in time \(t\), and \(A\) is the area of exposed membrane in cm².

Steady state flux (\(J_{ss}\)) is the amount of drug which passes through the membrane at a constant rate; \(J_{ss}\) is observed after the lag time when the amount continues to build up. At steady state, the drug concentrations measured at successive sampling timepoints are not significantly different. Permeability constant (\(K_p\)) can be calculated from the following relationship:

\[ K_p = \frac{Q}{A \cdot t \cdot (C_o - C_i)} \]  
Eq. 2

where \(Q\) is the quantity of compound transported through the membrane in time \(t\), \(C_o\) and \(C_i\) are the concentrations of the compound on the donor side and the receptor side of the
membrane respectively, and A is the area of exposed membrane in cm$^2$. The units of $K_p$ are cm/min or cm/hr [46].

**Figure 1-8**: The important components of a Franz cell. Modified from: http://www.permegear.com

**Figure 1-9**: Franz- diffusion cell setup used in the study.
1.5.7. Skin Irritation test: Skin irritation is a reversible damage to the skin following the application of a chemical substance for up to 4 h. It is generally assessed in laboratory animals. The idea of an in vitro skin model irritation assay is based on the premise that irritant chemicals are able to permeate the stratum corneum by diffusion and are cytotoxic to cells in the percutaneous layer. Irritant materials are identified by their ability to decrease cell viability. Also, the release of inflammatory mediators (e.g., Interleukin 1 alpha) could be found and used as an additional measure of skin irritation. There are also human skin models such as e.g. EpiDerm™ and EPISKIN™ models [47].

1.6. Advantages of PLO gels as a vehicle for drug delivery [27]:

PLO gels have numerous advantages over other transdermal drug delivery systems:

- Higher stability compared to other topical products.
- Manufacturing PLO gels could be cost-effective and it is easy to manufacture PLO gels in smaller and larger batches.
- Ability to deliver both hydrophilic and lipophilic drugs.
- Higher solubility of both hydrophilic and lipophilic active pharmaceutical ingredients.
- Exhibit higher drug permeability across the skin membrane.
- Ability to deliver drugs into the systemic circulation.
- Less greasy than ointments and can be easily removed from the skin.
1.7. Transdermal delivery of drugs using PLO gels

PLO gels have gained much attention due to their ability deliver drugs both locally and systemically after topical application [27]. Many medications such as chlorpromazine, lorazepam, morphine, and haloperidol have been formulated into topical gel formulations either alone or in combination products such as ABH (lorazepam, diphenhydramine, and haloperidol) gel [48-50]. Transdermal PLO gel formulations are applied with the goal of cutaneous absorption to achieve systemic activity [28]. The table below lists different medications incorporated into PLO gels for either local or systemic effects.

1.1. Lists of drugs compounded as PLO gel for systemic and local deliveries

<table>
<thead>
<tr>
<th>Drug</th>
<th>Study Model- Ex vivo skin model (or) Experimental subjects</th>
<th>Study Methods</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methimazole</td>
<td>Healthy cats; n=6 (3 male, 3 female)</td>
<td>A randomized triple crossover protocol to determine bioavailability of methimazole from PLO gel and compared with i.v. and oral routes of administration.</td>
<td>Methimazole absorption by the transdermal route was poor and variable relative to the i.v. and p.o. routes. Only one cat showed 100% transdermal bioavailability relative to p.o route.</td>
<td>[51]</td>
</tr>
<tr>
<td>Methimazole</td>
<td>Cats diagnosed with hyperthyroidism n=44 (17 cats oral group and 27 cats transdermal group)</td>
<td>To determine the safety and efficacy of the transdermal methimazole with oral methimazole for the control of hyperthyroidism in cats.</td>
<td>Generally, efficacy of transdermal methimazole is not as high as per oral methimazole after 2 weeks of treatment and it is associated with much less GI side effects compared to the oral route. Also, it is a good therapeutic alternative in the treatment of feline hyperthyroidism as it shows the same effect as oral dose after 4 weeks of treatments.</td>
<td>52</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Methimazole</td>
<td>Retrospective study n= 13 cats with hyperthyroidism</td>
<td>PLO gel was applied to the inner pinna of the ear in doses ranging from 2.5 mg/cat q 24 h to 10.0 mg/cat q 12 h. Clinical and laboratory data from the cats were retrospectively evaluated by telephone conversations with the veterinarians who managed the cases.</td>
<td>Methimazole PLO gel showed clinical improvement. No adverse effects were noticed after several months of treatment with PLO gel.</td>
<td>53</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Healthy cats (n=4)</td>
<td>Parallel study: 3 groups of 4 cats to assess bioavailability, pharmacokinetics, and safety of fluoxetine PLO gel for transdermal delivery and compare it with oral delivery.</td>
<td>Fluoxetine PLO gel (15%) formulation showed good absorption through the skin of cats into the systemic circulation. However, the relative bioavailability for transdermal administration is approximately 10% compared to the p.o. route.</td>
<td>54</td>
</tr>
<tr>
<td>Drug</td>
<td>Description</td>
<td>Study Design</td>
<td>Results</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Healthy cats n= 5</td>
<td>Pilot crossover study as each cat received both transdermal PLO gel and oral treatments in a random order to compare serum concentration of dexamethasone after single dose of both treatments.</td>
<td>There is no significant absorption of dexamethasone after administration of PLO gel as all samples were below the limit of detection (LOD).</td>
<td>[55]</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Carrageenan-induced rat paw edema n=24 rats</td>
<td>Evaluate and compare the <em>in vitro</em> and <em>in vivo</em> anti-inflammatory effects of the ricinoleic acid PLO gel system with isopropyl palmitate PLO gel. Ketoprofen was used as a model drug.</td>
<td>Both <em>in vitro</em> and <em>in vivo</em> studies have shown better anti-inflammatory property of 10% ketoprofen ricinoleic acid PLO gel compared to isopropyl palmitate PLO gel.</td>
<td>[35]</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Healthy human subjects, n= 8</td>
<td>Open-label crossover study as the volunteers received an oral ketoprofen capsule or topical 20 % ketoprofen PLO gel.</td>
<td>When ketoprofen administered as PLO gel, it had low relative bioavailability and high variability compared to oral route.</td>
<td>[56]</td>
</tr>
<tr>
<td>Ketoprofen and Testosterone</td>
<td>3 human skin samples</td>
<td>Testing <em>in vitro</em> and <em>ex vivo</em> percutaneous absorption of ketoprofen and testosterone from PLO gel compared to Pentravan cream by using Franz-Diffusion cells.</td>
<td>The absorption of Pentravan cream for both drugs was found to be more than PLO gel formulations.</td>
<td>[57]</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>14 patients with lateral epicondylitis.</td>
<td>A randomized, double-blind, crossover study to assess the effectiveness of diclofenac as a PLO gel for treatment of lateral epicondylitis.</td>
<td>A 2% diclofenac PLO gel provided an effective reduction of pain associated with lateral epicondylitis.</td>
<td>[58]</td>
</tr>
<tr>
<td>Drug</td>
<td>Participants</td>
<td>Study Design</td>
<td>Results</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------</td>
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<td>------------------------------------------------------------------------------</td>
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<td>-----------</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>74 patients with osteoarthritis of the knee.</td>
<td>A double blind, randomized, placebo, designed as a parallel group to evaluate using diclofenac as a PLO gel for treating osteoarthritis of the knee.</td>
<td>There was a significant improvement of quality of life of osteoarthritis patients as they experienced less pain after diclofenac PLO gel administration.</td>
<td>[59]</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>Healthy human subjects, n=12</td>
<td>To assess ondansetron PLO gel for transdermal delivery and assess inflammatory and hyperalgesia.</td>
<td>Topical application of PLO gel reduced the inflammatory effect induced by capsaicin injection.</td>
<td>[60]</td>
</tr>
<tr>
<td>Amitriptyline and buspirone</td>
<td>Healthy cats, n=6</td>
<td>Crossover design study to assess the relative bioavailability of amitriptyline and buspirone PLO gels compared to single oral dose.</td>
<td>Systemic absorption of amitriptyline and buspirone PLO gels administered by the transdermal route was poor compared with per oral route.</td>
<td>[61]</td>
</tr>
<tr>
<td>Promethazine</td>
<td>Healthy human subjects, n=15</td>
<td>Randomized, open-label, and crossover study to assess the bioavailability of promethazine PLO gel as 15 subjects were administered PLO gel and after 21 days, 10 of the subjects were given promethazine as an intravenous injection.</td>
<td>Systemic absorption of topically applied promethazine PLO gel happened as the amount of the drug was detected from plasma samples.</td>
<td>[62]</td>
</tr>
<tr>
<td>Morphine</td>
<td>Human subjects, n=5</td>
<td>Randomized, placebo-controlled, double-blind, crossover study to evaluate the bioavailability of morphine administered as topical PLO gel or</td>
<td>The amount of morphine could not be quantified after PLO gel administration, which is considered low bioavailability compared to</td>
<td>[49]</td>
</tr>
<tr>
<td>Drug</td>
<td>Study Details</td>
<td>Absorption Details</td>
<td>References</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>10 patients administered PLO gel, 5 patients administered orally, 1 patient was given placebo topically</td>
<td>To measure blood methadone levels achieved after topical PLO gel and oral administration to hospice patients. Methadone PLO gel in doses less than 45 mg/day did not result in trough methadone blood concentrations associated with analgesia. Also, the placebo response may explain the perceived benefit of methadone PLO gel in doses less than 45 mg/day. The evaluation of systemic absorption of methadone-PLO gel in doses more than 45 mg/day is guaranteed.</td>
<td>[63]</td>
<td></td>
</tr>
<tr>
<td>ABH gel</td>
<td>Healthy volunteers, n=10</td>
<td>In vivo study using ABH PLO gel formulation which is composed of lorazepam (Ativan®), diphenhydramine (Benadryl®), and haloperidol (Haldol®) incorporated in PLO applied to healthy volunteer’s wrists. Sufficient absorption of drugs was not observed from the PLO gel. All drugs concentration measurements were below sub-therapeutics values.</td>
<td>[64]</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Human subjects, n=11</td>
<td>A 25 mg chlorpromazine HCl in PLO gel administered to 10 subjects’ wrists and 100 mg chlorpromazine administered to one subject’s wrist with the goal to evaluate the absorption of the drug transdermally. All samples were under limit of detection which indicated that chlorpromazine would not permeate through skin membrane.</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Dialysis membrane and</td>
<td>Flurbiprofen PLO gel was formulated and in vivo study. The permeability profile with dialysis</td>
<td>[1]</td>
<td></td>
</tr>
</tbody>
</table>
**1.8. Controversies surrounding PLO gels**

PLO gels have exhibited great efficacy in transdermal delivery by enhancing the permeability of certain drugs especially in hospice patients and patients undergoing palliative care [27]. PLO gels are helpful in patients with swallowing difficulties and in patients suffering from acute and delayed chemotherapy-induced nausea and vomiting. Transdermal drug delivery using PLO gels has many advantages including ease of application and dose adjustment, simplicity, stability, and use of inexpensive generic drugs. Many drugs such as chlorpromazine, lorazepam, morphine, and haloperidol and combination drugs such as ABHR (lorazepam, diphenhydramine, and haloperidol, metoclopramide) have been formulated in PLO gel base for transdermal delivery [1, 49, 50]. In some cases, PLO gel formulations are applied with a goal of cutaneous absorption to achieve systemic activity [28]. To some extent, PLO gels are capable of enhancing the drug permeability through the stratum corneum and delivering the drug into systemic circulation via
dermal blood flow. However, certain controversies exists with the use of some compounded PLO gel drug preparations. While some reports have suggested the beneficial effects of drugs delivered in PLO gels, others questioned their efficacy.

Marketed transdermal products undergo thorough scrutiny by the Food and Drug Administration (FDA) for demonstration of efficacy. But, PLO gels are prepared by compounding pharmacies and these products do not require FDA approval [49]. Most compounding pharmacies advertise their willingness to provide formulations without proper clinical data and evidence based studies regarding the efficacy of PLO gels. Recently, the effectiveness and absorption of drugs into the bloodstream from PLO gels has been investigated by many groups on a case-by-case basis [1,49,50]. In this section, we intend to highlight some observational studies that demonstrate the effectiveness of PLO gels along with some clinical studies that negate the drug absorption from extemporaneously compounded PLO gels administered topically to patients.

1.8.1. Promethazine

Promethazine HCl is a phenothiazine derivative, widely used for treating nausea and vomiting in both adults and children. Most antiemetics are delivered via oral, injectable, and rectal routes; however, transdermal route could be a better alternative in hospice patients in the end stages of life [66]. In one study, researchers performed an in vivo study on 15 healthy humans at a tertiary care facility to determine the bioavailability of promethazine in a topical PLO gel. Fifty milligram of promethazine in PLO gel was applied once onto the skin of the nondominant
wrist and the application area was covered with an adhesive bandage. The results were compared with a single dose of intravenous promethazine (25 mg). Topical promethazine resulted in lower systemic concentrations when compared to the injectable administration, with an absolute bioavailability of 2%. Mean area under the curve after topical and intravenous administrations were found to be 16.63 ng.mL/hour and 407.15 ng.mL/hour, respectively. All patients who received the intravenous dose experienced sedation, while only 50% of the subjects who received topical promethazine experienced sedation. Despite low serum concentrations from topical promethazine PLO gel, this study demonstrated systemic concentrations after topical application [62]. Further clinical studies should be conducted to identify the antiemetic efficacy of promethazine PLO gel in a larger patient population.

### 1.8.2. Morphine

Morphine is an opioid analgesic drug and it is widely used in patients for treating cancer-related pain. Elderly patients with swallowing difficulties or intestinal obstruction require transdermal delivery of morphine. Topical administration of 0.1% morphine hydrochloride loaded into a PLO gel has been reported to provide rapid pain relief in patients receiving palliative care [67, 68]. A few studies have been conducted to evaluate both local and systemic effects of morphine. Wilken et al. [69] studied the analgesic response of topical morphine to control arthritis chronic pain and also detected the presence of morphine in the urine. Patients were instructed to apply the morphine gel to either the upper arm or subclavicular area twice daily until a pain goal of 1-2 out of 10 was achieved. After attaining the required pain control a
24-hour urine collection was obtained and morphine levels were analyzed. This study concluded that topical morphine provides good analgesic response in chronic arthritic patients, which was further supported by urinary morphine concentrations ranging from 31 to 191 ng/mL. Based on these literature findings, the PLO gel formulation of morphine applied to the wrist has been considered as a suitable alternate for systemic delivery. However, studies published in subsequent years contradicted the above findings. In 2008, Paice et al. [49] studied the bioavailability of topically administered morphine in five healthy volunteers. One milliliter of 10 mg/ml morphine PLO gel was applied to the wrist and 17 blood samples were collected from 5 minutes to 10 hours. The authors were unable to detect morphine in the blood samples collected and morphine at all timepoints was below the lower limit of quantification namely, 0.5 ng/ml. A morphine concentration of 10 ng/mL is needed for effective analgesic action [70]. The authors concluded that morphine PLO gel is unlikely to provide systemic concentrations and relief from cancer-related pain. A different study conducted on human cadaver skin reported similar findings and concluded that morphine is a poor candidate for transdermal drug delivery [71]. Even studies using animal models could not detect drug concentrations in plasma [72]. The complete lack of bioavailability of topically administered morphine has been attributed to the intact epidermis and the inability of the PLO gels to enhance the permeation of morphine to the required extent [72-74]. This contradictory finding warrants that further investigation be conducted with larger patient populations.

1.8.3. Methadone

Methadone is an opioid medication popularly used as an analgesic in hospice patients with
dementia for pain management. A transdermal formulation of methadone in PLO gel became available through compounding pharmacies based on anecdotal reports from the nursing staff. An *in vitro* study evaluated the absorption of methadone through mouse and human skin for its transdermal drug delivery. Methadone absorption was found to be higher in the hairless skin of a mouse than human cadaver skin [75]. This study demonstrated the feasibility of transdermal methadone administration. Despite the rampant use of transdermal methadone PLO gel in hospice patients, no clinical studies were conducted to assess the systemic absorption or clinical efficacy of topical methadone for systemic pain until recently. In 2011, Sylvester et al. investigated the serum concentrations of methadone following topical administration (10–45 mg/day in PLO gel) in 10 hospice patients and oral administration (15–40 mg/day) in 5 patients. This study aimed to compare methadone serum concentrations after oral and topical application. It was reported that 18 of 20 serum methadone concentrations after topical administration were below 10 ng/mL, while 25.8 ng/mL was observed in 1 subject who received the highest topical dose (45 mg/day). These blood concentrations are considerably less than the oral methadone administration ranging 62 to 393 ng/mL[63]. This study concluded that methadone applied topically as PLO gel in doses <45 mg/day failed to produce the required concentrations in plasma and further clinical studies are needed for doses >45 mg/day.

### 1.8.4. ABH gels

A hospice patient’s quality of life is greatly diminished by the disease state and medication related side effects. The physical condition of patients and medication related side effects such as nausea and vomiting demand the creation and use of alternative products in such patients. A
PLO gels containing a combination of antiemetic drugs such as lorazepam (A),
diphenhydramine (B), and haloperidol (H), also known as ABH gel, has been widely used for
treating nausea and vomiting. It is not readily available on the market and must be formulated
by a compounding pharmacist. A usual dose would be 1 g or (1 ml) applied to the skin of the
inner wrists every four to six hours. The letter “A” is for lorazepam(Ativan®). The antiemetic
activity of benzodiazepines is not clear, yet it represses the limbic system and decreases
cortical central nervous system input into the vomiting center of the central nervous system.
The letter “B” is for diphenhydramine (Benadryl®). It functions as an antihistamine and
anticholinergic agent, and diminishes the activity of the vestibular system. Also, it can diminish
extrapyramidal antagonistic impacts from dopamine antagonists. The letter “H” represents for
haloperidol(Haldol®), a strong dopamine antagonist. ABH gel is actually well tolerated by a
high percentage of patients. If ABH gel is powerful at alleviating nausea, it could be an
essential and moderately less costly treatment. One observational study, conducted two trials
in adult patients suffering from chemotherapy-induced nausea and vomiting. In Trial I, topical
administration of ABH gel decreased nausea and vomiting in 17 out of 23 patients with
chemotherapy induced nausea and vomiting. Trial II was carried out in 10 patients and the
treatment was found to be effective in all patients [1]. In a different study, 10 healthy
volunteers between the age of 25 - 58 years were recieved the standard 1.0 ml dose of ABH gel
on the volar surface of their wrists. In another study, authors tested the cutaneous absorption of
ABH gel in healthy adults. In this study, none of the lorazepam (A) or haloperidol (H) was
absorbed into the blood stream and diphenhydramine (B) had insufficient quantities to be
effective for nausea and vomiting. No lorazepam or haloperidol was detected in blood samples
collected after 0, 30, 60, 90, 120, 180, and 240 minutes, while diphenhydramine was detected in only 5 of 10 patients [2]. Fletcher et al., conducted a randomized, double-blind, placebo-controlled, crossover, noninferiority clinical trial to study the effectiveness of ABH gels compared it with a placebo in cancer patients with nausea. This study concluded that ABH gel did not decrease vomiting events better than the placebo (P = 0.34) and ABH gels should not be used in cancer patients experiencing nausea in its current form [76]. Similar results have been published with ABHR gels. The letter “R” is (Reglan®) for metoclopramide. This agent works both as a dopamine antagonist and to enhance slowed GIT transit time. The side effects anticipated from this mixture are generally drowsiness, lethargy, confusion and muscle jerks. The above discussion clearly indicates that investigation studies looking at the absorption and plasma concentrations of medications after topical administration do not support the results from observational studies.

1.8.5 Chlorpromazine

Chlorpromazine is a dopamine antagonist with additional anti-adrenergic, anti-serotonergic, anti-cholinergic and anti-histaminergic properties. It is widely used in the treatment of nausea and vomiting. The commercially available oral liquid of chlorpromazine (Thorazine®) was discontinued for safety reasons. Since then there has been an increased use of chlorpromazine PLO gel for managing symptoms such as nausea, vomiting, agitation and delirium. However, there is no supporting data for the percutaneous absorption of chlorpromazine following topical application. The physicochemical and pharmacokinetic properties of chlorpromazine have encouraged compounding pharmacists to formulate the drug
in PLO gels for hospice patients. However, very few studies have evaluated the transdermal absorption of chlorpromazine in humans. In an in vitro study, Figueroa, A., et al. studied the permeability of chlorpromazine across pig skin with the absorption following passive diffusion and iontophoresis. Iontophoresis procedures involve using gentle electric current to improve skin penetration of drugs. This work concluded that iontophoresis is a useful tool in improving the transdermal delivery of chlorpromazine for treating chronic psychosis [77]. Weiland et al. studied the transdermal absorption of chlorpromazine PLO gel in 11 healthy adults between 18 and 70 years of age. A dose of 25 mg of chlorpromazine hydrochloride in PLO gel was applied to wrists and 100 mg was applied to 1 subject's wrist. This study concluded that chlorpromazine was below the detection limit (10 ng/ml) in all 11 subjects' blood samples at 1, 2, and 4 hours after topical application [50].

1.9. Need for the transdermal delivery of antiemetics in hospice patients

Hospice provides care for chronically ill and terminally ill patients, attending to their medicinal, emotional and spiritual needs. A hospice team usually includes: the patient, patient’s family members, patient’s physician, home health aides, hospice physician, caregivers, nurses, pharmacists, counselors or social workers, and some volunteers. A hospice team’s primary goal is to transform the death experience in patients and to offer “death with dignity and freedom from pain”. The concept of hospice does not act only as palliative care for the patients, but provides emotional, social, and spiritual support for the patients and their families. These types of support will positively impact patient’s life and provide solace to their families before and after death. Hospice caregivers are willing to improve the quality of life for the whole
The role of a compounding pharmacist as a member of the hospice team is very important because they provide medical support in formulating medications into alternative dosage forms depending on the patient’s needs. When traditional therapy fails to meet the needs of an individual patient, a compounding pharmacist prepares alternate dosage forms such as topical gels, suppositories, oral suspensions, sublingual concentrates and troches [81]. Some diseases states which may require alternate medications include pain management, anxiety, nausea and vomiting, and wound care [82-84]. For example, nausea is a common unpleasant effect that induces a vomiting sensation and requires an alternative route of administration for anti-emetics, especially in hospice patients [85]. Hospice patients might experience nausea and vomiting due to several reasons including bowel constriction, stimulation of the cranial chemotrigger zone, gastroparesis, anxiety, and cholinergic effects from drugs [81]. Such patients require pharmacist intervention for preparing alternative dosage forms for anti-emetic drugs in the form of a suppository or transdermal gel [86-88]. Anti-emetics are generally compounded in PLO gels for transdermal administration.
Chapter 2

Aims and Objectives of the Thesis Research

2.1. Introduction

Transdermal delivery represents an attractive alternative to oral and parenteral delivery of drugs due to its accessibility and extensiveness [3-5]. The general difficulties that exist in administering drugs to nauseous and unconscious patients via the oral routes led researchers to look for alternative methods such as the topical route. In transdermal delivery, active ingredients are delivered across the skin for local and/or systemic effects. The skin is the largest organ in the human body and it mainly consists of three layers: the epidermis, dermis, and hypodermis. The skin protects the human body against penetration of foreign molecules and evaporation of water. The protective effects of the skin are mainly due to the impermeable, multi-layered epidermis [6, 7]. The epidermis is further divided into four distinct layers, namely, stratum corneum (outer most), stratum granulosum, stratum spinosum, and stratum germinativum (inner most) [6, 8]. The stratum corneum is composed of flattened cornified cells embedded in a lipid intercellular matrix. The barrier properties of the stratum corneum prevents absorption of approximately 90% of the transdermal drugs [6, 7]. Nearly 30 years ago, nicotine patches for smoking cessation revolutionized the field of transdermal drug delivery. Later on several other products were approved including, nitroglycerin for angina, clonidine for
hypertension, scopolamine for motion sickness, and estradiol for estrogen deficiency. Around the world, the transdermal business market is approximately $31.5 billion and the annual market in the US is more than $3 billion [89, 90].

On the other hand, a significant challenge for transdermal delivery is to expand the variety of drugs that can be delivered by this route. A very limited number of drugs are delivered by this route because of the low penetration of drugs through the human skin (especially the major barrier stratum corneum layer), which restrains daily drug dosage to around 10 mg with an acceptable size patch. Besides, transdermal drug delivery is not perfect for all substances, most of the drugs transdermally applied have to be small in molecular weight, highly lipophilic and should require small doses [3, 5, 91, 92]. The properties that make drug molecules ideal for drug delivery are listed below in Table 2.1.

**Table 2.1: Ideal properties of drug candidate for transdermal drug delivery. Modified from [93].**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Should be low (&lt; 20mg/day)</td>
</tr>
<tr>
<td>Half-life</td>
<td>10 hours or less</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>&lt; 400 Da</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>Log P (octanol-water coefficient) between 1.0 and 4</td>
</tr>
<tr>
<td>Skin permeability coefficient</td>
<td>&gt; 0.5 X 10^{-3} cm/h</td>
</tr>
<tr>
<td>Skin reaction</td>
<td>Non irritating and non-sensitizing</td>
</tr>
<tr>
<td>Oral bioavailability</td>
<td>Low</td>
</tr>
<tr>
<td>Therapeutic index</td>
<td>Low</td>
</tr>
</tbody>
</table>
Regardless of the plenteous points of interest for transdermal delivery systems, there are a few deterrents to its boundless application.

Chlorpromazine is widely used as an antiemetic in hospice patients for relieving symptoms such as vomiting and nausea at the end stages of life [50]. The oral dose of chlorpromazine for nausea and vomiting varies between 10 to 25 mg administered every 4 to 6 hours. Chlorpromazine is readily absorbed from the GI tract and its bioavailability varies between 10-80% due to first-pass metabolism by the liver. Other characteristics of chlorpromazine such as molecular weight (355.33 Da) and logP value (3.16 at pH 7.4 and 5.3 for the uncharged molecule) encouraged compounding pharmacists to look for an alternative administration approach such as transdermal drug delivery [94]. However, studies evaluating the transdermal absorption of chlorpromazine through animal skin in vitro or in humans in vivo are limited. Moreover, inconsistent results have been reported in the literature with respect to the systemic absorption of chlorpromazine after topical application in the form of PLO gels [50, 95]. There is a clear need for further research to determine the percutaneous absorption of chlorpromazine from PLO gels and its suitability in topical application.

2.2. Objectives of the study

The present research aims to examine the penetration and permeation profile of chlorpromazine hydrochloride in PLO gels across porcine ear skin and human abdominal skin. PLO gels were prepared using isopropyl palmitate or ricinoleic acid as oils, lecithin as a penetration enhancer, and poloxamer 407 as a stabilizer and hydrophilic base component. The objectives of the present study were to:
(a) develop and characterize PLO gel formulations of chlorpromazine hydrochloride with ricinoleic acid or isopropyl palmitate as a component in the oil phase.

(b) develop and validate a HPLC (high performance liquid chromatography) method for chlorpromazine hydrochloride.

(c) study the in vitro permeation of chlorpromazine hydrochloride from isopropyl palmitate PLO gel across porcine and human skin using Franz diffusion cells and compare the data with ricinoleic acid PLO gel.

(d) assess the theoretical plasma concentration of chlorpromazine from flux values.
Chapter 3

Chemicals Used

3.1. Chlorpromazine hydrochloride

![Structure of chlorpromazine hydrochloride]

Figure 3.1: Structure of chlorpromazine hydrochloride

Chlorpromazine is a Phenothiazine (Dopamine agonist) and it is used as antiemetics and antipsychotic agents. The oral dose of chlorpromazine for nausea and vomiting varies between 10 to 25 mg administered every 4 to 6 hours. Chlorpromazine is readily absorbed from the GI tract and its bioavailability varies between 10-80% due to first-pass metabolism by the liver. Other characteristics of chlorpromazine such as molecular weight (355.33 Da) and logP value (3.16 at pH 7.4 and 5.3 for the uncharged molecule) [94].
3.2. Ricinoleic acid

Ricinoleic acid is an unsaturated omega-9 fatty acid (Fig. 3.2) that naturally occurs in a mature Castor plant. Ricinoleic acid is known for its analgesic and anti-inflammatory properties following acute or repetitive local application [75].

![Figure 3.2: Structure of ricinoleic acid](image)

3.3. Isopropyl Palmitate

Isopropyl palmitate is the ester of isopropyl alcohol and palmitic acid (Fig. 3.3).

It is used as an emollient, moisturizer, thickening agent, and anti-static agent [96].

![Figure 3.3: Structure of isopropyl palmitate](image)
3.4. Lecithin

Lecithin is a yellow-brownish fatty substance extracted from animal and plant tissues. It consists of phosphoric acid, choline, fatty acids, glycerol, glycolipids, triglycerides, and phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol (Fig. 3.4). It used in the pharmaceutical products manufacturing as a wetting, stabilizing agent and a choline enrichment carrier, which helps in emulsification and encapsulation, and it is a good dispersing agent. Lecithin is used in manufacturing intravenous fat infusions and as a food additive [97].

![Figure 3.4: Structure of lecithin](image)
Chapter 4

Evaluation of the Percutaneous Absorption of Chlorpromazine Hydrochloride from PLO Gels Across Porcine Ear and Human Abdominal Skin

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ABSTRACT

Objectives: The overall objective of this work is to determine the percutaneous absorption of chlorpromazine hydrochloride from PLO gels and verify the suitability of topically applied chlorpromazine PLO gels for use in hospice patients in relieving symptoms such as vomiting and nausea during the end stages of life. The aims of the present study were to (a) prepare and characterize pluronic lecithin organogels (PLO gels) of chlorpromazine hydrochloride using isopropyl palmitate or ricinoleic acid as oil phase, (b) assess the in vitro percutaneous absorption of chlorpromazine hydrochloride through porcine ear and human abdominal skin from isopropyl palmitate and ricinoleic acid PLO gels, and (c) assess the theoretical plasma concentrations of chlorpromazine from flux values.

Methods: PLO gels of chlorpromazine hydrochloride were successfully prepared using isopropyl palmitate or ricinoleic acid as the oil phase and characterized for pH, morphology, stability, viscosity, thermal analysis using differential scanning calorimetry (DSC), in vitro drug release and stability. In vitro permeability studies were performed across pig ear and human abdominal skin using isopropyl palmitate PLO gel and compared with the ricinoleic acid PLO gel.

Results: The pH and viscosity of both PLO gels prepared with isopropyl palmitate and ricinoleic acid were comparable. The thixotropic property of ricinoleic acid PLO gel was found to be better than the isopropyl palmitate PLO gel. Both formulations were found to be stable at 25°C, 35°C, and 40°C for up to 60 days. The permeation of chlorpromazine hydrochloride was higher from
ricinoleic acid PLO gel than isopropyl palmitate PLO gel and pure drug solution. Theoretical steady state plasma concentrations ($C_{ss}$) of chlorpromazine from pure drug solution, isopropyl palmitate PLO gel and ricinoleic acid PLO gel were found to be 1.05, 1.20, and 1.50 ng/ml, respectively. PLO gels only marginally increased the flux and theoretical $C_{ss}$ of chlorpromazine. However, theoretical $C_{ss}$ values of chlorpromazine were much below the required therapeutic concentration for antiemetic activity in hospice patients.

**Conclusion:** From this study it is clearly evident that PLO gels fail to deliver the required systemic levels of chlorpromazine following topical application. To achieve better chlorpromazine hydrochloride skin permeation and thus higher concentration of chlorpromazine in plasma, following topical application on the skin, efficient permeation enhancers should be used.
INTRODUCTION

Chlorpromazine is a phenothiazine antipsychotic drug with anti-adrenergic, anti-cholinergic, anti-histaminergic, and anti-serotonergic properties [98-100]. Upon oral administration, chlorpromazine is known to undergo degradation in the gastrointestinal (GI) lumen and first pass metabolism by the liver resulting in variable bioavailability [77]. Despite these effects, chlorpromazine is mostly administered orally in the form as tablets or solutions. Chlorpromazine is also widely used as an antiemetic in hospice patients for relieving symptoms such as vomiting and nausea during the end stages of life [50]. A topical gel formulation of chlorpromazine is used in relieving the symptoms of nausea and vomiting for terminally ill hospice patients with swallowing difficulties.

The transdermal route of administration avoids metabolism by the GI lumen and hepatic first-pass effect and could be used as an attractive, noninvasive administration route in hospice patients [92, 101]. However, due to the lack of commercial formulations, pharmacists serving on hospice interdisciplinary teams have recommended the use of transdermal pluronic lecithin organogels (PLO gels) of chlorpromazine for the convenience of patients and caregivers in drug administration [50]. Apart from chlorpromazine, combination products containing lorazepam, metoclopramide, morphine, haloperidol, and methadone, commonly referred to as ABHR compounded gel, is also used in treating nausea and vomiting [101]. PLO gel has been widely used in compounding pharmacies as a vehicle for enhancing transdermal permeability of many therapeutic medications since it modifies the skin barrier function to enhance drug permeation [25]. PLO gels are known to improve the absorption of both lipophilic and hydrophilic
medications by enhancing the skin permeation [25]. Transdermal drug delivery is meant for both local dermal delivery and systemic delivery [92]. For transdermal delivery, drug candidates should be potent with a suitable molecular weight of around 200–500 Da and a logP value between 1-3[102]. Studies using the systemic delivery of drugs with PLO gels have shown inconsistent results for drugs such as chlorpromazine [50], lorazepam, diphenhydramine, haloperidol [64], and morphine [49]. While some studies supported the drug absorption from extemporaneously compounded PLO gels others refuted [103, 104].

For example, topical morphine hydrochloride gel administration was reported to provide rapid relief of localized pain in six patients receiving palliative care [67] and diffuse chronic arthritic pain in a series of three patients [69]. However, in a recent randomized, placebo-controlled, double-blind, crossover study by Paice et al.[49], no quantifiable morphine concentrations were detected in the plasma of healthy adults following topical application of 10 mg/mL morphine PLO gel. Bleicher et al, [48] investigated the efficacy of topical ABH gel containing lorazepam, diphenhydramine, and haloperidol in reducing chemotherapy-induced nausea and vomiting (CINV) in two pilot trials. In Trial 1 consisting of 23 patients, 74% of the patients reported that use of the gel decreased their CINV within 30 minutes of its application. In trial 2, all 10 patients believed that the topical ABH gel treatment was effective. The authors concluded that topical ABH gel appears to have promise in treating CINV. Similar effects of topical ABH gel were observed in previously published reports in hospice patients [101]. However, one recent study by Smith et al., reported contrary findings: none of the drugs used in ABH gel exhibited sufficient absorption to be effective in the treatment of nausea and vomiting. Moreover, absorption of diphenhydramine was found to be erratic with subtherapeutic
concentrations [64].

Similar findings have been reported for chlorpromazine PLO gel. Chlorpromazine PLO gels are designed with the intent to achieve relevant systemic concentrations following topical application. Studies, to date, with regard to transdermal absorption of chlorpromazine alone and PLO gel formulations have been limited. Chlorpromazine, as a free base, has limited water solubility and its pKa value is 9.20. Despite having an optimal log P value (3.16 at pH 7.4 and 5.3 for the uncharged molecule), the pKa value and molecular weight (355.33 Da) of chlorpromazine deters its permeation across the skin [94, 95].

A study conducted by Luppi et al., [95] concluded that chlorpromazine can be transdermally administered by using hydroxypropylmethylcellulose films and drug permeation through the skin could be modulated by the addition of permeation enhancers, in particular an oleic acid – polysorbate 80 mixture. Alternate methods such as electrophoresis have also been used to increase the permeation of chlorpromazine. Alvarez-Figueroa et al., [77] studied the in vitro iontophoretic transdermal delivery of chlorpromazine across pig skin. While the authors observed the penetration of chlorpromazine through passive diffusion, iontophoresis significantly enhanced the penetration of chlorpromazine. Weiland et al., [50] studied the bioavailability of chlorpromazine from a topical gel formulation in healthy adults. Chlorpromazine in PLO gel was applied at a dose of 25 mg to the wrists of 10 subjects and 100 mg to the wrist of one subject. This study demonstrated that chlorpromazine was undetectable in blood at 1, 2, and 4 h after topical application. While some studies supported the transdermal use of chlorpromazine from PLO gels [105] and hydroxypropylmethylcellulose films, other
researchers did not find systemic blood concentrations at therapeutic levels even for doses of 25 mg and 100 mg [50]. Therefore, there is a clear need for further research to determine the percutaneous absorption of chlorpromazine from PLO gels and its suitability for topical application.

Very few studies looking at the *in vitro* transdermal permeation of chlorpromazine hydrochloride from PLO gels exist in the literature. The paucity of data incited us to examine the percutaneous absorption of chlorpromazine from PLO gels across porcine ear and human abdominal skin. The objectives of the present study were (a) to prepare and characterize PLO gel formulations of chlorpromazine hydrochloride using isopropyl palmitate or ricinoleic acid as oil phase, (b) to assess the *in vitro* percutaneous absorption of chlorpromazine hydrochloride through porcine ear and human abdominal skin from isopropyl palmitate and ricinoleic acid PLO gels, and (c) to assess the theoretical plasma concentration of chlorpromazine from flux values.

**MATERIALS AND METHODS**

*Materials*

Ricinoleic acid (Lot 2DYB0) was obtained from TCI America (Portland, Oregon). Lecithin Soya fine powder (Lot C147506) was acquired from Fisher Scientific (Pittsburgh, Pennsylvania). Chlorpromazine hydrochloride USP (Lot 11120552) was received from LETCO Medical (Decatur, Alabama). Poloxamer 407 NF (Lot C153723) was obtained from PCCA (Houston, TX). Distilled deionized (DI) water was used for the preparation of PLO gels. High Performance Liquid Chromatography (HPLC) grade solvents such as acetonitrile (Lot SHBF1021V), methanol (Lot 122044), and trimethylamine (Lot 133825) were provided by Fisher Scientific.
(Pittsburgh, Pennsylvania).

**Preparation of drug loaded PLO gels**

The polymer solution was prepared by dissolving poloxamer in DI water to obtain a 20% (w/v) solution. To enhance the dissolution of the polymer, the poloxamer solution was kept under refrigerated conditions (4°C) overnight. The oil phases were prepared by combining lecithin/isopropyl palmitate and lecithin/ricinoleic acid in a 50:50 (w/w) mixture. The mixtures were left overnight to allow for the complete dissolution of the lecithin in isopropyl palmitate or ricinoleic acid. Chlorpromazine hydrochloride (10% w/v) was solubilized in the 20% ploxamer solution. The PLO gel was prepared by mixing 1 part of oil phase with 4 parts of aqueous phase (20% w/v poloxamer) using a vortex mixer (VORTEX–T, Genie® 2). Butylated hydroxytoluene (0.1% w/w BHT) was used as an antioxidant. Chlorpromazine hydrochloride loaded PLO gels were evaluated for pH, morphology, stability, viscosity, thermal analysis using differential scanning calorimetry (DSC), Fourier Transform Infrared (FT-IR) spectroscopy, and *in vitro* drug release and *in vitro* permeability.

**Determination of pH**

For the determination of pH, 1 g of drug loaded PLO gel was dispersed in 25 ml of distilled deionized water and the pH was determined using a Metler Toledo pH meter (Mettler-Toledo Ingold Inc., Billerica, MA USA). The pH meter was calibrated with standard buffer solutions of pH 4, 7, 10 before each use. This study was performed in triplicate and average pH values were reported.
**Determination of viscosity**

A Brookfield RVDV-I Prime digital viscometer (Brookfield Engineering Laboratories, Middleboro, MA) was used with SC4-29 Spindle to find out viscosities of PLO gel. All tests were performed at room temperature, 25°C. The viscosities for the chlorpromazine hydrochloride PLO gels prepared with isopropyl palmitate and ricinoleic acid were evaluated at varying speeds of 5, 10, 20, and 50 RPM.

**Morphology**

STEM (HITACHI HD-2300 A, Ultra-thin Film Evaluation System, Hitachi High Technologies America, Pleasanton, CA) was used in evaluating the morphology of PLO gels. Samples were prepared by placing a small drop of both isopropyl palmitate and ricinoleic acid PLO gel formulations on a copper grid. The samples were then stained using 2% phosphotungstic acid solution (Fisher Scientific, Pittsburgh, PA). The copper grids were kept undisturbed overnight for drying. Finally, the samples were viewed using STEM.

**Differential scanning calorimetry (DSC)**

DSC analysis was carried out for pure drug and drug loaded PLO gels of isopropyl palmitate and ricinoleic acid to examine the change in the rate of heat absorbed by chlorpromazine hydrochloride after dissolving in PLO gels. All samples (5-10 mg) were sealed and placed in aluminum crucibles using the Mettler MT 5 microbalance. DSC studies were done at a 10°C/min heating rate over a wide range (10 - 300°C) using a DSC 822° Mettler Toledo instrument (Mettler Toledo GmbH, Schwerzenbach, CH) fitted with a TSO801RO sample robot and a TSO800GC1 Gas control attached to a Nitrogen gas cylinder. Star e software V8.10 was used to take the scans.
Nitrogen gas was purged at a rate of 10 ml/min.

*Fourier Transform Infrared (FT-IR) spectroscopy*

FT-IR spectra of the pure drug, drug loaded PLO gels of isopropyl palmitate and ricinoleic acid, and blank PLO gels isopropyl palmitate and ricinoleic acid were taken using a FTS 4000 FTIR spectrometer (Varian Excalibur Series UMA 600 FTIR, Digilab, USA) equipped with a germanium crystal. A resolution of 16 cm$^{-1}$ was used and 16 scans were co-added for each spectrum in the range of 500 to 4,000 cm$^{-1}$.

*HPLC chromatographic conditions*

A high-performance liquid chromatography (HPLC) method was developed and validated for drug content determination. Samples were analyzed using a Waters Alliance e2695 separation module (Milford, MA) equipped with a 2998 PDA detector. A Waters symmetry® C18 column 5 µm (4.6 x 250 mm) was used with a mobile phase composed of a ternary composition (A:B:C) (42:27:31) pumped at a flow rate of 1 ml/min. The mobile phase (A) was prepared by dissolving 3.42g sodium acetate trihydrate and 2 ml trimethylamine in 970 ml of purified water, then the pH adjusted to 4.5 with glacial acetic acid. The mobile phases (B) and (C) were methanol and acetonitrile, respectively. The retention time for chlorpromazine ($\lambda_{\text{max}} = 254$ nm) was found to be 9.786 minutes. Chlorpromazine hydrochloride stock solution (1 mg/ml) was used in preparing calibration curve standards. Each standard concentration was analyzed in triplicate. A calibration curve was made by plotting the average area against the amount of drug (ng) to determine the drug content. A straight line ($y = 5140.1x - 18319$) was obtained with a correlation coefficient ($r^2$) value of 0.9998 and the standard curve region was from 0.390 µg/ml to 50 µg/ml (Fig. 4.1).
The HPLC method was validated as per regulatory requirements. The percentage recovery of chlorpromazine ranged from 97.25% to 100.33%. The intra- and inter-assay precisions of chlorpromazine were satisfactory; the RSD was less than 2%. The intra-day precision (measured by %RSD) was found to be in the range of 0.08% to 0.53%. The limit of detection of chlorpromazine was found to be 91.1ng/ml.

![Calibration curve of chlorpromazine](image)

**Figure 4-1**: Calibration curve of chlorpromazine

*Stability indication assay*

Chlorpromazine was exposed to different stress conditions such as thermal, acid, alkaline, oxidation, and photolytic in order to generate degradation byproducts of the drug. These conditions included heat (70°C), acid hydrolysis (0.1N hydrochloric acid), base hydrolysis...
(0.1N sodium hydroxide solution), oxidation (2% hydrogen peroxide solution), and photolysis with UV light. Samples were later analyzed to ensure that there was no interference between the degradation products and pure drug peak.

**Stability study**

Samples of chlorpromazine hydrochloride loaded PLO gel formulations were stored in glass vials at 25ºC, 35ºC, and 45ºC. At regular time intervals of 0, 7, 14, 21, 28, and 35 days, PLO gels were analyzed for drug content. An appropriate amount of drug loaded PLO gel was dissolved in 1 ml of methanol and further diluted for analysis. The stability study was done in triplicate.

**In vitro drug release study**

*In vitro* release of chlorpromazine hydrochloride loaded in PLO gels of isopropyl palmitate and ricinoleic acid was performed in Franz- diffusion cells using a cellulose membrane (Spectra pore, MWCO 1000Da) and Smart M-membrane. The cellophane membrane was clamped between the donor and the receptor chamber of the diffusion cell. The receptor chamber was filled with freshly prepared PBS (pH 7.4) solution. The receptor chamber was stirred with a magnetic stirrer at 300 rpm. The samples (300 µl aliquots) were collected at predetermined time intervals and replaced with an equal volume of fresh buffer. Samples were then analyzed for drug content using HPLC at 254 nm and the cumulative amount of drug released across both membranes was determined as a function of time.
In vitro permeability of chlorpromazine hydrochloride across porcine ear and human abdominal skin

Porcine ears of 2 month old pigs were obtained from a nearby slaughterhouse (Kastel's Slaughter House & Processing Center, Riga, MI). Human abdominal skin of a 36 years old caucasian woman was obtained from a local plastic surgeon at the Regency Park Surgery Center (Toledo, OH). The hair on the skin samples was removed gently with a razor. After hair removal, subcutaneous fat was carefully removed with a scalpel and the thickness of the skin was measured with a digital electronic micrometer, Mitutoyo Corporation, Japan. During the transport and cleaning procedures the tissues were placed in an ice cold PBS buffer (pH 7.4) solution. Permeability studies were initiated approximately 3-4 h after harvesting. Around 2 cm² skin specimens were cut and mounted between the receptor and donor chambers of Franz-diffusion cells (PermeGear Inc., Hellertown, PA) to evaluate the permeability of chlorpromazine hydrochloride from pure drug solution and PLO gels. The cells were placed on a magnetic stirrer and the water jacket temperature was maintained at 34.2±1°C with a circulating water bath. The receptor chamber was filled with PBS. The tissues were equilibrated for one hour before initiating the study. The outer surface of the skin was placed towards the donor chamber (chamber for placing the drug). The permeability study was initiated by placing 25 mg/ml (0.07M) of pure drug solution or equivalent drug loaded PLO gels of isopropyl palmitate and ricinoleic acid. An aliquot (300µl) was withdrawn at regular time points for up to 24 hours, and replaced with an equal amount of fresh PBS. Permeability studies were performed in triplicate. After 24 hours, the Franz diffusion cells were dismantled and the remaining drug was carefully removed from the donor chambers. The mass balance was evaluated by analyzing the drug content in the remaining formulation, washing samples,
receptor chamber solution, and dermal and epidermal layers. Drug concentrations were analyzed using an HPLC attached to a PDA detector. The mass balance recovery should be in the range of 100±20, any cell outside this range was excluded. The apparent permeability ($P_{\text{app}}$) of chlorpromazine hydrochloride from PLO gel formulations was calculated using Eq. 1:

$$\text{Permeability} \ (P_{\text{app}}) = \frac{\text{Flux}}{C_d} \quad \text{Eq. 1}$$

Flux ($J$) is calculated by dividing the slope obtained by plotting the cumulative amount of chlorpromazine hydrochloride penetrated ($M$) through the skin vs time ($t$) with the cross-sectional area of the membrane ($A$) exposed to the drug. $C_d$ is the initial drug concentration in the donor chamber [46].

Statistical analysis

All the experiments were carried out in triplicate. Values are expressed as mean ± standard deviation (SD). The statistical analysis of the data was carried out using a Student's $t$-test, and $p$-values<0.05 were considered statistically significant.

RESULTS AND DISCUSSION

PLO gels are widely used in compounding pharmacies to customize medications in order to deliver antiemetic agents such as chlorpromazine to hospice patients for relieving symptoms such as vomiting and nausea during the end stages of life. Despite widespread use of chlorpromazine PLO gels, a clear understanding of whether or not chlorpromazine enters the systemic circulation in therapeutic concentrations is lacking. In this study, we made an attempt
to understand the percutaneous absorption of chlorpromazine hydrochloride from PLO gels across porcine ear and human abdominal skin. PLO gels of chlorpromazine hydrochloride were prepared using isopropyl palmitate or ricinoleic acid as oil phase. Isopropyl palmitate acts as an emollient, moisturizer and penetration enhancer [106]. Our group has recently suggested the use of ricinoleic acid as an alternative to isopropyl palmitate, especially for delivering nonsteroidal anti-inflammatory drugs such as ketoprofen [34, 35]. Better thixotropic properties were observed for PLO gels prepared using ricinoleic acid compared to traditional PLO gels prepared using isopropyl palmitate [107]. Therefore, in this study we compared the percutaneous absorption of chlorpromazine hydrochloride from isopropyl palmitate and ricinoleic acid PLO gels. Chlorpromazine hydrochloride PLO gels (10 %) were formulated successfully using isopropyl palmitate or ricinoleic acid as the oil phase. Both PLO gel formulations generated a smooth feeling when rubbed onto the skin surface and both had the same consistency. The pH of both ricinoleic acid and isopropyl palmitate PLO gels were found to be in the range of 6.2-6.7.

**Viscosity**

The viscosity of PLO gels was evaluated at various shear stresses using a Brookfield RVDV-I Prime Digital Viscometer (Brookfield Engineering Laboratories, Middleboro, MA) with SC4-29 Spindle. Both PLO gels exhibited a similar viscosity pattern. Figure 4.2 represents the apparent viscosity versus speed RPM (shear stress) curves. This study resulted in a series of viscosity values depending on the shear rate with a clearly evident pseudoplastic behavior. The viscosities for the drug loaded PLO gels of isopropyl palmitate and ricinoleic acid at 5 rpm were found to be 33,300 cps and 49,000 cps, respectively. Both formulations represented a
non-Newtonian behavior with pseudoplastic flow. The shear thinning behavior of PLO gels is represented by the downward sloping curves. In addition, a significant decrease in viscosity was observed with isopropyl palmitate PLO gel, while ricinoleic acid PLO gel exhibited only a slight viscosity loss.

**Figure 4-2:** Viscosity data of chlorpromazine hydrochloride (10%) PLO gels of isopropyl palmitate and ricinoleic acid.

*Morphology*

The TEM and SEM images of drug loaded PLO gels containing ricinoleic acid showed a vesicular framework structured due to the presence of lecithin in the organogel (Fig. 4.3. a, b, c, and d). These results clearly indicate that ricinoleic acid is able to form PLO gels similar to traditional PLO gels formulated with isopropyl palmitate. Also, the figures clearly indicate that
drug particles are homogeneously distributed in the gel formulation. The TEM images clearly indicate that ricinoleic acid is able to form PLO gels similar to traditional PLO gels formulated with isopropyl palmitate. The TEM study also verified the formation of unilamellar vesicles[108].

Figure 4-3.a: Transmission Electron Microscopy image of PLO gel with ricinoleic acid
**Figure 4-3.b:** Scanning Electron Microscopy image of PLO gel with ricinoleic acid

**Figure 4-3.c:** Transmission Electron Microscopy image of PLO gel with isopropyl palmitate

**Figure 4-3.d:** Scanning Electron Microscopy image of PLO gel with isopropyl palmitate
**Differential Scanning Calorimetry**

DSC study was carried out to verify the absence of any un-dissolved chlorpromazine hydrochloride in PLO gels. DSC thermograms of chlorpromazine hydrochloride PLO gel with isopropyl palmitate and ricinoleic acid are illustrated in (Fig. 4.4.a-d). Chlorpromazine hydrochloride exhibited a sharp endothermic peak at around 200°C, which corresponds to its melting point. This is consistent with the data reported in the literature and the small peak at 260°C is a result of thermal decomposition. The thermograms of blank PLO gels of isopropyl palmitate and ricinoleic acid showed a broad endothermic peak at 110°C due to release of water molecules present inside the gel formulations. Drug loaded PLO gels exhibited a small shift in endothermic peaks from 110°C to around 125°C and the drug peak was completely absent[1]. This indicated the absence of any undissolved chlorpromazine hydrochloride in the PLO gels and that the drug was completely in its solubilized form [109, 110]. No unknown endothermic peaks resulting from drug-polymer incompatibility and drug polymorphism were observed in thermograms of drug loaded PLO gels.

**a- Chlorpromazine hydrochloride thermogram**

![Thermogram of Chlorpromazine Hydrochloride](image-url)
b – Blank isopropyl palmitate PLO gel

c – Blank ricinoleic acid PLO gel
d – Drug Loaded isopropyl palmitate PLO gel

![DSC thermogram of drug loaded isopropyl palmitate PLO gel]

e – Drug loaded ricinoleic acid PLO gel

![DSC thermogram of drug loaded ricinoleic acid PLO gel]

**Figure 4-4.** DSC thermograms of pure drug (a), blank PLO gels (b and c), and drug loaded PLO gels (d and e).
**Fourier Transform Infrared (FT-IR) spectroscopy**

FT-IR spectra for chlorpromazine hydrochloride, blank and drug loaded PLO gels are shown in (Fig. 4.5). The FT-IR spectrum of chlorpromazine hydrochloride is confirmed by the presence of the following characteristic peaks: aromatic and aliphatic C-H stretching at 3025 cm$^{-1}$ and 2950 cm$^{-1}$, aromatic C-C and C-N stretching at 1487 cm$^{-1}$ and 1655 cm$^{-1}$, C-S-C absorption at 750 cm$^{-1}$ [111]. FT-IR spectrum of blank PLO gels with isopropyl palmitate showed CH$_2$ stretching at 2800-3000 cm$^{-1}$ [112, 113]. In drug loaded PLO gel with ricinoleic acid, chlorpromazine hydrochloride characteristic peak at 1655 cm$^{-1}$ was absent, which indicated the absence of uncomplexed chlorpromazine hydrochloride in the PLO gel formulation. The complete disappearance of chlorpromazine hydrochloride characteristic peak at 3353 and 1655 cm$^{-1}$ in PLO gel could be attributed to the dissolution of functional groups of chlorpromazine hydrochloride inside PLO gels [25].
Figure 4-5: FTIR spectra of pure drug, blank PLO gels, and chlorpromazine hydrochloride loaded PLO gels.
**Stability Indication Assay**

A stability indicating method was developed for chlorpromazine hydrochloride. The retention time for chlorpromazine (λ<sub>max</sub> = 254 nm) was found to be 9.786 minutes as seen in (Fig. 4.6). Chlorpromazine hydrochloride was stressed with acid (1 N HCl) and base (1 N NaOH) for 8 hours, oxygen (2% H<sub>2</sub>O<sub>2</sub>), heat (70°C), and light (UV light) for 21 h. Stressed samples were measured using the HPLC method. No interference was observed between the chlorpromazine peak and degradant peaks. A sample chromatogram of chlorpromazine in 2% H<sub>2</sub>O<sub>2</sub> is shown below in (Fig. 4.7). An ideal stability-indicating method should allow the quantification of standard drug alone and also separate its degradation products. The developed HPLC method was shown to be suitable for analyzing stability samples. Drug loaded PLO gels were kept at 25°C, 35°C and 45°C and analyzed for drug content at regular intervals of 0, 7, 14, 21, 28, 35, and 60 days. All PLO gel formulations were stable under all three temperatures storage conditions. Table 4.1 represents that chlorpromazine hydrochloride was chemically stable for at least two months at 25°C, 35°C and 45°C. The study confirms the chemical compatibility between the drug and PLO gel excipients.

**Table 4.1:** Percent drug content of gel formulations after 60 days (n=3). Values expressed as mean ± standard deviation (SD)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25°C</td>
</tr>
<tr>
<td>Isopropyl palmitate PLO gel</td>
<td>99.73±2.80</td>
</tr>
<tr>
<td>(% drug content)</td>
<td></td>
</tr>
<tr>
<td>Ricinoleic acid PLO gel</td>
<td>101.41±0.53</td>
</tr>
<tr>
<td>(% drug content)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4-6: Sample HPLC chromatogram of chlorpromazine

Figure 4-7: Representative chromatography of chlorpromazine in 2% H₂O₂ after 10 mintues.
In vitro drug release study

In vitro release of chlorpromazine hydrochloride from PLO gels of isopropyl palmitate and ricinoleic acid were performed using Franz-diffusion cells with cellulose (Spectra pore, MWCO 1000Da) and Smart M-membranes. This study provided the basic information concerning the release and transfer of chlorpromazine hydrochloride across artificial membranes. It is also possible to identify how isopropyl palmitate and ricinoleic acid might alter the release of drug from PLO gels, which in turn would affect the drug permeation through the skin (Figs 4.8 and 4.9). The cumulative amounts of drug quantified in the receptor medium after 24 h from PLO gels of isopropyl palmitate and ricinoleic acid across the cellulose membrane were 75.19 ± 3.94 µg and 195.86 ± 14.09 µg, respectively. The cumulative amounts of chlorpromazine quantified in the receptor medium after 24 h from PLO gels of isopropyl palmitate and ricinoleic acid across the Smart M-membrane were 24.43 ± 4.45 µg and 32.25±5.41 µg, respectively. The release and transfer of chlorpromazine hydrochloride across cellulose and Smart M-membranes were found to be slightly higher with ricinoleic acid. This study indicated that chlorpromazine hydrochloride was released better in with ricinoleic acid as the oil phase, resulting in enhanced drug diffusion. The results are comparable to our lab’s previous work which showed higher permeation of ketoprofen with ricinoleic acid PLO gel compared to a traditional isopropyl palmitate PLO gel [35, 36].
Figure 4-8: *In vitro* diffusion of chlorpromazine hydrochloride across the cellulose membrane from isopropyl palmitate PLO gel and ricinoleic acid PLO gel. Values are expressed as mean, \(n=3\)

Figure 4-9: *In vitro* diffusion of chlorpromazine hydrochloride across the Smart M-membrane
from isopropyl palmitate PLO gel and ricinoleic acid PLO gel, Values are expressed as mean, (n=3)

Table 4.2: Cumulative amount/area (µg/sq.cm) ± SD after 24 hours of in vitro diffusion across cellulose and Smart M-membrane (n=4). SD: Standard Deviation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Average Cumulative amount/area (µg/cm²) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artificial cellulose membrane</td>
</tr>
<tr>
<td>Isopropyl palmitate PLO gel</td>
<td>75.19± 3.94</td>
</tr>
<tr>
<td>Ricinoleic acid PLO gel</td>
<td>195.86± 14.09</td>
</tr>
<tr>
<td>F1 value</td>
<td>33.77</td>
</tr>
<tr>
<td>F2 value</td>
<td>61.22</td>
</tr>
</tbody>
</table>

Percutaneous absorption of chlorpromazine hydrochloride across porcine ear and human abdominal skin

The cumulative amount of chlorpromazine hydrochloride permeating through the porcine ear skin is shown in Fig.4.10. A majority of the applied dose was retained on the skin and less than 0.5% of the dose actually permeated through the skin from the pure drug solution and PLO gels. PLO gels only marginally increased the drug permeation across the pig ear skin. The amount of drug penetrated into the epidermal and dermal layers from PLO gels was almost twice that of the pure drug solution. This indicated that PLO gels helped in the penetration of chlorpromazine into the epidermal and dermal layers; however, they failed to show required permeation of the drug into the receptor chamber. Overall mass balance was within the acceptable range of 100 ± 20% (Table 4.3). Permeability values (P x 10⁵) of the drug from pure drug solution, isopropyl palmitate PLO gel, and ricinoleic acid PLO gel were found to be 4.54 ± 0.98, 5.43 ± 0.55, and 6.48 ± 0.45 cm/hour, respectively (Table 4.4). Ricinoleic acid
PLO gel exhibited slightly higher permeation of the drug when compared to the pure drug and isopropyl palmitate PLO gel.

Figure 4-10: Permeation profile of chlorpromazine hydrochloride across the excised porcine ear skin from pure drug solution, isopropyl palmitate PLO gel, and ricinoleic acid PLO gel. Values are expressed as mean, (n=3)
Table 4.3: Total absorption and mass balance of chlorpromazine hydrochloride results across porcine ear skin. Percutaneous absorption and permeation values from pure drug and PLO gels into and through the intact porcine ear skin over 24 hours from a single application. Values expressed as mean ± standard deviation as a percentage of the applied dose.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Percent drug not permeated</th>
<th>Percent drug permeated</th>
<th>Mass Balance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remaining in the donor chamber</td>
<td>Remaining in the Epidermis and Dermis</td>
<td>Washing caps and Parafilm</td>
</tr>
<tr>
<td>Isopropyl palmitate PLO gel</td>
<td>66.07 ± 4.50</td>
<td>30.04 ± 2.12</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>Ricinoleic acid PLO gel</td>
<td>52.40 ± 3.17</td>
<td>35.41 ± 4.50</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>Pure Drug</td>
<td>82.65 ± 4.7</td>
<td>16.29 ± 6.70</td>
<td>0.25 ± 0.02</td>
</tr>
</tbody>
</table>

Table 4.4: Apparent permeability ($P_{app}$), flux of chlorpromazine hydrochloride across the excised porcine ear skin, lag time, and the cumulative amount permeated after 24 hours ($Q_{24}$). Values are expressed as mean ± standard deviation, n = 3.

<table>
<thead>
<tr>
<th>Formulation (10% chlorpromazine hydrochloride)</th>
<th>$P_{app}$ (cm/h)</th>
<th>Flux (µg/cm²/h)</th>
<th>Lag time (h)</th>
<th>$Q_{24}$ (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl palmitate PLO gel</td>
<td>5.06E-05 ± 0.9</td>
<td>1.27 ± 1.5</td>
<td>4.0 ± 0.1</td>
<td>30.00 ± 1.03</td>
</tr>
<tr>
<td>Ricinoleic acid PLO gel</td>
<td>6.49E-05 ± 1.1</td>
<td>1.62 ± 2.7</td>
<td>4.5 ± 0.1</td>
<td>38.17±1.15</td>
</tr>
<tr>
<td>Pure Drug</td>
<td>4.54E-05 ± 1.3</td>
<td>1.14 ± 3.4</td>
<td>4.7 ± 0.2</td>
<td>20.10±1.05</td>
</tr>
</tbody>
</table>

Similar results were observed with human abdominal skin. The cumulative amount of drug permeated through the human abdominal skin is shown in Fig.4.11. A majority of the applied
dose was retained on the skin and less than 0.5% of the applied dose actually permeated through the skin from the pure drug solution and PLO gels. Only a marginal increase in the drug permeation was observed with PLO gels. The amount of drug penetration into the epidermal and dermal layers of the human abdominal skin from PLO gels was almost twice that of the pure drug solution. Overall mass balance for chlorpromazine hydrochloride from the human abdominal skin permeation study was within the acceptable range of 100 ± 20% (Table 4.5). Permeability values (P x 10^{-5}) for chlorpromazine from pure drug solution, isopropyl palmitate PLO gel, and ricinoleic acid PLO gel were found to be 4.54 ± 0.2, 7.27 ± 0.33, and 8.98 ± 0.49 cm/hour, respectively (Table 4.6). Ricinoleic acid PLO gel exhibited slightly higher permeation of the drug when compared to the pure drug and isopropyl palmitate PLO gel. A slightly higher drug permeation was observed across the human abdominal skin as compared with pig ear skin. However, no significant difference was observed in the flux values and lag time of the pure drug and PLO gels between pig ear skin and human abdominal skin. This study also indicates that pig ear skin could be used as an alternative to human skin for in vitro permeation studies.
Figure 4-11: Permeation profile of chlorpromazine hydrochloride across the human abdominal skin from pure drug solution, isopropyl palmitate PLO gel, and ricinoleic acid PLO gel. Values are expressed as mean ± standard deviation, (n=3).

Table 4.5: Total absorption and mass balance of chlorpromazine hydrochloride across human abdominal skin. Percutaneous absorption and permeation values from the pure drug and PLO gels through intact human abdominal skin over 24 hours from a single application. Values expressed as mean ± standard deviation as a percent of the applied dose.

<table>
<thead>
<tr>
<th>Formulation (10% chlorpromazine hydrochloride)</th>
<th>Percent drug not permeated</th>
<th>Percent drug permeated</th>
<th>Mass Balance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remaining drug in the donor chamber</td>
<td>Remaining drug in Epidermis and Dermis</td>
<td>Washing caps and parafilms</td>
<td></td>
</tr>
<tr>
<td>Isopropyl palmitate PLO gel</td>
<td>65.95±4.00</td>
<td>35.72±4.2</td>
<td>0.32±0.02</td>
</tr>
<tr>
<td>Ricinoleic acid PLO gel</td>
<td>57.74±1.25</td>
<td>33±4.94</td>
<td>0.33±0.04</td>
</tr>
<tr>
<td>Pure Drug solution</td>
<td>74.78±3.38</td>
<td>16.29±6.70</td>
<td>0.24±0.02</td>
</tr>
</tbody>
</table>
Table 4.6: Apparent permeability ($P_{\text{app}}$), flux of chlorpromazine hydrochloride across the human abdominal skin, Lag time, and the cumulative amount permeated after 24 hours ($Q_{24}$), Values are expressed as mean ± standard deviation, $n = 3$.

<table>
<thead>
<tr>
<th>Formulation (10% chlorpromazine hydrochloride)</th>
<th>$P_{\text{app}}$ (cm/h)</th>
<th>Flux (µg/cm²/h)</th>
<th>Lag time (h)</th>
<th>$Q_{24}$ (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl palmitate PLO gel</td>
<td>7.27E-05</td>
<td>1.82</td>
<td>3.3 ± 0.10</td>
<td>30.93 ± 2.41</td>
</tr>
<tr>
<td>Ricinoleic acid PLO gel</td>
<td>8.98E-05</td>
<td>2.24</td>
<td>2.8 ± 0.12</td>
<td>41.50 ± 4.21</td>
</tr>
<tr>
<td>Pure drug solution</td>
<td>4.54E-05</td>
<td>1.60</td>
<td>3.9 ± 0.31</td>
<td>27.13 ± 2.11</td>
</tr>
</tbody>
</table>

Theoretical calculation of chlorpromazine plasma concentration from flux values

The required steady state concentration of chlorpromazine in plasma of humans is 100-300 ng/ml [114]. Using the results obtained from in vitro permeation studies through human abdominal skin, the $C_{\text{ss}}$ of chlorpromazine attained after topical application was calculated. The following equation could be used to calculate the expected steady state plasma concentration of chlorpromazine with an application area of 25 cm²:

$$C_{\text{ss}} = J_{\text{ss}} * A / CL$$

Eq.2

where $J_{\text{ss}}$ is the flux value of chlorpromazine hydrochloride from permeation study, $A$ is the application of 25 cm², and $CL$ is chlorpromazine body clearance (38 L/h)[115]. By using the above values, $C_{\text{ss}}$ was calculated for the pure drug solution and PLO gels (Table 4.7)
Table 4.7: Theoretical steady state concentrations of chlorpromazine in the plasma based on the flux values from the *in vitro* human abdominal skin permeation study.

<table>
<thead>
<tr>
<th>Formulation (10% chlorpromazine hydrochloride)</th>
<th>Flux (\text{Flux} \ (\mu g/cm^2/h))</th>
<th>C\text{ss} \text{Theoretical steady state plasma concentration (ng/ml)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl palmitate PLO gel</td>
<td>1.82</td>
<td>1.20</td>
</tr>
<tr>
<td>Ricinoleic acid PLO gel</td>
<td>2.24</td>
<td>1.50</td>
</tr>
<tr>
<td>Pure drug solution</td>
<td>1.60</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Theoretical steady state concentrations for chlorpromazine from the pure drug and PLO gels were much below the required steady state concentration for their therapeutic activity. Based on the permeability data across the human abdominal skin, it is concluded that chlorpromazine hydrochloride does not have sufficient permeability through the skin in order attain the required systemic concentrations needed to effectively manage nausea and vomiting in hospice patients. The results obtained in this study corroborate with the published results by Weiland et al., which reported that a dose of 25 mg of chlorpromazine in PLO gel applied to wrists did not produce detectable concentrations for chlorpromazine in all 11 subjects' blood samples at 1, 2, and 4 hours [50]. The lag time of chlorpromazine hydrochloride from pure drug solution and PLO gels varied between 2.5 - 4 hours, which might be a reason for the delay in the onset of plasma concentrations. From this study it is clearly evident that PLO gels fail to deliver required systemic levels of chlorpromazine following topical application.

**Conclusion**

In this study, we evaluated the percutaneous absorption of chlorpromazine hydrochloride from PLO gels with ricinoleic acid or isopropyl palmitate. The results obtained from this study
clearly indicate that PLO gels fail to deliver the required systemic levels of chlorpromazine following topical application. Permeability studies indicated higher penetration of chlorpromazine hydrochloride from PLO gel with ricinoleic acid than isopropyl palmitate. However, the theoretical \( C_{ss} \) values calculated from \textit{in vitro} permeability data were far below the required therapeutic concentrations for the antiemetic effect. To achieve better chlorpromazine hydrochloride skin permeation and thus higher concentrations of chlorpromazine in plasma, following smaller application surface area on the skin, efficient permeation enhancers should be used.
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