TOPICAL FORMULATIONS FOR NEUROPATHIC PAIN

by

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To my parents and siblings

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ABSTRACT

Neuropathic pain affects a significant portion of the population. It is chronic in duration, and resistant to many conventional oral analgesics. Topical gabapentin and amitriptyline in various strengths and bases have been compounded by specialized pharmacies for years. Although there is some evidence of efficacy in the literature, there are also controversies as well because of lack of drug release, permeation and stability data. In this thesis, stability of amitriptyline and gabapentin in three different commonly used topical bases were investigated at three different temperatures over a 90-day period. *In vitro* drug release and permeation studies were also conducted using Franz diffusion cells. Data from these studies provided new evidence that will improve compounding services for neuropathic pain and patients' quality of life.

LIST OF ABBREVIATIONS AND SYMBOLS USED

%CV	Percentage coefficient of variance
®	Registered Trademark
AMIT	Amitriptyline
BUD	Beyond use date
cm^2	Centimeter squared
CNS	Central Nervous System
EC	Emollient cream®
FDA	Food and Drug Administration
GBP	Gabapentin
h	Hour(s)
HPLC	High Precision Liquid Chromatography
IASP	International Association for the Study of Pain
ICH	International conference on harmonization
IVRT	*In vitro* release testing
LB	Lipoderm® base
LOD	Limit of detection
LogP	Partition coefficient
LOQ	Limit of quantification
Min	Minutes
mL	Milliliter
NAPRA	National Association of Pharmacy Regulatory Authorities
NMDA	N-Methyl-D-aspartic acid
OECD	Organization for Economic Co-operation and Development
PBS	Phosphate buffered saline
PCCA	Professional Compounding Centers of America
PLO	Pluronic lecithin organogel
QC	Quality Control
R^2	Coefficient of determination
RT	Room temperature
Sec	Seconds
TCA	Tricyclic antidepressant
USP	United States Pharmacopoeia
VG	Versabase® gel
µg/mL	Micrograms per milliliter
µL	Microliter

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CHAPTER 1- INTRODUCTION

Pain, which is both an unpleasant sensory and emotional experience, is an important element of the body's defense system [1]. It is a complex subjective process that is also impacted by psychological factors such as beliefs about pain, anxiety and past experiences. Most often, pain serves as a warning for a potential or actual noxious stimulus and hence allows individuals to elicit the appropriate response [2].

The process of pain transmission and sensation begins when free nerve endings, called nociceptors, are exposed to some sort of harmful chemical, thermal, or mechanical stimuli [2]. Nociceptors are the free nerve endings of primary afferent fibers, which transmit signals from the periphery to the central nervous system (CNS) [3]. Additionally, these nerve ending are widely distributed within the epidermis of the skin, deep tissues, musculoskeletal system and internal organs [3]. Primary afferent neurons are unique compared to the typical neurons in the body in that they are pseudo-unipolar [4]. This means that the neuron contains an axon that has split into two biochemically similar branches, one of which extends to the periphery while the other to the spinal cord. This is advantageous as pharmacologic agents can be directed to either or both terminals to influence pain transmission [4].

1.1 NEUROPATHIC PAIN

More than 2 million Canadians suffer from some form of neuropathic pain [5]. Neuropathic pain, as defined by the International Association for the Study of Pain (IASP), is "pain caused by a lesion or disease of the somatosensory system" [1]. Unlike other types of pain, which alerts and protects an individual from a noxious stimulus, neuropathic pain serves no useful purpose [6]. The pain is a problematic condition

because it is usually chronic in nature and resistant to many analgesics [7]. It can be due to multiple etiologies such as surgery, injury, herpes zoster, diabetes, ischemia, infection, chemotherapy, radiotherapy, malignancy, and central nervous system diseases [8]. Although the mechanisms underlying neuropathic pain are complex, multi-factorial, and still poorly understood, there are proposed peripheral and central mechanisms that attempt to explain the pathogenesis [4,6].

In individuals without neuropathic pain, nerve fibers in the periphery, prior to reaching the spinal cord are isolated from one another and do not affect neural activity of each other [6]. However, after an injury or lesion occurs in the peripheral nervous system, it is thought that new nerve projections form and connect injured neurons with nearby uninjured neurons [6,7]. Some of these uninjured nerve fibers, may normally be involved in the detection of non-noxious stimuli such as tactile discrimination [3]. Hence, with these new nerve connections, what would normally be a non-painful stimulus may thereby produce pain [6]. Additionally, a known characteristic of neuropathic pain is increased neuronal excitability and firing. This, in part, is due to an increased expression of voltage gated sodium channels in the peripheral nerves. Another mechanism that may have role in neuropathic pain is the increase in the expression of the a2δ subunit of calcium channels on peripheral neurons which plays a big role in hypersensitivity and allodynia [4]. The reduced pain threshold (allodynia) and amplified response to painful stimuli (hyperalgesia), characteristic of neuropathic pain is partly due to increased N-Methyl-D-aspartic acid (NMDA) receptor excitability [9]. The magnesium ion, which normally blocks the NMDA receptor is removed, hence the channel remains open longer and is activated by inputs that are normally subthreshold [9].

1.2 CONVENTIONAL TREATMENTS FOR NEUROPATHIC PAIN

The most recent Canadian guidelines for the management of chronic neuropathic pain recommends use of oral anticonvulsants or tricyclic antidepressants (TCAs) as first line pharmacologic therapy [5]. The anticonvulsants most commonly used are gabapentin and pregabalin, while the TCAs used include amitriptyline, nortriptyline, imipramine, desipramine, and doxepin [5].

Oral gabapentin and pregabalin act by inhibiting voltage gated calcium channels in the presynaptic nerve, hence resulting in a significant reduction in excitatory neurotransmitter release [10,11]. Both medications are widely dispensed in community pharmacies and have become more popular due to demonstrated efficacy, minimal drug-drug interactions and overall better patient tolerability [12]. Common side effects of both medications include dizziness, somnolence, fatigue, and peripheral edema [12]. Although these side effects may be reduced by careful dose titration, geriatric patients have an increased risk of falls and injury, especially if they are on concomitant medications with similar side effect profile [13].

TCAs are an old class of medications used for insomnia, various psychiatric conditions and neuropathic pain [14]. TCAs are known as “dirty drugs” due to their non-specific actions on multiple receptors such as: norepinephrine, histamine, muscarinic, α_1-adrenergic and voltage gated sodium channels [15]. Although this class of medications is helpful in relieving pain associated with various neuropathies, the dose is often limited by side effects that may be intolerable [5]. The most common side effects include: dizziness, somnolence, orthostatic hypotension, urinary retention, dry mouth, constipation, tachycardia, confusion and weight gain [5,14].

1.3 TOPICAL TREATMENTS FOR NEUROPATHIC PAIN

Although the oral route is the most common mode of administering medications, it may not always be desirable due to higher rates of side effects, drug-drug interactions, and confusing dosing regimens. Using the skin as an alternative or concurrent route of administration has many advantages such as direct delivery of analgesics to the peripheral nerves, no first pass metabolism, and lower rates of adverse effects [2]. Topical analgesics are intended to produce their effects near the site of application without systemic side effects. Hence tissue concentration should be high with low serum concentration levels. Additionally, topical pain medications allow pharmacists to combine multiple medications in one cream, which significantly simplifies pain regimens. It is thought that a multi-modal approach to pain treatment where multiple agents with different mechanisms of action are utilized can increase efficacy and lower adverse effects [16].

As mentioned previously, voltage gated sodium channels and calcium channels in the peripheral nerves play an important role in pain transmission [4]. Given these channels are abundant in the integumentary system, it makes sense to use topical agents to target them. Two common oral drugs, amitriptyline and gabapentin, already recommended as first line treatment for neuropathic pain act on these targets. Amitriptyline acts as an inhibitor of voltage gated sodium channels in the peripheral nerves and was found to be a more potent anesthetic than bupivacaine [17,18]. Gabapentin, on the other hand, acts as a calcium channel blocker, NMDA receptor antagonist as well as other peripheral mechanisms that are not currently clear [11,19]. Topical agents containing either gabapentin or amitriptyline have shown clinical efficacy, however little to no data exists on the impact of dosage form formulation on drug performance [20,21,22,23].

1.4 BARRIERS TO TOPICAL ANALGESIC ABSORPTION

Drug transport through the skin is a multi-factorial process involving both physical and chemical barriers [24]. The biggest physical barrier and rate limiting step to drug absorption is the *stratum corneum* which is 5 to 15 μm thick [25]. The *stratum corneum* is the outermost layer of the skin composed of dense flat keratinized dead epidermal cells, called corneocytes, organized into closely packed layers [26]. The corneocytes are arranged in an analogous manner to a "brick and mortar" wall where the cells represent the bricks and the mortar are made up of intercellular lipids [27]. Where intercellular absorption is the major route of drug permeation, the oil-water partition coefficient, concentration, and aqueous solubility of a drug are important elements to overcome this barrier [26]. The thickness of the *stratum corneum* can vary significantly based on body site, age, and sex [25]. Once the drug penetrates the *stratum corneum* by passive diffusion, it can partition into the aqueous viable epidermis and upper dermis where it can target peripheral nerves [24].

There are several factors that allow for favorable topical drug absorption. Having a higher drug concentration per surface area of skin generally increases permeation as there is a greater concentration gradient [26]. Additionally, the drug having a greater affinity to the skin compared to the vehicle is also important [26]. Although increased lipophilicity aids in passive diffusion through the *stratum corneum*, the drug should have some aqueous solubility to diffuse through the epidermis [24]. Otherwise, if the drug is too lipophilic, it may be retained in the *stratum corneum.* Finally, a molecular weight of less than 400 Da is considered ideal for topical administration [26].

1.5 *IN VITRO* MODEL FOR TESTING DRUG RELEASE AND PERMEATION OF TOPICAL FORMULATIONS

Franz cell assay is a type of *in vitro* skin permeation assay that is commonly used in formulation development and commercial scale-up procedures. It is one of the performance tests recommended by the United States Pharmacopeia and the organization for Economic Co-operation and Development (OECD) guideline 428 as a measure of drug release and permeation potential of semi-solid formulations [28].

Franz cells are composed of an upper and a lower borosilicate glass piece (Figure 1). In the literature, the upper part is most commonly called the donor chamber, however, it is also called a cell top or cell cap. The cell's entire lower component is called the cell body with the innermost portion of the cell referred to as the receptor chamber. A magnetic stir bar may be placed in the receptor chamber to ensure that receptor fluid's content is uniformly distributed. The sampling port is attached to the receptor chamber and is where samples are drawn from during the permeation experiment. The top area of the cell body is called the orifice and is where the membrane, which can be human skin, animal skin, or an artificial membrane is placed. The Franz cell donor chamber and the cell body are held together with a metal clamp. The area where the donor chamber and cell body meet is the joint. There are several different types of joints available commercially, including flat ground, flat flange, O-ring, and spherical [29]. The most commonly used connection is the flat ground joint which is also known as an O-ring joint [29]. Due to the way the O-ring joint is shaped, it holds and compresses a thin membrane smaller than 4 mm over the top of the receptor chamber quite well.

Franz cells are available as "jacketed" and as "unjacketed" forms. Figure 1 depicts

a jacketed Franz cell. The term "jacketed" refers to the outer glass layer surrounding the receptor chamber. The jacket may be attached to a water circulator via the inlet and outlet glass tubes to control the temperature of the receptor chamber. This is especially important if a researcher wants to mimic *in-vivo* conditions. In the case of topical skin absorption, the temperature is maintained at 32 ±1°C as this is typically the temperature of the skin surface [28].

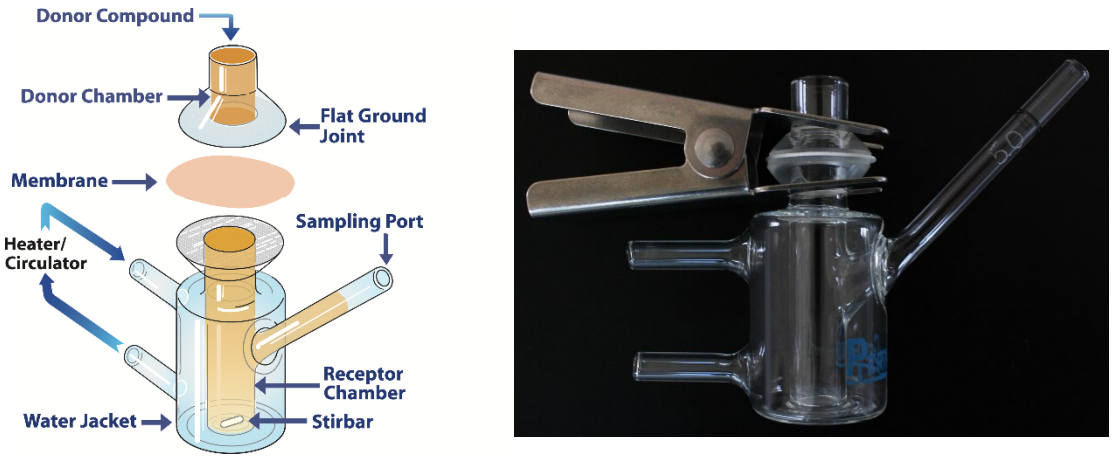

Figure 1. Jacketed Franz cell with flat ground joint. Picture provided with permission from PermeGear Inc (Hellertown, PA, USA).

1.5.1 *In Vitro* Release Testing (IVRT)

Measuring drug dissolution from various dosage forms is important in both quality control and formulation development [30]. In the case of semi-solid formulations, dissolution testing is more commonly known as *in vitro* release testing (IVRT). The purpose of drug release testing is to characterize the rate, extent and mechanism by which a drug leaves the formulation [31]. Several factors such as drug particle size, drug solubility in the formulation, formulation rheology, physical properties of the base and excipients, temperature, pH and other drug physicochemical properties can affect the drug

release profile which have the potential to influence the clinical performance of the overall product. [30].

IVRT is conducted by applying test formulations on an inert membrane and measuring drug diffusion in the receptor chamber at various time points [31]. The pores of the membrane fill with receptor fluid allowing the active ingredient in the semi-solid formulation to move into the receptor chamber [30]. The purpose of a membrane, in this case, is to provide physical support as well as a barrier between the semi-solid base and the receptor medium [30]. The membrane must be inert, have high porosity, have minimal thickness, have no chemical interaction with the formulation, not be susceptible to degradation due to presence of certain excipients, as well as offer minimal resistance to the diffusion of the active drug [30]. In this type of experiment, barriers to liberation of a drug from its formulation may include strong binding to the base, and long time-frame required for release [30]. The selection of the membrane is often made based on the active drug's LogP as well as the nature of the membrane itself. Based on literature information, cellulose-based membranes are the most widely used [30].

1.5.2 Kinetics of IVRT

There are several mathematical models that describe drug release from dosage forms. The five main ones include zero order, first order, Higuchi, Hixon-Crowell and Korsmeyer-Peppas models [32]. IVRT data is fitted into these models and regression analysis conducted to generate a coefficient of determination (R^2). The model that results in the highest R^2 value is likely to be the mechanism of drug release. The equations and data simulation approaches using these models is demonstrated in Table 1.

For products following zero order kinetics, constant drug release occurs with time, regardless of initial or existing product concentration [32]. However, with first order

kinetics, the release rate over time is dependent on concentration [32]. The Higuchi model describes diffusion out of a non-degrading matrix system following Fick's first law of diffusion [33]. The model has several assumptions that include: (a) Concentration of the active drug is higher than the solubility of the drug, (b) Thickness of the base it is dispersed in is larger than the molecular size of the drug, (c) Swelling of the matrix is negligible, and (d) Diffusion is constant and perfect sink conditions occur [32,34]. The Hixon-Crowell model, also known as the cube root rule describes products where the surface area and diameter of the drug matrix changes with time [34]. This is commonly seen in oral tablets. The last model to be described is the Korsmeyer-Peppas model, which is a nonspecific model that can involve multiple release mechanisms [35]. To more clearly characterize the mechanism, the slope of the linear regression equation is used. If the value is <0.5, the formulation likely follows Fickian diffusion. Fickian diffusion involves particles moving from an area of high to low concentration [36]. However, if it is greater than 0.5 and less than 1, then the release mechanism likely follows non-Fickian or anomalous transport [35]. If the value is 1, then the release mechanism is likely to be zero-order.

Table 1. Mathematical kinetic models for drug release from semi-solid formulations.

Kinetic Model	Equation	Simulation method
Zero order	$Q_t = Q_0 + K_0 t$	Cumulative percentage drug released vs time
First order	$Log(Q_t) = \log(Q_0) + K_1 t/2.303$	Log cumulative percentage drug remaining vs time
Higuchi	$Q_t = k_H \sqrt{t}$	Cumulative percentage drug release vs square root of time
Hixon-Crowell	$Q_t^{1/3} - Q_0^{1/3} = K_s t$	Cube root of drug percentage remaining vs time
Korsemeyer-Peppas	$M_t/M_\infty = K_{KP} t^n$	Log of percentage drug released vs log time

Q_t = amount of drug dissolved at time t; Q_0 = drug present at t = 0; K_0 = zero order rate constant; t = time; k_1 = first order rate constant; k_H = higuchi release constant; K_s = release rate constant; $Mt/M\infty$ = fraction of drug released at time t; n = release exponent; K_{KP} = rate constant [32,34].

1.5.3 Drug Permeation Testing

Information on dermal absorption and rate of absorption between different strengths and formulation compositions is important in the drug manufacturing process. The most accurate information is obtained using human *in vivo* data. However, given the strict regulations, cost, and time associated with pursuing such an endeavor, alternative approaches are used [37]. *Ex-vivo* human abdominal skin, obtained after plastic surgery may be used to mimic *in vivo* conditions, however, human skin is expensive and usually difficult to acquire [38]. Animal skin from rats, pigs, rabbits, guinea pigs, and mice may be used as an alternative to human skin as a predictor of drug permeation [37]. Amongst all the animals listed, pig skin, particularly the outer ear, is the most widely used [37]. The reasoning behind this choice is the fact that pig skin is readily available and cheap [37]. Also, the thickness of the *stratum corneum*, number of hairs per cm^2 of skin, and the thickness of the epidermis most closely resemble human skin [37]. Caution should be used not to wash the animal skin with scalding water, as is customarily done after slaughter at an abattoir, as this breaks down the barrier properties of the *stratum corneum*

[39]. Where animal skin is not readily available, membranes that functionally mimic the human skin, such as Strat-M® may be used.

1.5.4 Strat-M®

Strat-M® (Merck Millipore, USA) is an artificial membrane consisting of multiple layers of polyether sulfone and polyolefin [40]. The top layer is the least porous and is impregnated with lipids while the layers below become increasingly more porous and thicker [40]. These multiple layers, particularly the top layer, creates a morphology like that of human skin [40]. A comparison between human skin and Strat-M®, which has a total membrane thickness of 325 μm, is shown in Figure 2. This membrane was reported to have good *in vitro-in-vivo* correlation (IVIC) compared to human skin with a variety of chemicals ranging in molecular weights between 151 and 288 g/mol and LogP between -0.90 to 3.53 [38]. Advantages of using Strat-M® involve less lot to lot variability, no requirement for ethics approval, and it is readily available [41]. A major limitation of Strat-M® is that drug molecules trapped in each of the layers cannot be quantified after permeation studies [36].

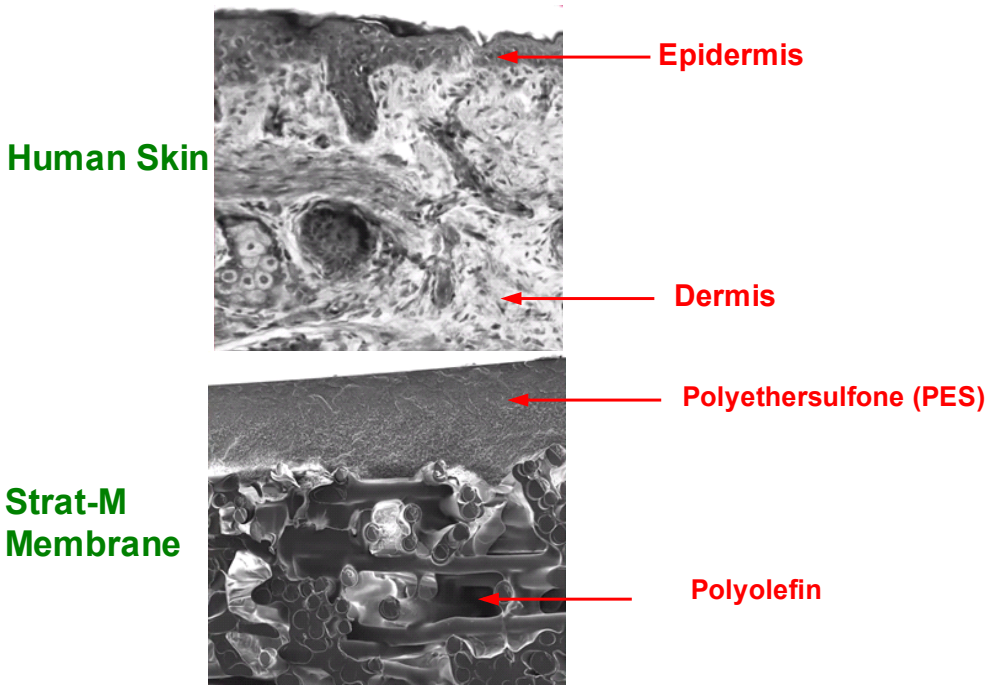

Figure 2. Comparison of Strat-M® membrane cross section with layers of human skin. Picture provided with permission by Millipore Sigma, a division of Merck KGaA (Darmstadt, Germany).

1.6 OBJECTIVES AND SCOPE

To date, there is no well-designed study that analyzes the topical absorption, pharmacokinetics and stability of commonly compounded topical neuropathic pain medications (gabapentin and amitriptyline). Furthermore, there are no studies on ways to improve local absorption of these compounds, while reducing potential systemic bioavailability. Moreover, there are currently no published studies supporting the recommendations on how long patients can store and use these compounded products based on the specific base used. The aim of this project was to assess stability, *in vitro* release and skin permeation of gabapentin and amitriptyline when administered topically. Such data have the potential to provide the scientific evidence needed by pharmacists and physicians for recommending compounding vehicles, storage conditions and duration/dose of topical therapy. To achieve the stated aim above, our studies were divided into specific objectives being: (1) Establish beyond use dates for topical gabapentin compounded with Lipoderm® base, Versabase® gel, and Emollient cream® (2) Establish beyond use dates for topical amitriptyline compounded with Lipoderm® base, Mediflo® PLO gel, and Emollient cream® (3) Characterize mechanism and extent of gabapentin and amitriptyline release based on concentration and topical base used (4) Determine rate and extent of gabapentin and amitriptyline permeation through simulated human skin, Strat-M®. Each objective was addressed in a specific chapter as summarized below:

Chapter 2: Stability assessment of topical gabapentin extemporaneously compounded with Lipoderm® base, Versabase® gel, and Emollient cream®.

Chapter 3: Stability assessment of topical amitriptyline extemporaneously compounded with Lipoderm® base, Mediflo®30 PLO gel, and emollient cream®.

Chapter 4: *In vitro* release profile and permeation of gabapentin across simulated human skin Strat-M® when compounded with Lipoderm® base, Versabase® gel, and Emollient cream®.

Chapter 5: *In vitro* release profiles and permeation of amitriptyline compounded with Lipoderm cream®, Emollient cream®, and Mediflo® 30 PLO gel.

The challenges and prospects of compounded topical pain formulations, significance of our work and future research opportunities in this area are discussed in Chapter 6.

CHAPTER 2- STABILITY ASSESSMENT OF TOPICAL GABAPENTIN EXTEMPORANEOUSLY COMPOUNDED WITH LIPODERM® BASE, VERSABASE® GEL, AND EMOLLIENT CREAM®.

2.1 INTRODUCTION

Gabapentin, 1-(aminomethyl) cyclohexaneacetic acid (Figure 3), is an antiepileptic medication currently prescribed for the treatment of partial seizures and used off-label in Canada for peripheral neuropathic pain [42]. It is a white crystalline compound with a molecular weight of 171.34 g/mol and a log P of -1.10 [43]. It is a zwitterion at physiological pH and has two pK_a values of 3.68 and 10.70 [43]. It is freely soluble in water and degrades by intramolecular cyclization to form a γ-lactam [44]. In a clinical setting, topical gabapentin is used alone or in combination with other active pharmaceutical ingredients for neuropathic pain [22,23]. It is structurally like the endogenous neurotransmitter GABA, however, its mechanism of action when applied topically is mainly through blockade of calcium channels and NMDA receptors on peripheral nerves [45]. Chronic neuropathic pain results in increased sensitivity and/or expression of these channels in the periphery and is associated with allodynia and hyperalgesia. Therefore, blockade of these channels by administering topical gabapentin is expected to reduce these processes. The choice of a base is very important when compounding any active pharmaceutical ingredient as it not only impacts its release and permeation characteristics, but also the stability, which may ultimately impact efficacy of the product [46].

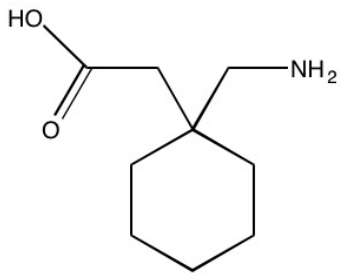

Figure 3. Chemical structure of gabapentin.

Drug stability is an important factor to consider before dispensing any compounded preparation [47]. As per Canada's National Association of Pharmacy Regulations (NAPRA), pharmacists are legally mandated to assign a beyond use date (BUD) for all dispensed non-sterile preparations [47]. A BUD is the date after which a compounded preparation should no longer be used by the patient [47]. Manufacturer information, available literature on degradation mechanism(s), compatibility, stability, microbial growth, and packaging, regulatory agencies/institutional guidelines, and professional judgement may be used as guides in establishing a conservative BUD [47,48]. Currently, compounding pharmacists use the United States Pharmacopoeia (USP) beyond use date of 30 days for topical gabapentin compounds [48]. However, it is unknown if these formulations are stable beyond this point. Furthermore, stability of this compound under various potentially deleterious compounding (e.g. acidic and basic pH) and storage (elevated temperature) conditions is not known.

Literature search through PubMed, Google Scholar, and Embase indicated that there is one stability study for topical gabapentin (4, 6, and 10%) combined with other active pharmaceutical ingredients in Lipoderm®, Lipobase® and PLO gel at room temperature [49]. This paper was published after this study was commenced. It was found that gabapentin was not stable in Lipoderm® base after 28 days when combined with

amitriptyline and ketoprofen [49]. In this study, a stability-indicating HPLC method described in the USP was used to assess the stability of gabapentin (10%) in Lipoderm® base, Versabase® gel, and Emollient cream® at room temperature, 4°C and at 40°C. These three bases were selected as they are widely used and gabapentin is compounded in each of these bases in pharmacy practice. The purpose of this study was to determine if the 30-day BUD is valid in all three storage conditions and bases.

2.2 MATERIALS AND METHODS

2.2.1 Materials

Gabapentin USP (Lot: CC006973), Lipoderm® cream (Lot: 7048812) and emollient cream (Lot: 7038834) were kindly donated by Professional Compounding Centers of America (PCCA) (Huston, TX, USA). Versabase® gel (Lot: 7155191) was purchased from PCCA. Ethoxy diglycol (Lot:14164-5104) was acquired from Galenova; Québec, Canada). Monobasic ammonium phosphate (Lot: 034242) and acetonitrile of analytical grade (Lot: 171203) were purchased from Fisher Scientific (NJ, USA). Sodium perchlorate (Lot: 10193677) was from Alfa Aesar (Ward Hill, MA, USA). Perchloric acid 70% (Lot: 16I086507) was obtained from VWR (Mississauga, ON, Canada). Deionized water (18 Ω) was purified in the lab using a Barnstead Nanopure II filtering system. Ecolojars® containers of adjustable volume for storing topical compounded drugs were purchased from Mckesson Canada (Moncton, NB, Canada).

2.2.2 Instrumentation

A Varian-920 Liquid Chromatograph with a quaternary gradient pump, autosampler with a 50 µL sample loop, a UV-Vis detector and Galaxie chromatographic software were used for sample analysis. Chemical separation was achieved using a stationary phase consisting of a Phenomenex Luna® 5 µm C18(2) 100 Å, LC Column 250

x 4.6 mm (Serial No 256867-12). A VWR Hybridization oven was used to maintain a storage temperature of 40^{o}C.

2.2.3 Chromatographic Conditions

A United States Pharmacopeia stability-indicating assay method was adapted and optimized for analyzing the compound [50]. The mobile phase consisted of a buffer and acetonitrile in a ratio of 76:24, v/v. The buffer was prepared by mixing 0.58 g of monophasic ammonium phosphate and 1.83 g of sodium perchlorate in 900 mL deionized water. The pH was adjusted to 1.8 using perchloric acid and made up to 1 L with deionized water using a volumetric flask. The mobile phase was vacuum filtered through a 0.22 µm nylon filter and degassed for 20 minutes. The mobile phase was run overnight at 0.1 mL/min to condition the HPLC column. During separation, the column was kept at 40^{o}C while the flow rate was maintained at 1 mL/min. Chromatographic conditions are summarized in Table 2.

Table 2. Chromatographic conditions for the detection and assay of gabapentin.

Column	Phenomenex Luna® 5 µm C18(2) 100 Å, LC Column 250 x 4.6 mm
Mobile Phase	Buffer, acetonitrile (76:24)
Flow Rate	1 mL/min
Detection	UV-Vis at 215 nm
Injection	20 µL
Column Temperature	40 ^{o}C
Run time	6 minutes

2.2.4 High Performance Liquid Chromatography Assay Validation

Gabapentin solutions were prepared to simulate stressful conditions (e.g. high temperature, basic/acid pH conditions and modified oxidation states) to facilitate forced degradation and for validating the assay as a stability-indicating method. Degradation of

gabapentin was evaluated by preparing a 10 mg/mL gabapentin solution in deionized water. A volume of 0.5 mL of the solution was added to vials with aqueous NaOH (0.1 M, 0.5 mL), aqueous HCl (0.1 M, 0.5 mL) and hydrogen peroxide (3%, 0.5 mL). These three solutions were stored at room temperature for 4 hours. A fourth vial containing gabapentin diluted to 5000 µg/mL with deionized water was heated at 80^{o}C for 4 hours. The four solutions were later scanned by HPLC for peaks.

The chromatographic method was validated for linearity of response, limit of quantification, limit of detection, as well as precision and accuracy of the method. The concentrations (x-axis) were plotted against the peak AUC and regression analysis was performed to generate a calibration curve. The range was determined by finding the highest and lowest concentrations of standard solutions where there is acceptable linearity, accuracy, and precision. Accuracy of the method was determined by scanning three samples of gabapentin (10%) in each Lipoderm® base, Versabase® gel and Emollient cream® diluted in deionized water and calculating percentage recovery. The intra-day precision or repeatability of the method was estimated by calculating the mean percentage coefficient of variation (%CV) of three QC standards (low QC = 100 µg/mL, intermediate QC = 1000 µg/mL, and high QC = 2500 µg/mL) scanned in triplicate. Inter-day precision was determined by scanning the QC standard samples in triplicates on three different days and calculating the mean %CV. Values under 5% were considered acceptable [51]. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the equations 1 and 2 [52]:

$$\mathrm{LOQ} = \frac{10 \times \mathrm{SD}}{\mathrm{S}} \quad \textbf{.....Equation 1}$$

$$\mathrm{LOD} = \frac{3.3 \times \mathrm{SD}}{\mathrm{S}} \quad \textbf{.....Equation 2}$$

Where S = the slope of the calibration curve and SD = standard deviation of the y-intercept.

2.2.5 Compounding and Sample Preparation

To prepare the formulations, an appropriate amount of gabapentin was weighed out and triturated to produce a fine powder using mortar and pestle. Required amount of ethoxy diglycol was added to the fine powder and levitated to produce a smooth paste. Each of the bases to be used was added to the prepared paste using the principles of geometric dilution. The cream was transferred to a jar and mixed using a Gako Unguator® electronic mortar and pestle (Norman, OK, USA) and processed once through an Exakt 50 ointment mill (Oklahoma City, OK, USA). The resulting gabapentin (10%) in Lipoderm® base, Versabase® gel, and Emollient cream® were placed in nine Ecolojars® for each compound, for a total of 27 jars. Three jars of each type of formulation were stored at room temperature, 4^{o}C and 40 ^{o}C, respectively. Samples were taken at days 0, 14, 28, and 90 and immediately stored in a -80 ^{o}C freezer for later analysis.

On the day of analysis, samples were thawed for 1 hour at room temperature, diluted with deionized water and extracted using a Fisher Scientific digital vortex mixer (Fisher Scientific®, ON, Canada) for 30 minutes at 1500 RPM. The pH of the samples was measured in triplicate using a calibrated pH meter. Samples were scanned for degradation peaks using HPLC and gabapentin potency was calculated based on AUC values observed. Changes in mean pH values as well as potency at the different time points were compared to zero time point by two-way analysis of variance (ANOVA) with Tukey's multiple comparison post-hoc test using GraphPad Prism® software version 7.0 (GraphPad Software Inc., San Diego, CA, USA) with a 95% confidence interval. P values <0.05 were considered statistically significant.

2.2.6 Physical Evaluation of Topical Compounds

The organoleptic properties consisting of physical appearance, colour, homogeneity, phase separation, texture and immediate skin feel were monitored and qualitatively described at each sampling time point.

2.3 RESULTS

The chromatographic method was specific for gabapentin (retention time 4.08) as no other peaks were detected as shown in Figure 4. Under acidic, alkaline, oxidizing, and thermal conditions, peaks were successfully monitored with no degradation peak interfering with gabapentin (Figure 5), hence, confirming this method is adequate for stability assessment. The retention times of A, B, and C were 4.05, 4.06, and 4.06 with no gabapentin peak observed in D.

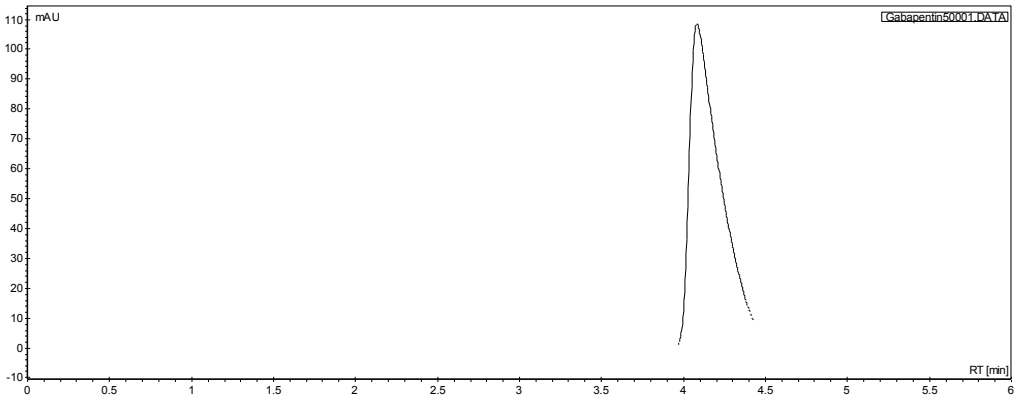

Figure 4. Chromatogram for gabapentin 5000 µg/mL solution.

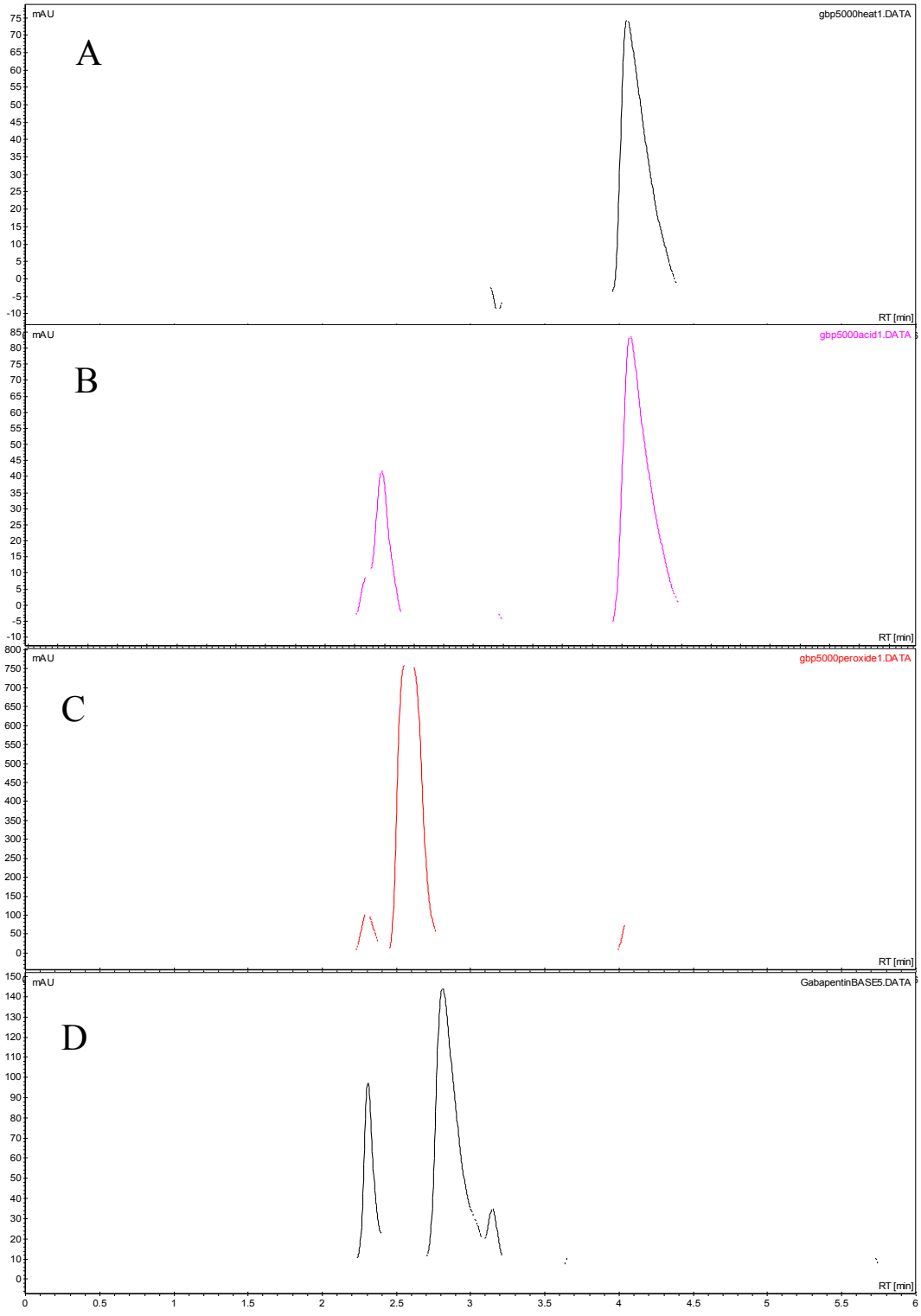

Figure 5. Chromatograms of gabapentin in forced degradation samples under heat (A), acidic (B), oxidizing (C) and basic (D) conditions.

The standard curve, characterized by the equation y = 0.2816x - 1.5765, was linear across the range of 78.125 to 5000 mg/mL (R^2 = 0.9999) as depicted in Figure 6. The %CV of the intraday and interday assays were 1.0% and 0.9 %, respectively. This indicates high method intraday and interday precision. Sample recovery for gabapentin was 99.1 ± 1.2%, 102.3± 0.8%, and 103.6 ± 1.3% in Lipoderm® cream, Versabase® gel, and Emollient cream®, respectively. The limit of detection and quantification for the assay was 18.15 and 55.01 µg/mL, respectively.

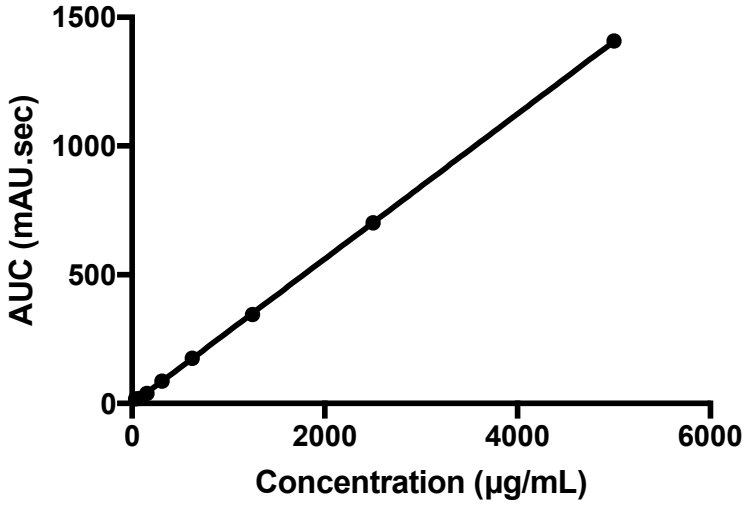

Figure 6. Linearity of gabapentin in the range of 39 to 5000 µg/mL.

The pH of the gabapentin (10%) in Lipoderm® base at 40°C was significantly lower at 90 days ($p<0.0001$). This can be seen quite clearly in Figure 7. Similarly, there was also a significant decrease in pH for gabapentin compounded in Emollient cream® ($p<0.0001$). At 40°C, no changes in pH were observed in the Versabase® gel ($p>0.05$). Storage at 4°C

Figure 8), resulted in a significant decrease in pH for gabapentin (10%) in Lipoderm® base at 90 days ($p<0.0001$), and an increase in pH of gabapentin (10%) in Versabase® gel ($p<0.0001$). No significant change was observed between initial and final time points for gabapentin (10%) in Emollient cream® ($p<0.05$).

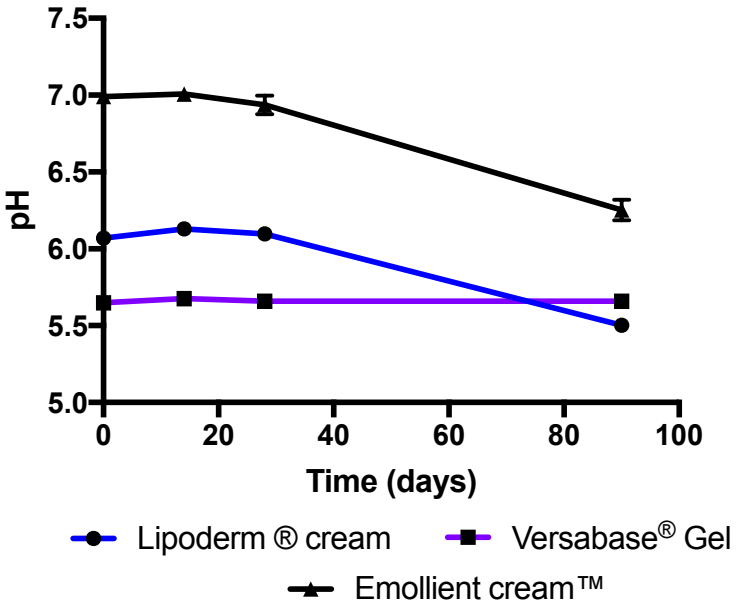

Figure 7. pH change of compounded gabapentin (10%) in Lipoderm® cream, Versabase® gel and Emollient cream® over 90 days at 40° C. Data represent mean ± SD, n = 3.

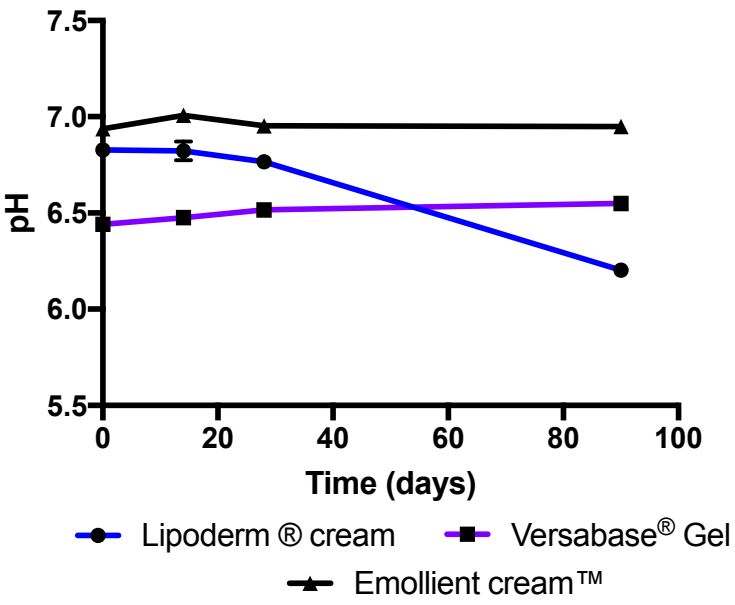

Figure 8. pH change of compounded gabapentin (10%) in Lipoderm® cream, Versabase® gel and Emollient cream® over 90 days at 4° C. Data represent mean ± SD, n = 3.

At room temperature (Figure 9.), pH of gabapentin (10%) in Lipoderm® base remained stable until 28 days ($p<0.05$), but, significantly decreased after 90 days ($p<0.0001$). No changes in pH were observed in the Versabase® gel at room temperature ($p>0.05$) at day 28. However, the 90-day time point was excluded as physical instability of the preparation made it impractical to obtain pH measurements. There was a significant decrease in pH observed for gabapentin compounded in Emollient cream® at the 90-day time point ($p<0.0001$).

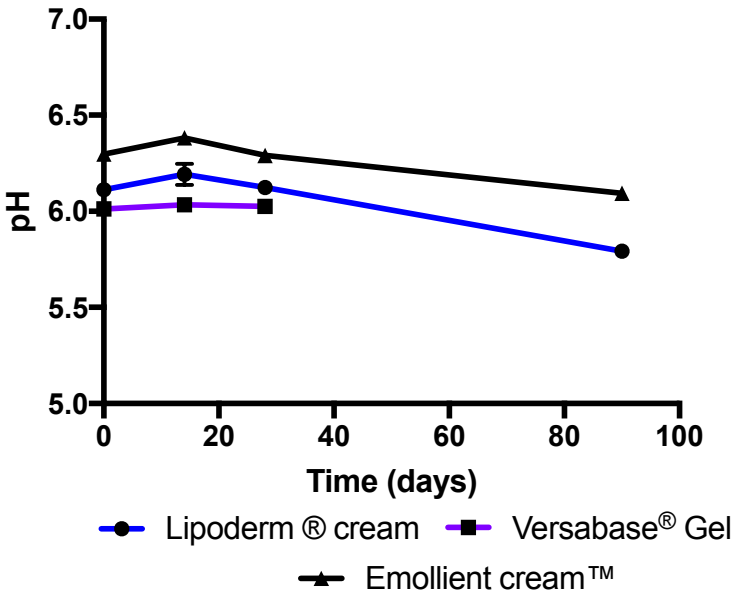

Figure 9. pH change of compounded gabapentin (10%) Lipoderm® cream, Versabase® gel and Emollient cream® over 90 days at room temperature. Data represent mean ± SD, n = 3.

The potency of gabapentin or percentage of initial concentration remaining in the topical formulations at each of the time points and storage conditions are summarized in Table 3**.** The potency of gabapentin in Lipoderm® base significantly increased after 28, and 90 days,respectively when stored at room temperature. Similarly, it also significantly increased at 14, 28 and 90 days when stored at 40 ^{o}C ($p<0.05$). However, no significant changes in potency was observed when stored at 4 ^{o}C ($p>0.05$). When formulated in Versabase® gel, no significant change in potency was noticed over the 90-day period and temperature conditions investigated ($p>0.05$). In contrast, there was significant differences between each of the time points in all storage conditions when Emollient cream® was used as a base. This is particularly pronounced when day 0 is compared to day 90.

Table 3. Potency of gabapentin (10%) in Lipoderm® base, Versabase® gel and Emollient cream®. Mean±SD (n = 3).

	Temperature	Percentage of Initial Concentration Remaining			
		Day 0	Day 14	Day 28	Day 90
Lipoderm® Base	RT	99.1± 1.2	102±1.4	106±3.7	146 ± 4.2
	4°C	99.1 ± 1.2	100.1± 2.3	99.3± 0.6	98.2 ± 2.3
	40°C	99.1± 1.2	119.6 ±4.2	136± 2.3	140± 1.8
Versabase® gel	RT	102.3± 0.8	117± 4.2	128.5± 3.2	NA
	4°C	102.3± 0.8	92.3± 1.3	99.3± 4.1	96.5 ± 0.4
	40°C	102.3± 0.8	88.4± 37.0	99.1± 22.8	102±22.4
Emollient cream®	RT	103.6± 1.3	96.8± 0.5	117.5± 3.5	148.2± 0.6
	4°C	103.6± 1.3	91.5± 1.3	106.5±2.1	108.8± 1.6
	40°C	103.6± 1.3	115.1± 2.8	140.5± 2.1	151.6± 1.5

RT = Room Temperature

The organoleptic properties consisting of physical appearance, colour, homogeneity, phase separation, texture and immediate skin feel were observed throughout the 90 days and are summarized in Table 4. At room temperature, the organoleptic properties for gabapentin compounded in Lipoderm® base at room temperature remained relatively consistent for up to 28 days, but showed signs of physical instability in other bases and storage temperatures.

Table 4. Qualitative description of organoleptic properties of compounded topical gabapentin (10%).

		Lipoderm® Base	Versabase® gel	Emollient® cream
Day 14	RT	Same as time 0.	Same as time 0.	Cream is more viscous and feels slightly gritty and leaves small, flat crystalline substance on skin after application.
	4 °C	Same as time 0.	Feels slightly gritty when applied to the skin.	Feels slightly gritty when applied to the skin.
	40 °C	Gritty and paste-like.	Small white crystalline aggregates formed. Cannot place on skin as aggregates are abrasive.	Cream feels slightly gritty and leaves small, flat crystalline substance on skin after application.
Day 28	RT	Same as time 0.	Large white crystalline aggregates formed. Cannot be spread on skin.	Cream is more viscous and feels slightly gritty and leaves small, flat crystalline substance on skin after application.
	4 °C	Creamy and spreadable. Slightly gritty with visible small crystals. Had to be mixed before it became spreadable.	Small white crystalline aggregates formed. Cannot spread on skin as aggregates are abrasive.	Cream feels slightly gritty and leaves small, flat crystalline substance on skin after application.
	40 °C	Gritty and paste-like.	Large white crystalline aggregates formed. Cannot be spread on skin.	Cream is more viscous, gritty and leaves small, flat crystalline substance on skin after application.
Day 90	RT	Gritty and paste-like.	Gel has become "gummy-like" with a large, solid white, crystalline aggregates. (See Figure 10).	Cream is more viscous and feels slightly gritty and leaves small, flat crystalline substance on skin after application.
	4 °C	Gritty and more viscous however it is spreadable. Had to be mixed before it became spreadable.	Small white crystalline aggregates formed. Cannot spread on skin as aggregates are abrasive.	Cream feels slightly gritty and leaves small, flat crystalline substance on skin after application.
	40 °C	Gritty and paste-like. Does not spread on skin at all.	Separation of gel with a large, solid white, crystalline aggregate.	Pasty, very gritty, cannot spread on skin.

RT = Room Temperature

Crystallization of the compound was visible when compounded in Versabase® gel and Emollient cream® as early as the 14-day mark. Crystallization was quite pronounced in the Versabase® gel as depicted in Figure 10.

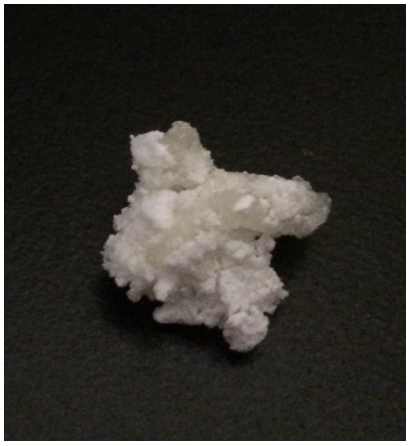

Figure 10. Compounded gabapentin (10%) in Versabase® gel stored at room temperature for up to 90 days. Physical degradation due to crystalization is visible.

2.4 DISCUSSION

Establishing reliable beyond use dating (BUD) based on the literature, pharmaceutical science knowledge and relevant guidelines, is a legal requirement for the compounding pharmacist [47]. Hence, generating reliable data to support or refute current BUD estimates based on the USP guidelines is an immense help for the practicing community pharmacist.

The first step to conduct stability studies is to develop or modify a stability indicating analytical assay [53]. A method is considered stability indicating if the degradant products appear as a separate peak from the intact drug [53]. Degradation products are attained by exposing the active pharmaceutical ingredient to conditions expected to degrade the drug such as high heat, acid exposure, base exposure, UV radiation, and peroxide exposure [53]. The method used for the analysis of gabapentin samples was

stability indicating as there were no interfering peaks under all four harsh conditions as seen in Figure 5.

It is clear from Figure 5 that gabapentin is susceptible to degradation based on extremes of pH (high and low). A known mechanism of gabapentin degradation in the literature is through intramolecular cyclization to form a lactam by nucleophilic attack of the amine on the carboxylate group followed by dehydration [44]. At the pH ranges in Figures 7-9, gabapentin exists as a zwitterion and is in equilibrium with the species thought to be the reactive form which allows for the proton transfer from the amine to the carboxylate, forming a lactam [44]. Additionally, the rate of lactam formation is least likely to occur in the pH range of 5.5-6 [44]. Given that the pH values recorded in this study were predominately above this range, the poor stability of gabapentin may be attributed to lactam formation.

Acceptable potency of a pharmaceutical active product is between 90 and 110% of the labelled claim [54]. Gabapentin (10%) compounded in Lipoderm® base was stable for 28 days at room temperature. After this time, it had a pasty and gritty consistency unsuitable for patient use. The potency of the product was also much higher than the 110% maximum requirement set by the FDA, which is possibly due to excessive water evaporation and shrinkage of the base [54]. At 40^{o}C, gabapentin was not stable at all with a gritty and paste like appearance at the 14-day mark. This may have occurred because of increased rates of lactamization at higher temperatures and lower humidity and moisture [44]. Although the potency of this formulation remained within 90 to 110% over the 90-day period at 4^{o}C, the cream had visible crystals. This may be explained by decreased gabapentin solubility at lower temperatures, which resulted in visible crystallization [55].

Gabapentin (10%) in Versabase® gel was not stable at all temperatures as the organoleptic properties are not suitable for patient use. The consistency of the product was gritty with visible large white crystals of gabapentin. Crystallization of a drug from a hydrogel base is often a sign that the drug is not fully soluble in that formulation [56]. Although initially gabapentin appeared to be well incorporated into the base, as the water content within the gel decreased, the formulation became saturated with gabapentin beyond its maximum solubility point resulting in crystallization [57]. The very large standard deviation observed in the percent potency of gabapentin stored at 40°C (Table 3) was likely due to a lack of drug uniformity because of drug crystallization. Additionally, at higher temperatures, the structure of the gel likely changed. With the evaporation of water the structure of the gel shrunk due to the surface tension of the remaining water pulling the polymer chains together [58]. The Ecolojars® used for storage were not airtight and could have facilitated a more rapid decline of water content in the base which consequently may have increased lactamization of gabapentin.

Gabapentin 10% in Emollient cream® also displayed poor physical stability with the presence of flat translucent crystals in all three storage conditions. Physical instability particularly at 40°C where significant water evaporation occurred, resulted in a paste-like base. The increase in observed gabapentin potency is likely due to decrease in water content resulting in a more concentrated product.

2.5 CONCLUSIONS

Compounded preparations of gabapentin 10% in Lipoderm® base were stable in Ecolojars® for 28 days at room temperature. The potency was higher than the acceptable range beyond this point as well as at 40 °C. Changes in the organoleptic properties were

observed at both high and low temperatures of 4^{o}C and 40 ^{o}C. Hence, this compound should not be stored in those conditions. Gabapentin 10% was not stable in both Versabase gel® as well as Emollient cream®. Therefore, based on this information, the beyond use date of currently dispensed gabapentin 10% formulation in Lipoderm® cream should not be extended beyond the currently used 30-day mark as well. Pharmacists that currently compound gabapentin in Versabase gel® and Emollient cream® may want to consider its lack of stability prior to dispensing it in the future. Additionally, the use of Ecolojars® does not appear to be appropriate for these preparations. Based on our data, we recommend the use of air-tight containers for compounded gabapentin formulations. Similar approach may be adopted for other products with comparable stability profiles.

CHAPTER 3- STABILITY ASSESSMENT OF TOPICAL AMITRIPTYLINE EXTEMPORANEOUSLY COMPOUNDED WITH LIPODERM® BASE, MEDIFLO®30 PLO GEL, AND EMOLLIENT CREAM®.

3.1 INTRODUCTION

Amitriptyline hydrochloride (HCl) is a tricyclic antidepressant (TCA) used clinically for neuropathic pain [59]. Semi-solid formulations containing amitriptyline have been reported to produce an analgesic effect when applied topically [20,21]. The topical mechanism of action may be due to sodium channel inhibition [60]. Amitriptyline is a white crystalline chemical with a molecular weight of 277.4 g/mol [61]. As seen in Figure 11, it is a tertiary amine dibenzocycloheptene TCA with a propylidene side chain extending from the carbocyclic ring in the centre [62]. The diarylpropylideneamine moiety makes is sensitive to photo-oxidation hence dispensing in a container that protects it from light is important [62]. The hydrochloride salt is freely soluble in water, alcohol, chloroform and methanol and is very lipophilic with a LogP of approximately 3 [61].

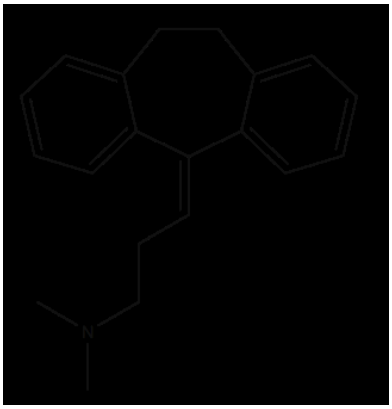

Figure 11. Chemical Structure of amitriptyline

Topical amitriptyline is not available as a commercial product and must be compounded for individual patient use. Pharmaceutical products manufacturers are strictly regulated by national authorities such as the Food and Drug Administration (FDA) or Health Canada. They are required to provide an expiry date for all their pharmaceutical products. Compounding pharmacists, however, are excluded from this regulation and can assign beyond use dates based on the published literature data and/or the United States Pharmacopeia (USP) guidelines [48]. The USP requires beyond use dating (BUD) for all compounded preparations dispensed by pharmacists [46]. When a drug is compounded in a water containing formulation intended for topical use, a beyond use date of no more than 30 days is assigned by the compounding pharmacist, unless stability data exists [48].

Literature search indicated that there are no reports on topical amitriptyline (10%) stability in Lipoderm® base, Mediflo®30 PLO gel, and Emollient cream®. The purpose of this study was to determine the stability of amitriptyline in Lipoderm® base, Mediflo® 30 PLO gel, and emollient cream over a 3-month period under various storage temperatures {room temperature (25^{o}C), refrigeration (4 ^{o}C) and hot temperature (40 ^{o}C)}. These bases were selected based on literature information and the fact that amitriptyline is compounded in community pharmacies with them. A higher strength of 10% was selected for stability studies due to case reports reporting greater efficacy and success compared to lower strengths [20,21].

3.2 MATERIALS AND METHODS

3.2.1 Materials

Amitriptyline HCl (Lot: CC006121), Lipoderm® Base (Lot: 7048812), and emollient cream (Lot: 7302343) were kindly provided by PCCA (Houston, Texas, USA). Mediflo® 30 PLO gel (Lot: I243P/B) was purchased from Medisca Inc. (St-Laurent, QC, Canada). Ethoxy diglycol was procured from Galenova (Lot: 14164-5104-5321). Acetonitrile (Lot: 171203) of analytical grade was purchased from Fisher Scientific (NJ, USA). Deionized water (18 Ω) was available in the lab using a Barnstead Nanopure II filtering system. Ecolojars® (Figure 12), containers of adjustable volume, were purchased from Mckesson Canada (Moncton, NB, Canada).

Figure 12. Ecolojars® containers used for storage of topical amitriptyline compounds

3.2.2 Instrumentation

A Varian 920-LC chromatography unit (Agilent Technologies, Mississauga ON, Canada) equipped with a low-pressure quaternary gradient pump with built-in 4-channel degasser, and a refrigerated auto sampler with a desktop computer loaded with Galaxie Chromatography Data System software was used for the analysis of amitriptyline. A CP224S Sartorius balance was used for standard and sample preparation. Chemical separation was achieved using a stationary phase consisting of a Waters u-Bondapak 10 µm C18 125 Å, LC Column 300 x 3.9 mm (Serial No 023033615252005).

3.2.3 Chromatographic Conditions

The HPLC assay used for the studies was a validated stability-indicating USP method [63]**.** The mobile phase consisted of a buffer and acetonitrile in a ratio of 58:42, v/v. The buffer was prepared by dissolving 11.04 g monobasic sodium phosphate in 900 mL of deionized water and the pH adjusted to 2.5 ±0.5 with 70% phosphoric acid. The solution was then diluted with water to a final volume of 1000 mL. It was then vacuum-filtered through a 0.22µm nylon filter and degassed for 20 minutes. The column was kept at ambient temperature while the flow rate was maintained at 2 mL/min. The injection volume for each sample was 20 µL. The detection wavelength was 254 nm and under the described chromatographic conditions the retention time of amitriptyline was 3.4 minutes.

3.2.4 High Performance Liquid Chromatography Assay Validation

Amitriptyline samples were placed under stressful conditions (heat, basic and acid) to facilitate forced degradation for validating stability-indicating nature of the selected assay method. Amitriptyline degradation was evaluated by preparing a 1000 µg/mL amitriptyline HCl solution in deionized water. A volume of 0.5 mL of the solutions was added to vials with aqueous NaOH (0.1 M, 0.5 mL) and aqueous HCl (0.1 M, 0.5 mL). These two solutions were stored at ambient temperature for 4 hours. A third vial containing only amitriptyline diluted to 500 µg/mL with deionized water was heated at 80^{o}C for 4 hours. The three solutions were later scanned with HPLC for peaks.

The chromatographic method was validated for specificity, linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) as per the International Conference on Harmonization (ICH) guidelines [52]. Specificity was assessed by comparing scans of the receptor medium alone, receptor medium mixed with the topical bases to be used and a standard solution of amitriptyline 1000 µg/mL. An

initial 2000 µg/mL stock solution was made by dissolving 20 mg of amitriptyline HCl powder in 10 mL of deionized water. Standard amitriptyline concentrations of 10, 25, 50, 100, 200, 400, 600, 800, and 1200 µg/mL were prepared from the stock solution. The concentrations (x-axis) were plotted against the area under the curve (AUC) and regression analysis was performed to generate a calibration curve. The range was determined by finding the highest and lowest concentrations of standard solutions where there is acceptable linearity, accuracy, and precision. Accuracy of the method was determined by injecting three quality control (QC) standard samples (50 µg/mL, 600 µg/mL and 1000 µg/ml) into the HPLC in triplicates and determining percentage recovery. Additionally, recovery of amitriptyline from Lipoderm® base, Mediflo®30 PLO gel, and Emollient cream® was also investigated. The intra-day precision or repeatability of the method was estimated by calculating percentage coefficient of variation (%CV) of three QC standards (low QC = 100 µg/mL, intermediate QC = 600 µg/mL, and high QC = 1000µg/mL) injected into the HPLC in triplicates. Inter-day precision was determined by injecting the QC standard samples in triplicates on three different days and calculating %CV. Values under 5% were considered acceptable [51]. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the equations 1 and 2 [52]:

$$\mathrm{LOQ} = \frac{10 \times \mathrm{SD}}{\mathrm{S}} \quad \text{.....}\textbf{Equation 1}$$

$$\mathrm{LOD} = \frac{3.3 \times \mathrm{SD}}{\mathrm{S}} \quad \text{.....}\textbf{Equation 2}$$

Where S = the slope of the calibration curve and SD = standard deviation of the y-intercept.

3.2.5 Compounding and Sample Preparation

To prepare the formulations, an appropriate amount of amitriptyline was weighed out and triturated to produce a fine powder using mortar and pestle. Required amount of ethoxy diglycol was added to the fine powder and levitated to produce a smooth paste. Each of the bases to be used was added to the prepared paste using the principles of geometric dilution. The cream was transferred to a jar and mixed using a Gako Unguator® electronic mortar and pestle (Norman, OK, USA) and processed once through an Exakt 50 ointment mill (Oklahoma City, OK, USA).The resulting amitriptyline (10%) in Lipoderm® base, Mediflo®30 PLO gel, and emollient cream were placed in Ecolojars®. Three jars containing each compound were stored at room temperature, 4°C and 40 °C, respectively. Samples were taken in triplicate after mixing the cream at days 0, 14, 28, and 90 and stored in a -80 °C freezer for later analysis.

On the day of analysis, samples were thawed for 1 hour at room temperature and diluted with deionized water and shaken using a Fisher Scientific digital vortex mixer (Fisher Scientific®, ON, Canada) for 30 minutes at 1500 RPM. The pH of each sample was measured and recorded using a calibrated pH meter. Samples were scanned for degradation peaks using HPLC and amitriptyline potency was calculated based on AUC values observed. Changes in mean pH values and potency at different time points were compared to time 0 by two-way analysis of variance (ANOVA). Tukey's multiple comparison post-hoc test was also performed using GraphPad Prism® software version 7.0 (GraphPad Software Inc., San Diego, CA, USA). P values <0.05 were considered statistically significant.

3.2.6 Physical Evaluation of Topical Compounds

The organoleptic properties consisting of physical appearance, colour, homogeneity, phase separation, texture and immediate skin feel were monitored and qualitatively described at each sampling time point.

3.3 RESULTS

3.3.1 HPLC Method Validation

The chromatographic method was specific for amitriptyline as no other peaks were detected. A clear peak obtained from injecting both the amitriptyline standard and sample solutions had a retention time of 2.8 minutes. Figure 13 is a representative chromatograph after injecting 20 µL of a 500 µg/mL amitriptyline solution.

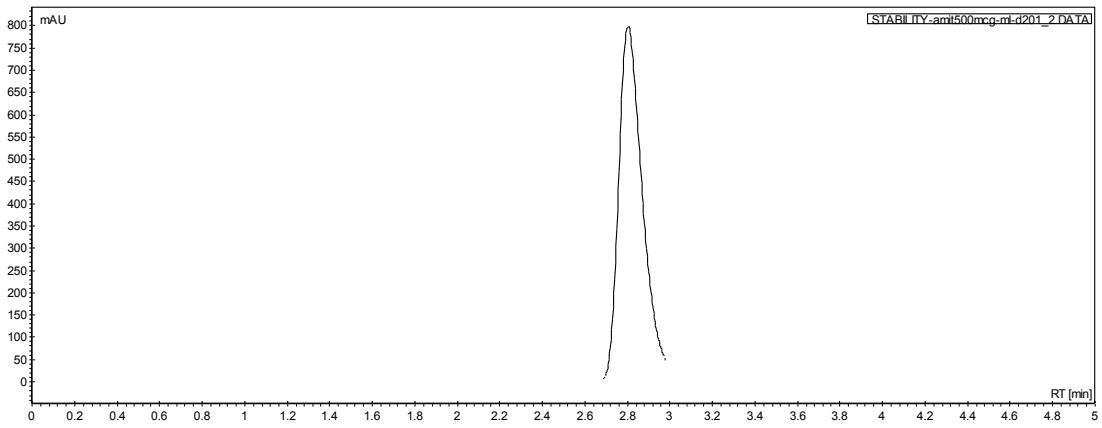

Figure 13. HPLC chromatogram for 20 µL of a 500µg/mL amitriptyline solution.

Linearity was confirmed using an analytical range of 10 µg/mL to 1200 µg/mL. A calibration curve was generated using the 9 standard solutions, as seen in Figure 14. The coefficient of determination (R^2) was 0.9996 and the equation for the standard curve was y = 14.806x + 91.401. The LOD and LOQ were calculated to be 1.54 and 5.10 µg/mL, respectively.

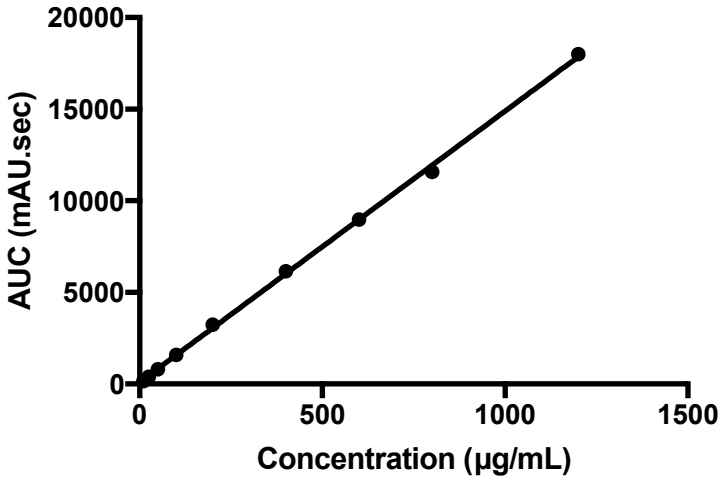

Figure 14. Linearity of amitriptyline assay within a concentration range of 10-1200 µg/mL.

The accuracy was acceptable with recovery of between 98.82- 100.53%. Results for the accuracy of this HPLC method are summarized in Table 5. Sample recovery, was determined to be $101.5 \pm 2.9\%, 101.4 \pm 3.1\%$ and $99.8 \pm 0.6\%$ for amitriptyline in Lipoderm® cream, Mediflo®30 PLO gel, and Emollient cream®, respectfully.

Table 5. Accuracy of amitriptyline assay method used for sample analysis

	50 µg/mL	600 µg/mL	1000 µg/mL
Amount recovered (µg/mL)	49.13 50 49.1	595.35 596.65 595.37	808.23 802.16 802.43
Mean	49.41	595.79	804.27
Standard deviation	0.51	0.74	3.42
%RSD	1.03	0.12	0.42
%Recovery	98.82	99.29	100.53

In the repeatability studies, the mean %CV for low (100 µg/mL), intermediate (600 µg/mL) and high standard (1000 µg/mL) QC amitriptyline solutions was 0.93, 0.78 and 0.42 %, respectively. The mean %CV for inter-day studies was 1.05%. All results obtained were within the acceptable limit of 5%. Precision data is summarized in Table 6.

Table 6. Intra-day and inter-day precision for amitriptyline (n = 3).

		Conc. (µg/mL)	Intra-day Precision			Inter-day Precision		
			Mean	SD	CV (%)	Mean	SD	CV (%)
Standard 1 100 µg/mL	Day 1	101.87 100.97 99.74	100.86	1.07	1.06	100.82	0.31	0.31
	Day 2	100.67 101.18 99.65	100.50	0.78	0.78			
	Day 3	102.21 100.79 100.34	101.11	0.98	0.97			
Standard 2 600 µg/mL	Day 1	595.35 596.65 595.37	595.79	0.74	0.12	593.96	6.20	1.04
	Day 2	608.99 596.93 591.18	599.03	9.08	1.51			
	Day 3	590.36 582.3 588.5	587.05	4.22	0.72			
Standard 1 1000 µg/mL	Day 1	1002.83 994.75 1000.52	999.37	4.16	0.42	1009.87	18.13	1.80
	Day 2	999.15 995.75 1003.41	999.47	3.83	0.38			
	Day 3	1025.5 1033.45 1033.48	1030.81	4.59	0.446			

3.3.2 Stability Indicating Method

Under acidic, alkaline and heat conditions, amitriptyline peaks were successfully monitored. There was no degradation peak that interfered with the parent peak as shown in

Figure 15. The retention time of amitriptyline in A, B, and C were 2.73, 2.8 and 2.74. This demonstrates that the assay can selectively monitor the amitriptyline peak under basic, acidic and high heat conditions.

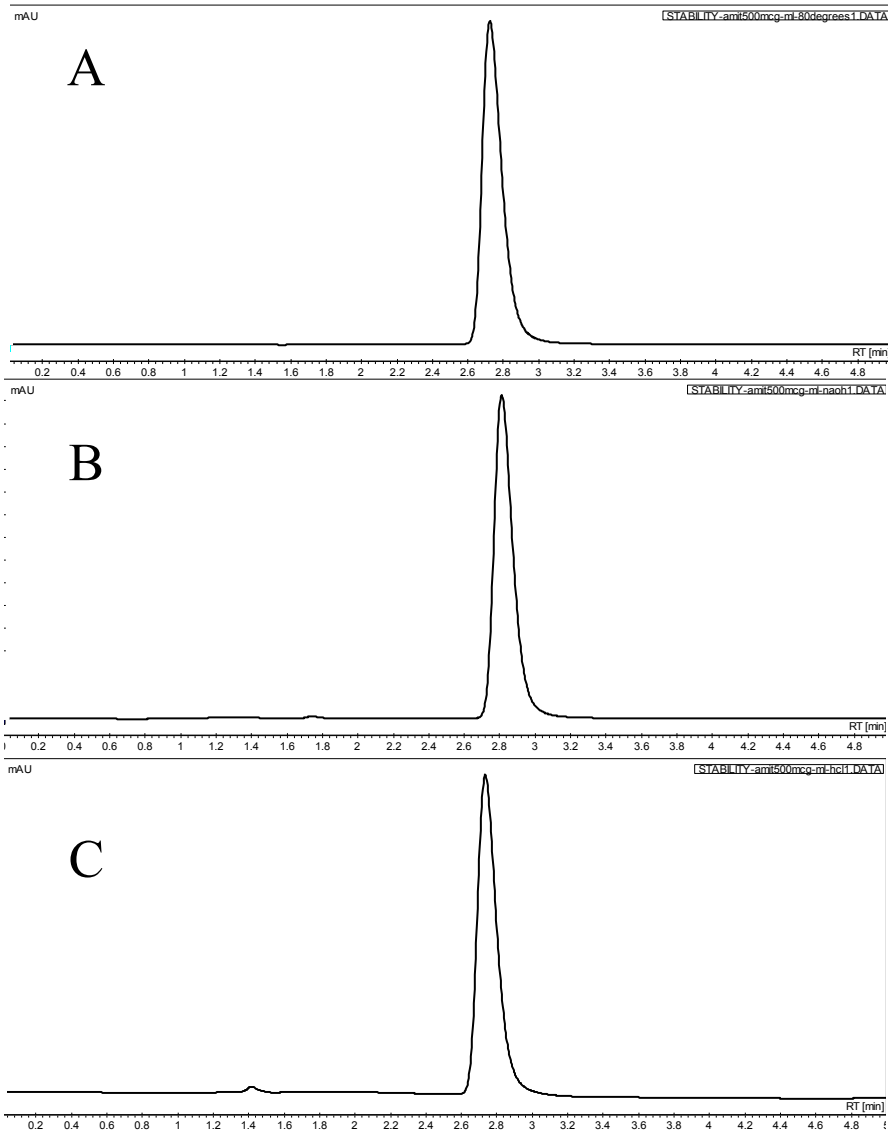

Figure 15. Amitriptyline forced degradation sample chromatograms under heat (A), basic (B), and acidic conditions (C).

The pH of all samples remained stable throughout the three-month duration as shown in Figure 16, Figure 17, and Figure 18**.** Statistical analysis of pH change of all topical preparation in each temperature condition revealed no significant changes in pH at all time points ($p>0.05$).

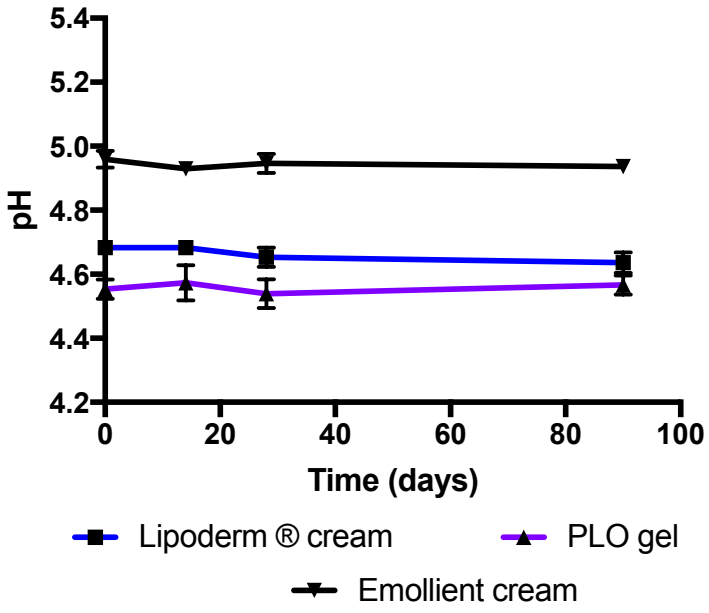

Figure 16. pH of Amitriptyline (10%) in Lipoderm® cream, Mediflow® PLO gel and emollient cream over 90 days at 40° C. Data represent mean ± SD, n = 3.

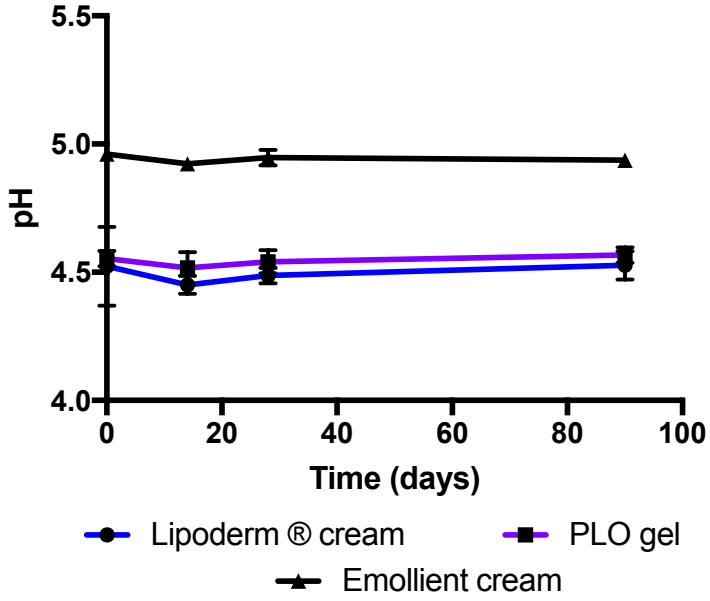

Figure 17. pH of Amitriptyline (10%) in Lipoderm® cream, Mediflow® PLO gel and emollient cream over 90 days at 4° C. Data represent mean ± SD, n = 3.

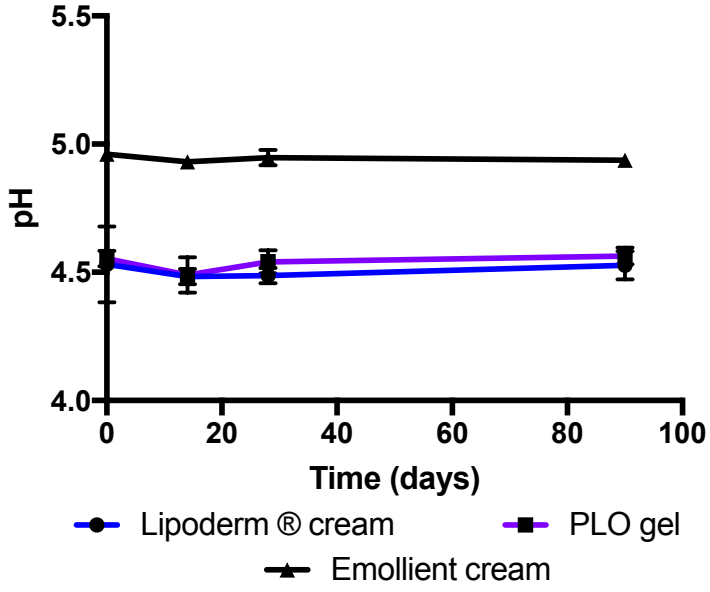

Figure 18. pH of Amitriptyline (10%) in Lipoderm® cream, Mediflow® PLO gel and emollient cream over 90 days at room temperature. Data represent mean ± SD, n = 3.

The potency of amitriptyline or percentage of initial concentration remaining in the topical formulations at each of the time points and storage conditions are summarized in Table 7. For amitriptyline (10%) in Lipoderm® base stored at room temperature as well as at 4°C, the drug potency significantly increases at day 90 when compared to day 1 ($p<0.05$). However, it remains within the acceptable potency of 90 to 110%. When the amitriptyline in Lipoderm® base preparation was stored at 40°C, there was a significant reduction in potency at day 14 ($p<0.05$), followed by a significant increase at both day 28 and 90, respectively ($p<0.05$).

Amitriptyline (10%) compounded in Mediflo® PLO gel displayed a significant decrease in potency when stored at both room temperature and 4°C ($p<0.05$). However, a significant increase in potency was observed when stored at 40°C ($p<0.05$). In contrast, amitriptyline compounded in emollient cream stored at all three temperatures had a statically significant decrease in potency ($p<0.05$).

Table 7. Potency of amitriptyline (10%) in Lipoderm® base, Mediflo® PLO gel and Emollient cream®. Mean±SD (n = 3).

	Temperature	Percentage of Initial Concentration Remaining			
		Day 0	Day 14	Day 28	Day 90
Lipoderm® Base	RT	101.5±2.9	103.95±1.6	102.2±2.7	109±0.5
	4°C	101.5±2.9	104±2.8	107±2.8	110.5±1.7
	40°C	101.5±2.9	78.7±3.9	90.4±4.2	110.95±1.1
Mediflo® PLO gel	RT	101.4±3.1	94.7±0.6	96.2±0.3	95.6 ±0.3
	4°C	101.4±3.1	93.4±3.5	92.8±4.8	94.8±0.2
	40°C	101.4±3.1	106.7±2.5	110.9±3.1	124.3 ±4.0
Emollient cream®	RT	99.8±0.6	61.8±0.2	72. 4 ±0.6	78.17±0.2
	4°C	99.8±0.6	54.4±0.4	60.1±2.4	59.7±4.9
	40°C	99.8±0.6	61.3±0.1	58.9±4.3	48.9 ±0.9

RT = Room Temperature

The organoleptic properties consisting of physical appearance, colour, homogeneity, phase separation, texture and immediate skin feel were observed throughout the 90 days and are summarized in Table 8. At room temperature, the organoleptic properties for amitriptyline compounded in Lipoderm® base at room temperature remained relatively consistent with only a slight increase in viscosity on day 90. The same was observed when compounded in Mediflo® 30 PLO. However, the gel was darker in colour. Crystallization of amitriptyline was visible when compounded in

emollient cream as early as on day 14, which implies that this base is not acceptable for compounding this drug.

Table 8. Qualitative description of organoleptic properties of topical amitriptyline (10%) compounds.

		Lipoderm® Base	Mediflo®30 PLO gel	Emollient cream®
Day 14	RT	Same as time 0.	Same as time 0.	Slightly more viscous and amitriptyline crystals visible. Gritty on the skin.
	4 °C	Same as time 0.	Same as time 0.	Same as time 0.
	40 °C	Same as time 0.	Slightly more viscous but spreadable on the skin.	Slightly more viscous and amitriptyline crystals visible. Gritty on the skin.
Day 28	RT	Same as time 0.	Same as time 0.	Same as day 14.
	4 °C	Same as time 0.	Same as time 0.	Same as time 0.
	40 °C	Slightly more viscous but spreadable on the skin.	More viscous but spreadable on the skin.	Same as day 14.
Day 90	RT	Cream is slightly more viscous however still spreadable and feels smooth on the skin.	Slightly more viscous, darker colour, but spreadable.	Much more viscous and amitriptyline crystals quite visible. Very gritty on the skin.
	4 °C	Cream is slightly more viscous however still spreadable and feels smooth on the skin.	Cream is slightly more viscous however still spreadable and feels smooth on the skin. Gel is more translucent rather than initially.	Cream is slightly more viscous however still spreadable and feels smooth on the skin.
	40 °C	More viscous, darker colour, and paste-like. Cannot spread on skin.	More viscous, darker colour, and paste-like. Cannot spread on skin.	More viscous and paste-like. Cannot spread on skin.

RT = Room temperature

3.4 DISCUSSION

In community practice, "potency" and "stability" are sometimes thought to refer to the same thing in terms of establishing beyond use dating. Acceptable potency of a pharmaceutical active product is between 90 and 110% of the labelled claim [54]. If a compounded product retains its potency throughout the set beyond use dating it cannot be thought to be stable as potency testing by itself is not an indication of stability of a product if the analytical method used is not stability indicating [53]. An analytical method is considered stability indicating if the degradant products appear as a separate peak(s) from the intact drug. Forced degradation of the active drug is achieved by subjecting the active ingredient to harsh conditions such as high heat, acid exposure, base exposure, UV radiation, and peroxide exposure [53]. The analytical method used for the analysis of amitriptyline samples was stability indicating as no interfering peaks were observed when exposed to forced degradation conditions. Additionally, amitriptyline in solution appeared stable at high temperatures as well as basic and acidic conditions, as seen in Figure 15.

Over the 90-day period described in the method section, amitriptyline remained stable in Lipoderm® base, as well as in Mediflo® 30 PLO stored at room temperature. However, by 90 days, there was a slight increase in viscosity, which may partially be due to the storage container used. The Ecolojar® which has replaced many of the old screw cap jars appears less airtight, which can contribute to evaporation of water contained in the bases, hence resulting in a more "potent" formulation as seen in Table 7. As physical changes were observed when these compounds were stored at 4^{o}C and 40 ^{o}C, dispensed products should be protected from prolonged exposure to high (such as in a vehicle) or cold temperatures (such as in a fridge). Amitriptyline was not stable in emollient cream at all, hence compounding in this base is not recommended. Amitriptyline has been reported

to chelate to metal ion impurities leaking from storage containers [64]. Presence of a chelating agent in a base, such as edetate disodium, is hypothesized to reduce amitriptyline decomposition in this manner [64]. Emollient cream® does not contain any chelating agent which may have contributed to poor amitriptyline stability in the base.

3.5 CONCLUSIONS

Compounded preparations of amitriptyline (10%) in Lipoderm® base, as well as Mediflo® 30 PLO were stable in Ecolojars® for 90 days at room temperature. Physical changes were observed at high and low temperatures, hence these compounds should be stored at room temperature and not exposed to temperatures as low as 4^{o}C and as high as 40 ^{o}C. Amitriptyline was not stable in Emollient cream®. The information generated from this study may be used to increase the beyond use date of 30 days currently used to 90 days, which can produce significant cost savings to patients as these products tend to be expensive.

CHAPTER 4- IN VITRO RELEASE PROFILE AND PERMEATION OF GABAPENTIN ACROSS SIMULATED HUMAN SKIN STRAT-M® WHEN COMPOUNDED WITH LIPODERM® BASE, VERSABASE® GEL, AND EMOLLIENT CREAM®.

4.1 INTRODUCTION

Neuropathic pain, as defined by the International Association for the Study of Pain (IASP), is "pain caused by a lesion or disease of the somatosensory system" [1]. It is a problematic condition because it is usually chronic in duration and resistant to many analgesics [7]. Oral gabapentin is one of the recommended first line pharmacologic therapies recommended for neuropathic pain [7]. It acts by inhibiting voltage gated calcium channels in the presynaptic nerve, hence resulting in a significant reduction in excitatory neurotransmitter release [10,11]. It also acts as an inhibitor of the N-Methyl-D-aspartic acid (NMDA) receptors in the periphery to reduce hyperalgesia and allodyna [45]. Common side effects of oral gabapentin include dizziness, somnolence, fatigue, and peripheral edema [5]. Although the oral route is commonly used for administering medications, it may not always be desirable due to side effects, drug-drug interactions, and inconvenient dosing regimens. Using the skin as an alternative or concurrent route of administration has many advantages such as direct analgesics delivery to the peripheral nerves, no first pass metabolism, and reduced systemic side effects [5].

Currently, topical gabapentin formulations must be compounded by specialized pharmacies, as no commercial product exists. Although there are many anecdotal stories of success with topical gabapentin, there is very little data in the literature evaluating its efficacy. In a retrospective study of 51 patients with either generalized or localized

vulvodynia, different concentrations of gabapentin cream (2%, 4%, 6%) in Lipoderm® base were applied over an 8-week period. After a minimum of eight weeks of therapy, mean general pain scores decreased from 7.26 to 2.49 (95% confidence interval: –5.47 to –4.07) [23]. A pain score reduction of 30% is considered of moderate clinical benefit, while a reduction of greater than 50% is a substantial improvement [65].

In a prospective study of 23 patients with severe post herpetic neuralgia and other neuropathic pain syndromes, researchers found that of the 23 patients who received and applied gabapentin cream (6%), 20 had benefited from the treatment, including 2 of the 3 women who had post herpetic neuralgia [22]. These patients' pain scores were reduced from 8.2± 1.4 to 5.6 ±1.7 after 1 month of gabapentin cream use. Additionally, in patients where the topical formulation was efficacious, onset of analgesia occurred within 30 minutes of application. The constituents of the topical base used in this prospective study were not described in the paper [22].

Gabapentin is a small molecule with a molecular weight of 171.34 g/mol and a logP of -1.10 [43]. Previous studies investigated the permeation of gabapentin (5 and 10%) in Lipoderm, but did not show its drug release characteristics. Furthermore, there are no published data on the release and permeation of gabapentin in other commonly used topical bases such as Emollient cream® and Versabase® gel. The purpose of this study was to investigate gabapentin release mechanisms from Lipoderm® Cream, VersaBase® Gel and Emollient Cream®, and its permeation characteristics across simulated human skin, Strat-M®.

The ingredients contained in Lipoderm® Cream, VersaBase® Gel and Emollient Cream® are listed in Table 9.

Table 9. Ingredients contained in PCCA Lipoderm® Cream, VersaBase® Gel and Emollient Cream® [66,67,68].

PCCA Lipoderm Cream®	PCCA VersaBase® Gel	PCCA Emollient Cream®
1. Purified Water 2. Preliposomic Phosphatidylcholine 3. Isopropyl Myristate Cetearyl Alcohol 4. and Ceteareth-20 5. Stearyl Alcohol 6. Cetyl Alcohol 7. Caprylic / Capric Triglycerides 8. Wheat Germ oil 9. Glycerin 10. Polydimethylsiloxane 11. Magnesium Aluminum Silicate 12. Xanthan Gum 13. Polyacrylamide 14. C13-C14 Isoparaffin 15. Laureth-7 16. Edetate Disodium 17. Butylated Hydroxytoluene 18. Phenoxyethanol 19. Methylchloroisothiazolinone/ Methylisothiazolinone	1. Purified Water 2. Ammonium Acryloyldimethyltaurate/ VP Copolymer 3. Aloe Vera 4. Edetate Disodium 5. Allantoin 6. Methylchloroisothiazolinone / Methylisothiazolinone	1. Purified Water 2. Base, Anhydrous 3. Sorbimacrogol Oleate 300 4. Butylated hydroxytoluene 5. Methylchloroisothiazolinone / Methylisothiazolinone

4.2 MATERIALS AND METHODS

4.2.1 Materials

Gabapentin USP (Lot: CC006973), Lipoderm® cream (Lot: 7048812) and Emollient cream® (Lot: 7038834) were kindly donated by PCCA (Huston, TX, USA). Versabase® gel (Lot: 7155191) was purchased from PCCA (Huston, TX, USA). Ethoxy diglycol (Lot:14164-5104) was acquired from Galenova (Québec, Canada). Ninhydrin (Lot: BCBP3847V) and *N,N*-Dimethylformamide anhydrous (Lot: SHBG9734V) were from Sigma-Aldrich (Oakville, ON, Canada). Chloroform (Lot:63906) was bought from Xenex Laboratories Inc. (Coquitlam, BC, Canada). Phosphate buffered saline (Lot: I2614) 10X was purchased from Sigma-Aldrich (Oakville, ON, Canada). Deionized water (18 Ω) was available in the lab using a Barnstead Nanopure II filtering system. Strat-M®

membrane (Lot: K3BA7673) was purchased from EMD Millipore (Billerica, MA, USA). Coarse cellulose filter paper were purchased from Fisher Scientific (Ottawa, ON, Canada). Cellulose acetate membranes (Lot: 530CDC) with a pore size of 0.47 µm were obtained from Geotech Environmental Equipment Inc. (Denver, CO, USA). Tuffryn® membrane filters (Lot: 64535) with a pore size of 0.45 µm were obtained from Pall Corporation (Ann Arbor, Michigan, USA). Six Hamilton 18G, 4" needles with a metal hub were purchased from Chromatographic Specialties Inc. (Brockville, ON, Canada).

4.2.2 Instrumentation and Other Components

Six, 9mm clear jacketed Franz Cells with flat ground joint and 5 mL receptor volume (Part number: 4G-01-00-09-05) and V-series stirrer (V6-CA-01) were purchased from PermeGear, Inc. (Hellertown, PA, USA). A Lauda Ecoline E100 heated water bath circulator (Lauda-Koenigshofen, Germany) was used to maintain the Franz cells at a specific temperature. A Cary 50 UV-Vis Spectrophotometer (Serial number 03037676; Varian Inc., CA, USA) and Cary WinUV software was used for analysis of gabapentin. Microcell quartz cuvettes (10 mm path length, 50 µL) were used for spectrophotometric analysis.

4.2.3 Spectrophotometric Method Validation for Quantification of Gabapentin

The spectrophotometric method used for studies described in this section was validated for linearity, accuracy, precision, limit of detection (LOD), and limit of quantification as per the International Conference on Harmonization (ICH) Q2 guidelines for validation of analytical procedures [69]. A stock solution of gabapentin 1 mg/mL was prepared by dissolving 10 mg of gabapentin powder in 10 mL deionized water. Additionally, a 0.2% ninhydrin solution was prepared by dissolving 20 mg ninhydrin in

10 mL of in *N,N*-Dimethylformamide. Both solutions were freshly prepared daily. Ten aliquots ranging from 0.01 to 5.0 mL of gabapentin 1 mg/mL solution were transferred into test tubes. To each test tube, 2 mL of ninhydrin 0.2% was added and the volume was made up to the 10-mL mark with deionized water. The final concentration of these solutions was 25, 50, 100, 150, 200, 250, 260, 270, 280, and 290 µg/mL. The solutions were heated in a water bath at 90±5 ^{o}C for 5 minutes then cooled to room temperature. The optimal volume of ninhydrin 0.2% solution, water bath temperature, and time to heat the solutions were previously optimized and validated in previous studies [70,71]. The absorbance of the solutions was then measured against a reagent blank at 405 nm. Gabapentin contains a primary aliphatic amino group, which reacts with the ninhydrin reagent by oxidative deamination of the amino group, followed by condensation of the reduced ninhydrin to form the characteristic purple colour [72]. Figure 19 shows the reaction scheme between gabapentin and ninhydrin [72].

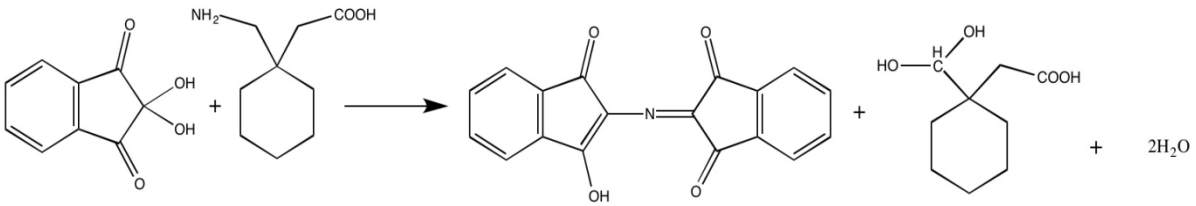

Figure 19-Chemical reaction between gabapentin and ninhydrin [72].

The concentrations (x-axis) were plotted against the absorbance values and regression analysis performed to generate a calibration curve. The range was determined by finding the highest and lowest concentrations of standard solutions where there is acceptable linearity, accuracy, and precision. Accuracy of the method was determined by scanning three quality control (QC) standard sample (50 µg/mL, 100 µg/mL and 250 µg/ml) against a blank and determining percentage recovery. The intra-day precision or

repeatability of the method was estimated by calculating percentage coefficient of variation (%CV) of three QC standards (low QC = 25 µg/mL, intermediate QC = 150 µg/mL, and high QC = 290µg/mL) and scanned in triplicate. Inter-day precision was determined by scanning the QC standard samples in triplicates on three different days and calculating %CV. Values under 5% were considered acceptable [51]. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on equations 1 and 2 below [52]:

$$\mathrm{LOQ} = \frac{10\times \mathrm{SD}}{\mathrm{S}} \quad \text{.....Equation 1}$$

$$\mathrm{LOD} = \frac{3.3\times \mathrm{SD}}{\mathrm{S}} \quad \text{.....Equation 2}$$

Where S = the slope of the calibration curve and SD = standard deviation of the y-intercept.

4.2.4 Preparation of Topical Compounded Gabapentin

Gabapentin was compounded with Lidoderm® Base, Versagel® base, and emollient cream®. Three different concentrations (1, 5, and 10%) of the drug were compounded by measuring the required amount of gabapentin powder into a plastic weigh boat, wetting with ethoxy diglycol until a thick paste was formed. Slowly the base was incorporated using the principles of geometric dilution until the compound appeared uniform and smooth. The various formulations were freshly compounded prior to permeation or release studies.

4.2.5 Gabapentin Extraction and Recovery

Gabapentin 10% was compounded with Lipoderm® cream, Emollient cream® and Versabase® gel. Additionally, a blank formulation containing the base as well as ethoxy diglycol was also prepared. For the 10% gabapentin in Versabase® gel formulation, 0.1g

was weighed and placed in a 10-mL falcon tube. To this, 10 mL of deionized water was added and the mixture was shaken on a Fisher Scientific digital vortex mixer (Fisher Scientific®, ON, Canada) for 15 minutes at 1500 RPM. Afterwards, the mixture was sonicated for 30 minutes at 60°C. A 0.5 mL sample was derivatized and analyzed using the method described in section 4.2.3. For gabapentin formulated in Lipoderm® and Emollient cream®, 0.1g was weighed and placed in 10 mL Pyrex centrifuge tubes. Thereafter, 5 mL of deionized water and chloroform were added to the sample, respectively. The same procedures used for shaking and sonicating the gel formulations were followed for the cream formulations. To further enhance separation of gabapentin from the cream bases, samples were centrifuged under refrigeration (4°C) for 30 minutes at 4000 RPM. The supernatant solutions were collected and analyzed using the same spectrophotometric method described above. Versabase® gel is fully soluble in water, while Lipoderm® and Emollient cream® are not, hence the different extraction methods. All experiments were done in triplicate and mean percent recovery and standard deviations were calculated.

4.2.6 *In Vitro* Drug Release and Permeation Studies

To characterize the release rate of gabapentin from different topical formulations, *in vitro* drug release studies were carried out in a Franz diffusion cell system with a diffusion area of 0.64 cm^2 and a receptor medium capacity of 5 mL. The system was maintained at a constant temperature of 32 $\pm$ 0.5 °C with a Lauda Ecoline E100 heated water bath circulator. PBS (5 ml, pH 7.4) was slowly added to each Franz cell through the receptor chamber orifice. Small magnetic stir bars were placed into the receptor chambers of each cell and the system was left to equilibrate for a minimum of 60 minutes.

To decide which membrane provides the least resistance to the diffusion of the active compound, cellulose filter paper, cellulose acetate and Tuffryn® membranes were soaked in PBS for 30 minutes. Each wetted membrane was placed on top of one receptor chamber with the Teflon® O-ring and donor chamber placed over the membrane and secured with a metal clamp. Gabapentin 1 mg/mL (0.5 mL) was added to the donor chamber, after which both the donor chamber chimney and sampling port were covered with parafilm. Receptor medium was stirred constantly at 650 RPM. The 4" blunt needles were used to withdraw 0.5 mL samples from the sampling port over a 6-hour period (0, 0.5, 1, 2, 3, 5, 6 hours). After each sampling, 0.5 mL of fresh PBS was added back into the receptor chamber to maintain sink conditions and PBS contact with the membrane. The samples were derivatized by adding 0.5 mL of 0.2% ninhydrin solution, 1.5 mL deionized water and heating in a 90 $\pm$5 ^{o}C for 5 minutes. Gabapentin concentration in each sample was quantified by spectrophotometry and the cumulative drug release calculated using equation 3. The mean percentage cumulative diffusion of gabapentin through the three membranes were calculated.

Cn = C'n + Vs/Vt (C'n-1+-----+C'1)……**Equation 3**

Where, Cn, C'n-1, C'1 = concentration at n, previous sample, and first sample, respectively. Vs, Vt = Volumes of sample in the donor and receiver compartments, respectively.

The membrane that demonstrated the least resistance to gabapentin diffusion and was cheapest to use was selected for all subsequent drug release experiments. The membrane and Franz cells were set up identically except, instead of using gabapentin solution in the donor chamber, 100 $\pm$0.5 mg of prepared topical gabapentin was applied

on the membrane using a glass rod. The mass of the glass rod was recorded before and after application of the compound to determine the exact quantity applied. One cell was reserved as a blank containing the base with ethoxy diglycol only. Both the sampling port opening and the donor chamber chimney were covered with parafilm. Samples (0.5 mL) were drawn at the 0.5, 1, 2, 3, 4, and 6-hour mark. The same volume of receptor medium taken during each sample draw was replaced with fresh PBS. Cumulative drug release was calculated using equation 4 [31]**:**

$Q = (C_n V + \sum_{i=1}^{n-1} Ci\ S)$……**Equation 4**

Where Cn is the concentration of drug determined at nth sampling interval; V is the volume of the Franz diffusion cell, $\sum_{i=1}^{n-1} Ci$ is the sum of concentrations of drug determined at sampling intervals 1 through n-1 and S is the surface area of the sample well.

Data obtained were fitted to the first order, second-order, Higuchi, Korsmeyer-Peppas, and Hixon-Crowell kinetic models to determine the mechanism of drug release from each of the formulations. The kinetic model yielding a linear function with an R^2 closest to 1 is the most likely mechanism of drug release. Where the release mechanism is unclear due to a high degree of linearity with multiple models, the slope of the Korsmeyer-Peppas model was used to clarify the mechanism.

Drug permeation studies were conducted in an identical manner to the drug release studies, except for the membrane utilized and the fact that the receptor or sampling port was not occluded. Strat-M®, which is an *in vitro* membrane model that functionally simulates drug permeation through human skin, was placed on the receptor chamber and did not require hydration prior to use. Each topical formulation was tested in

triplicate and flux and lag time were calculated. The steady-state flux was the slope divided by the diffusional area from the linear portion of the cumulative drug permeation graph, while lag time was the x-intercept. Additionally, after permeation experiments were completed, Strat-M® and the diffusion cell was rinsed with deionized water, prepared as described in section 4.2.5 and scanned to determine overall drug recovery.

4.2.7 Statistical Analysis

Microsoft® Office Excel 2011 was used to calculate *in vitro* release and permeation data. Differences between mean cumulative percent release or permeation at each time point was determined using statistical two-way analysis of variance, ANOVA, with Tukey's multiple comparison post-hoc test using GraphPad Prism® software version 7.0 (GraphPad Software Inc., San Diego, CA, USA) with a 95% confidence interval. P values <0.05 were considered statistically significant

4.3 RESULTS

4.3.1 Spectrophotometric Method Validation

Upon heating the standard gabapentin solutions prepared with 0.2% ninhydrin in *N,N*-Dimethylformamide, a blue-purple solution, also called ruhemann's purple, was formed. When scanned with the spectrophotometer, two clear peaks were found at 405 nm and 570 nm wavelengths (Figure 20).

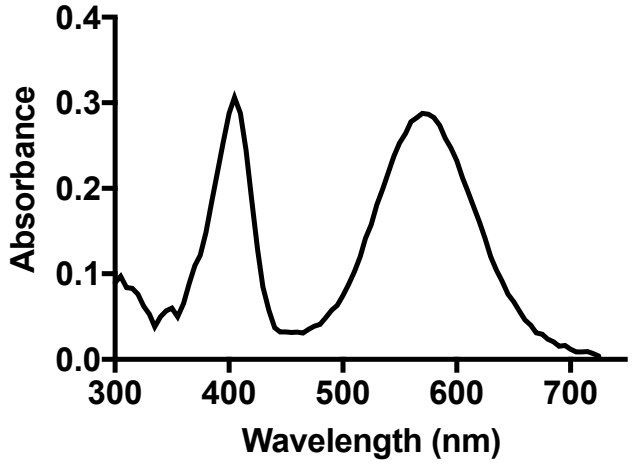

Figure 20. Absorption spectrum of reaction product of gabapentin 500 mcg/mL with ninhydrin 0.2% in *N,N*-Dimethylformamide.

As two peaks appeared, aqueous standard solutions of gabapentin were measured at both 405 and 570 nm against a blank. Linearity of the spectrophotometric method was confirmed for gabapentin using the analytical range of 25-290 µg/mL. Range was optimized based on concentrations expected in samples. A standard curve was then prepared by plotting known drug concentrations (x-axis) against UV absorbance values (y-axis) at 405 nm and 570 nm. To decide which peak absorbance value to use for future studies, a calibration curve was constructed using absorbance values obtained from scanning standard solutions at both 405 and 570 nm. A line of best fit was constructed and both were compared using the F-test on GraphPad Prism. It was found that the difference between slopes was not statistically significant (P = 0.0712). Hence, the linear regression equation y = 0.0007x-0.0171 (R^2 = 0.9910) found for the sample scans at 405 nm was used for future experiments. Linearity data is summarized in Figure 21.

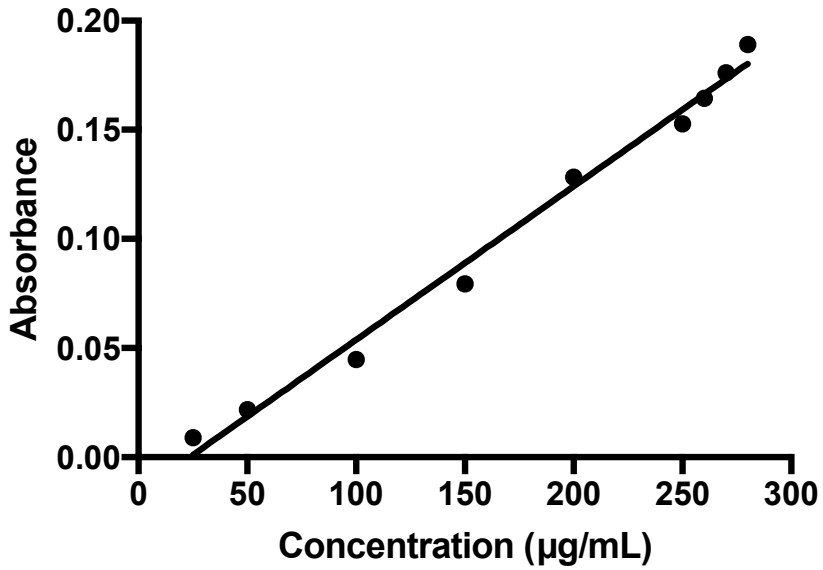

Figure 21. Linearity of gabapentin assay within a concentration range of 25-290 µg/mL.

The accuracy was acceptable with recovery being between 98.00 and 102.28%. Results for the accuracy of this method are summarized in Table 10. The LOD and LOQ were calculated to be 1.04 and 3.22 µg/mL, respectively.

Table 10-Accuracy of gabapentin quantification with Varian Cary 50 (n = 3).

	50 µg/mL	100 µg/mL	250 µg/mL
Amount Recovered (µg/mL)	51.1 49.9 52.4	103.0 99.9 102.4	243.6 242.4 249.0
Mean	51.1	101.8	245.0
Standard Deviation	1.3	1.7	3.5
%RSD	2.5	1.6	1.4
%Recovery	102.3	101.8	98.0

When conducting intra-day precision studies for this method, the mean percent coefficient of variation (%CV) for low (25 µg/mL), intermediate (150 µg/mL) and high (290 µg/mL) standard solutions was 3.38, 0.95, 1.06%, respectively. However, for inter-

day studies, mean %CV was 1.69 %. All results obtained were within the acceptable limit of 5%. Intra-day and inter-day precision data for gabapentin is summarized in Table 11.

Table 11-Intra-day and inter-day precision for gabapentin (n = 3).

		Conc. (µg/mL)	Intra-day Precision			Inter-day Precision		
			Mean	SD	CV (%)	Mean	SD	CV (%)
Standard 1 25 µg/mL	Day 1	37.14 38.57 38.14	37.95	0.73	1.93	38.90	1.19	3.06
	Day 2	37.29 40.14 38.14	38.52	1.46	3.80			
	Day 3	42.29 39.29 39.14	40.24	1.78	4.41			
Standard 2 150 µg/mL	Day 1	137.85 137.57 137.71	137.71	0.14	0.10	135.68	1.96	1.44
	Day 2	131.85 134.00 135.57	133.81	1.86	1.39			
	Day 3	136.28 133.43 136.85	135.52	1.83	1.35			
Standard 3 290 µg/mL	Day 1	298.14 301.71 303.86	301.24	2.89	0.96	300.64	1.75	0.58
	Day 2	298.86 304.86 302.29	302.00	3.01	0.99			
	Day 3	302.57 298.14 295.29	298.67	3.67	1.23			

4.3.2 Drug Extraction and Recovery of Gabapentin

The percent recovery of gabapentin from Versabase® gel, Lipoderm® cream, and Emollient cream® are 100.84% ± 2.73, 101.28% ±1.24, 104.86% ±3.29, respectively. Percent recovery of gabapentin is summarized in Figure 22.

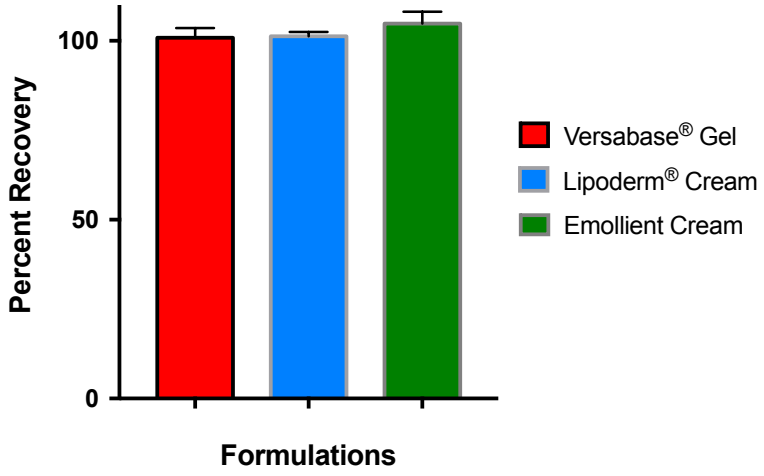

Figure 22. Percent recovery of gabapentin from Versabase®, Lipoderm® and Emollient cream®.

4.3.3 Selection of Membrane for Drug Release Studies

There was no statistically significant difference in gabapentin diffusion through cellulose filter paper, cellulose acetate membrane and Tuffryn® membranes as 100% of the drug was present in the receptor fluid at the first sampling point for all three membranes ($p<0.05$). Given that cellulose filter paper is the cheapest of all the three membranes, it was selected for all subsequent drug release experiments.

4.3.4 *In Vitro* Drug Release

Cumulative gabapentin (10%) release from Lipoderm® cream, Versabase® gel, and Emollient cream® is shown in Figure 23. There was no significant difference in cumulative release from all three bases at all time points ($p<0.05$). At three hours 100.9±4.5% gabapentin was released from Versabase® gel. However, at three hours, 83.3±6.1% and 88.5±16% were released from Lipoderm® base and Emollient cream®, respectively. It was not until 4 hours that mean percentage gabapentin release from Lipoderm® base reached 100%. In contrast, there was a delay in full release to 6 hours for Emollient cream®.

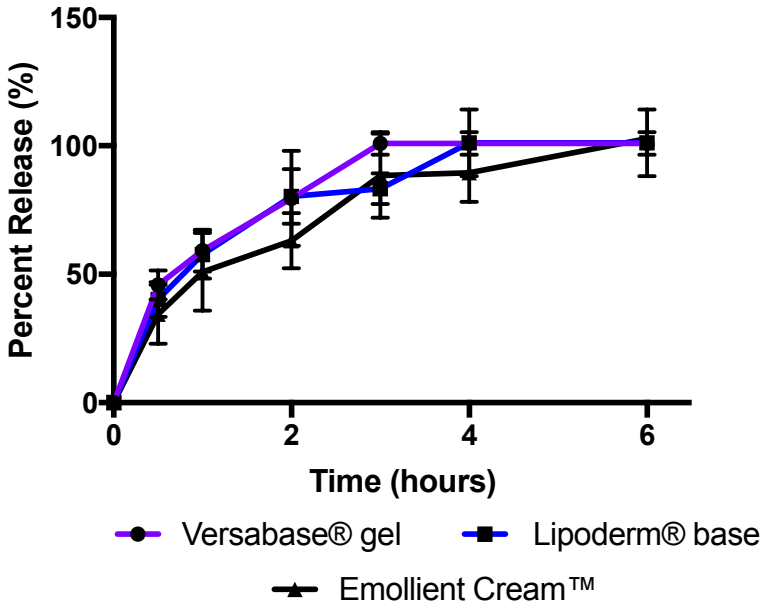

Figure 23. Cumulative gabapentin (10%) release from Versabase® gel, Lipoderm® cream, and Emollient cream®. Data represent mean ± SD, n = 3.

Cumulative percent release of gabapentin (5%) from all three bases followed an almost identical release pattern as seen with the 10% strength. The cumulative gabapentin (5%) release is illustrated in Figure 24. At three hours 100.9±2.9% gabapentin was released from Versabase® gel. However, at three hours, 84.2±1.5% and 74.4±2.1% was released from Lipoderm® base and Emollient cream®, respectively. The mean difference in drug release between Versabase® gel and the other two bases was significant ($p<0.0001$). At four hours, there was no significant difference in cumulative drug release between Versabase® gel and Lipoderm® base ($p>0.05$). However, drug release at 4 hours from Emollient cream® was approximately 25% less than Versabase® gel and Lipoderm® base ($p<0.0001$). There was no difference between all three bases at 6 hours ($p<0.001$).

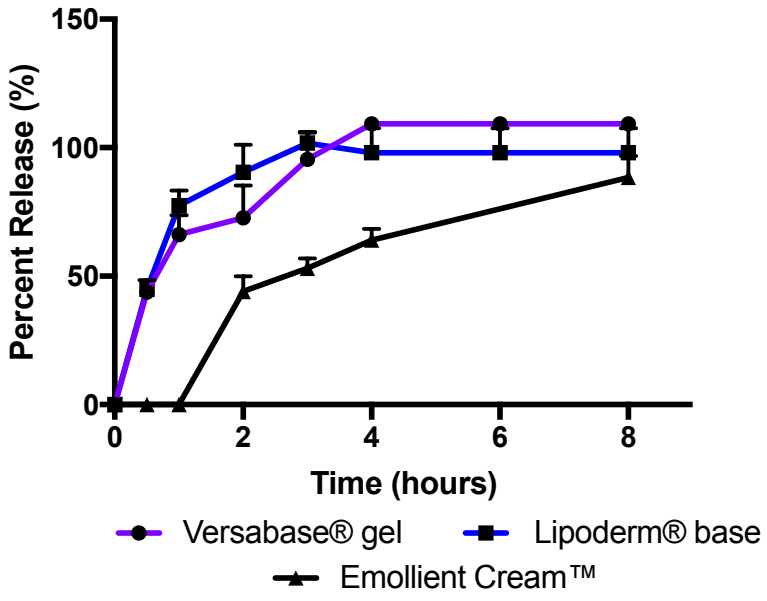

Figure 24. Cumulative gabapentin (5%) release from Versabase® gel, Lipoderm® cream, and Emollient cream®. Data represent mean ± SD, n = 3.

Gabapentin (1 %) release from Lipoderm® cream, Versabase® gel, and Emollient cream® is summarized in Figure 25. It is obvious from Figure 25 that there was significantly lower drug release from Emollient cream® until just before the 8-hour time point. The cumulative percent gabapentin release from Versabase® gel, Lipoderm® cream, and Emollient cream® after 4 hours was 109± 2.9%, 98.1± 9.5%, and 64.1± 4.4%, respectively. Like the 10% and 5% strengths, mean cumulative gabapentin release from Emollient cream® tends to be slower. Also, cumulative release from Emollient cream® at 8 hours was significantly less than Versabase® gel ($p<0.05$), but not significantly different from Lipoderm® base ($p>0.05$).

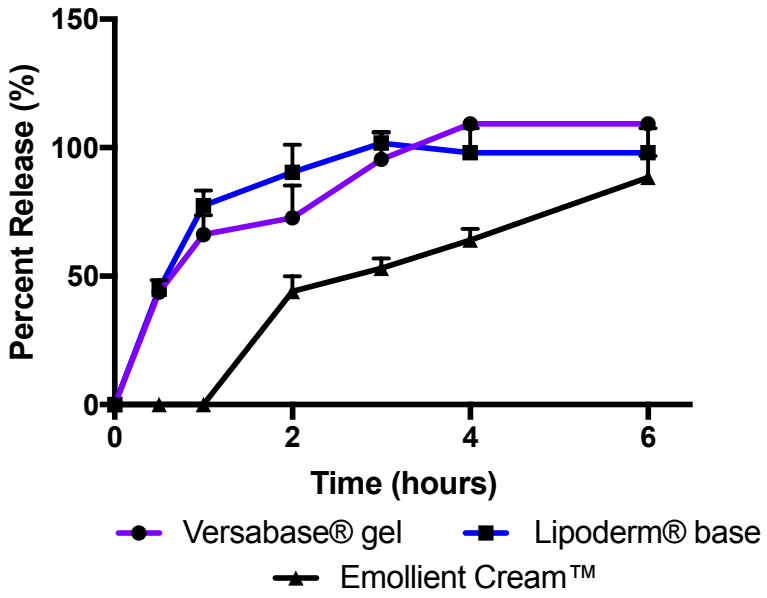

Figure 25. Cumulative gabapentin (1%) release from Versabase® gel, Lipoderm® cream, and Emollient cream®. Data represent mean ± SD, n = 3.

For drug release modeling (Table 12), the Higuchi model yielded the highest R^2 for the 3 concentrations of the drug compounded with Lipoderm® cream (LIPO), Versabase® gel (VG) and emollient cream® (EC). Although gabapentin appears to be released according to the Higuchi model, the Korsmeyer-Peppas model also resulted in relatively high R^2, which may imply drug release by multiple mechanisms. The slope of the linear regression equations for the Korsmeyer-Peppas model were used to confirm if other release mechanisms were involved. All slopes were under 0.5 implying release by Fickian diffusion.

Table 12. Squared correlation coefficient (R^2) following linear regression of drug release data using kinetic models.

Gabapentin % in vehicle	Zero Order R^2	First Order R^2	Higuchi R^2	Hixon-Crowell R^2	Korsmeyer-Peppas R^2
10% VG	0.8849	0.8564	0.9802	0.8380	0.9291
5% VG	0.8992	0.9239	0.9812	0.8893	0.9587
1% VG	0.8942	0.8809	0.9941	0.8696	0.9818
10% LIPO	0.8457	0.8797	0.9821	0.8415	0.9783
5% LIPO	0.8527	0.8645	0.9747	0.7998	0.9509
1% LIPO	0.7283	0.9542	0.9915	0.9356	0.9706
10% EC	0.8330	0.8944	0.9838	0.9042	0.9763
5% EC	0.7069	0.8866	0.9501	0.8901	0.9199
1% EC	0.6270	0.7941	0.9488	0.8101	0.9453

GBP = gabapentin; VG = Versabase® gel; LB = Lipoderm® Base; EC = emollient cream.

4.3.5 *In Vitro* Drug Permeation

The quantity of gabapentin that cumulatively crossed the Strat-M® membrane is an indication of the potential amount of the drug to cross the human skin. The permeation data for gabapentin (5 and 10%) in Lipoderm® cream, Versabase® gel, and Emollient cream® are shown in Figure 26 and Figure 27. Interestingly, gabapentin did not permeate Strat-M® from 1% formulations with any of the bases that were investigated. The difference in cumulative gabapentin 5 and 10% permeation in Lipoderm® base and Versabase® cream was not significant at most time points except after 12 hours for the 10% formulation; significantly more permeation with the Versabase® gel was apparent ($p<0.05$). However, significantly lower permeation was observed in the 10% strength at the 8, 12, and 24-hour time point for Emollient cream® compared to Versabase® gel ($p<0.05$), but no difference at these same time points relative to Lipoderm® base ($p>0.05$).

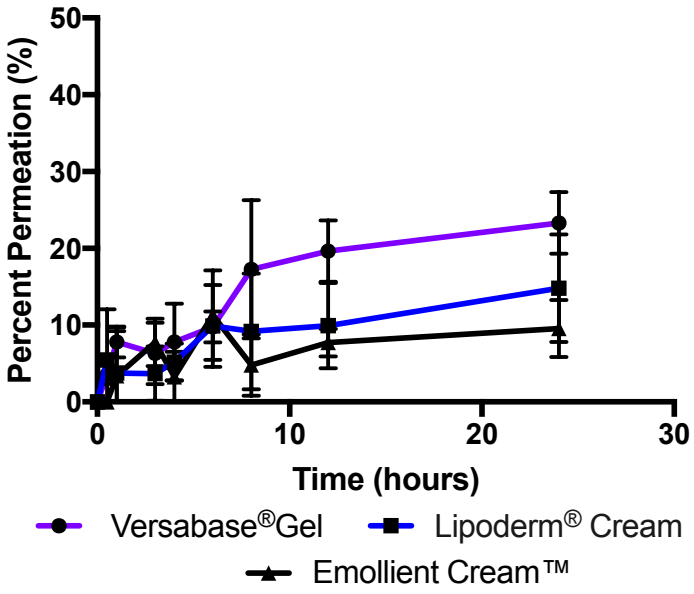

Figure 26. Cumulative gabapentin (10%) permeation across Strat-M. Data represent mean ± SD, n = 3.

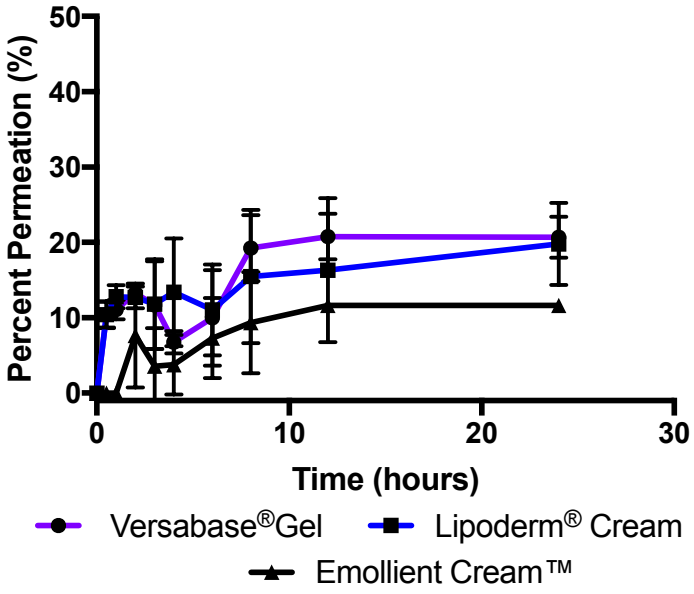

Figure 27. Cumulative gabapentin (5%) permeation through Strat-M®. Data represent mean ± SD, n = 3.

Total gabapentin permeation through Strat-M® over a 24-hour period is summarized in Figure 28. At a 10% strength, 23.3±4%, 14.8±7%, and 9.6±3.7% permeated through Strat-M® when compounded in Versabase® gel, Lipoderm® cream, and Emollient cream®, respectfully. There was significantly more permeation with Versagel® gel ($p<0.05$), but no difference between Lipoderm® cream, and Emollient cream® ($p>0.05$) was observed. At a 5% strength, 20.7±2.7%, 19.8±5.5%, and 11.7±0.1% permeated through Strat-M® when compounded in Versabase® gel, Lipoderm® cream, and Emollient cream®, respectfully. No difference was found when permeation between Versabase® gel and Lipoderm® cream were compared ($p>0.05$), but significantly less permeation with Emollient cream® ($p<0.05$) occurred. It was found that decreasing the strength from 10 to 5% did not result in any difference in total percent permeation ($p>0.05$). The total gabapentin recovery from Strat-M, the receptor medium and equipment was within acceptable limits (90-110%).

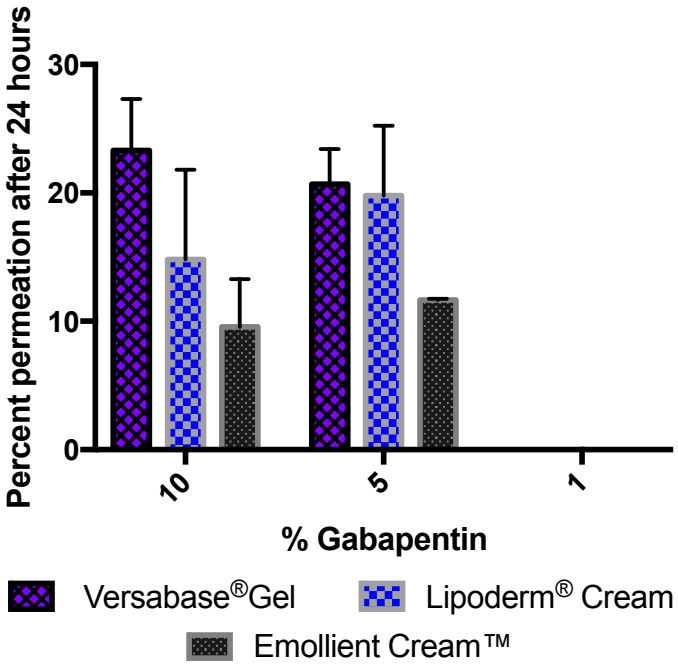

Figure 28. Total gabapentin (10, 5, and 1%) permeation through Strat-M® over a 24- hour period. Data represent mean± SD, n = 3.

Due to the barrier nature of the *stratum corneum* most compounds require some time to hydrate the skin before permeation (lag time). Lag time depends on the physicochemical properties of the molecules, level of skin hydration and formulation vehicle. In our study, 5 and 10% gabapentin compounded with Lipoderm® base permeated the skin rapidly (Table 13). For Emollient cream® permeation started after approximately 10 minutes. Gabapentin (5%) compounded with 5% Emollient cream and 5% Versabase gel required 60 and 120 minutes, respectively for permeation to occur. A strong correlation was observed between lag times and flux of the compound in formulations with various bases. Flux was the greatest for gabapentin formulated in Lipoderm® base and least for Versabase® gel. It decreased significantly in Emollient cream® and Versabase® gel as the concentration of gabapentin was decreased.

Table 13. Lag time and flux of gabapentin through Strat-M®.

Gabapentin % in vehicle	Lag time (minutes)	Peak Flux (mg/hr/cm^2)
10%Lipoderm® base	0	1.9328
5%Lipoderm® base	0	1.9337
10%Emollient cream®	10	1.8740
5%Emollient cream®	60	0.4639
10%Versabase® gel	5	1.5122
5%Versabse® gel	120	0.2458

4.4 DISCUSSION

Prior to conducting drug release or permeation testing, assay methods suitable for analysis of the gabapentin in PBS were explored. Complete method validation of the method is required to ensure accurate drug release and permeation results [31]. Gabapentin has very poor UV absorbance with an absorption maximum of around 210 nm. Initially, a simple spectrophotometric method for detecting the compound in deionized water was used [73]. Although a clear peak was detected at 205 nm with a 2.5 mg/mL gabapentin solution, the method had poor inter-day and intra-day precision with CV values over 20%. Another issue with this low maximal UV absorbance was the fact that some excipients present in the formulations also display some absorbance in this UV range. To avoid this interference as well as improve the specificity and accuracy of gabapentin detection, we derivatized the drug with a chlorophoric group that displays maximal UV absorption much higher than the solvents and excipients used in our studies. Hence, a previously validated spectrophotometric method involving derivatization of gabapentin with a solution of ninhydrin 0.2 % in *N,N*-Dimethylformamide was used [70,71,74,72].

In vitro drug release testing (IVRT) using a synthetic membrane and the Franz diffusion cell model provides valuable information on the product quality as well as the

release mechanism of the drug [31]. IVRT also serves as a tool to compare drugs compounded in different preparations [31]. Several factors such as drug particle size, physical properties of the base and excipients, temperature, and pH can affect the drug release profile [30]. This is crucial to understand as changes in the release profile has the potential to influence the clinical performance of the overall product. The drug release patterns of gabapentin in the three different bases and concentrations were explored in this study by entering data into different kinetic models. All compounds most closely followed the Higuchi model which describes diffusion out of a non-degrading matrix system following Fick's first law of diffusion [33]. However, there was also a relatively high R^2 observed with the Korsmeyer-Peppas model. This model is nonspecific and can involve multiple release mechanisms [35]. To more clearly characterize the mechanism, the slope of the linear regression equation is used. If the value is <0.5, the formulation likely follows Fickian diffusion. Fickian diffusion involves particles moving from an area of high to low concentration [36]. However, if it is greater than 0.5 and less than 1, then the release mechanism likely follows non-Fickian or anomalous transport [35]. In the case of topical gabapentin, it appears that diffusion was the only factor impacting drug release from the formulation. Therefore, the larger the concentration gradient, the higher the diffusion rate. Additionally, given the hydrophilic nature of gabapentin, its affinity towards the cellulose membrane and receptor fluid may also have played a role in the rate of diffusion.

It was observed that mean gabapentin release from Emollient cream® was generally slower than Lipoderm® cream and Versabase® gel. This may be due to the higher viscosity of Emollient cream® as release rate tends to be inversely proportional to viscosity [31]. In contrast, release rate from Versabase® gel, which is a hydrogel, was

quite rapid. This is not surprising because hydrogels rapidly absorb water and swell facilitating drug migration from the gel's core to the pores of the cellulose membrane and into the receptor medium [75].

Drug permeation studies were conducted using Strat-M®, which is a synthetic membrane model that functionally mimics the human skin [41,40]. It demonstrates a high *in vitro- in vivo* correlation (IVIVC) for a variety of active pharmaceutical ingredients and is easy to acquire [41,40,38]. It was observed that formulations containing 1% gabapentin displayed no detectable drug permeation in the receptor fluid. Therefore, compounding such a low strength is not likely to exert any sort of clinical effect. The cumulative amount permeating Strat-M® after 24 hours for gabapentin 5 and 10% compounded in Lipoderm® cream was approximately 15 and 20%, respectively. This value is significantly higher than what is reported in the literature where only 0.3% to 3.9% permeated into the receptor fluid [76,77]. The reasons for the discrepancy are many. Firstly, the study used human trunk skin which tends to be thicker and more difficult to permeate than other areas traditionally targeted with topical formulations [76]. Additionally, where part of the dermis was remaining, this could have served as an additional barrier in an *in vitro* model as typically the dermis is vascularized allowing for systemic absorption to occur *in vivo*. Another factor is that in this study, gabapentin was one of 6 active drugs present in the topical formulation, which may have impacted both its drug release as well as its permeability characteristics. It is also important to note that there was at least 10% of the drug retained within the epidermis and dermis which is where gabapentin exerts is therapeutic effect [76]. Given the nature of Strat-M®, quantifying drug retention in each layer is not possible, however, a conservative estimate of total drug capable of passing the *stratum corneum* is useful.

Roughly 20% of gabapentin permeated when compounded in Versabase® gel. Versabase® gel has been used in practice as a base for gabapentin although it is not reported in the literature. Due to poor gabapentin stability, as seen in chapter 2, its use is not practical. Gabapentin (5 and 10%) in Versabase® gel also had a higher lag time as well as lower flux than Lipoderm® base. The composition of the Lipoderm® base (e.g. permeation enhancers) may explain this result. The use of Emollient cream® as a base resulted in total gabapentin permeation after 24 hours of approximately 10%. The percent permeation may have been lowest due to lack of several permeation enhancers in the base.

4.5 CONCLUSIONS

Topical gabapentin is compounded routinely in specialized pharmacies. However, there is little to no literature on the drug release and permeation characteristics of these products as they are not commercially available. *In vitro* drug release and permeation studies conducted revealed full release of the active drug within the first 6 hours of application in all bases, however, no permeation through the Strat-M® occurred with 1% gabapentin formulations. Considering *in vitro* drug release, drug stability, and permeation data, gabapentin compounded in Lipoderm® base may be the most practical for patient use. However, further studies with human and animal skins are needed to confirm this.

CHAPTER 5- *IN VITRO* RELEASE PROFILES AND PERMEATION OF AMITRIPTYLINE COMPOUNDED WITH LIPODERM CREAM®, EMOLLIENT CREAM®, AND MEDIFLO® 30 PLO GEL.

5.1 INTRODUCTION

Amitriptyline is a tricyclic antidepressant often used as a first line agent in the treatment of neuropathic pain [13]. It is readily absorbed through the oral route with a bioavailability between 31-61% due to high first pass metabolism [62,78]. Its large volume of distribution (12-18 L/kg) and extensive tissue and protein binding (95%) can be partially explained by the fact that amitriptyline is a highly lipophilic compound with a logP of 3 [62,79]. Additionally, it has a small molecular weight of 313.86 g/mol [61]. It is thought to produce analgesia in neuropathic pain by acting as an inhibitor of voltage gated sodium channels in the peripheral nerves [17]. This mechanism of action is similar to local anesthetics, such as lidocaine, which explains the effectiveness of amitriptyline as a topical agent [59].

The use of compounded topical amitriptyline, as an adjunctive or alternative to oral therapies for peripheral neuropathic pain, has been increasing. Topical use has the advantage of avoiding many of the adverse effects associated with oral formulations such as dizziness, drowsiness, dry mouth, blurred vision, urinary retention, weight gain and tachycardia [80]. In the literature, evidence for efficacy is mixed, partially due to relatively low amitriptyline concentrations in tested topical preparations [81]. Lynch et. al conducted a double-blind, randomized, placebo-controlled 3-week study evaluating the efficacy of topical 2% amitriptyline, 1% ketamine, and a combination of the two [82,18]. They found no significant difference in pain scores between all three groups as well as no

detectable systemic absorption of amitriptyline. The main side effect experienced was skin irritation and redness, which was consistent with the results reported by Al-Musawai et al. [83]. Other controlled trials using low concentrations (≤5% amitriptyline) also reported similar outcomes [84,85,86]. For studies involving higher concentrations, more consistent results were obtained. For instance patients experienced significant pain relief in 3 uncontrolled case reports using amitriptyline concentrations ranging from 5 to 10% [21,20,87]. Although the patients who received topical amitriptyline 10% had a better pain response, one patient had trouble concentrating [21]. Other patients did not experience this adverse effect and the authors hypothesized that the patient may have been a poor metabolizer. Additionally, where there was no placebo comparator, it is difficult to ascertain if the adverse effect was fully attributable to the drug itself.

Drug release from formulation excipients and subsequent permeation through the *stratum corneum* is highly dependent on physicochemical properties, drug concentration and properties of the vehicle [88]. Many reports that examined the efficacy of topical amitriptyline alone have either used pluronic lecithin organogel (PLO) or undisclosed bases. Additionally, for those that used PLO, no rationale was given to justify this choice [89].

The aim of this study was to provide the scientific rationale that enables compounding pharmacists to choose appropriate bases that facilitate amitriptyline release and subsequent permeation across the skin. To achieve this aim, we compared the *in vitro* release characteristics of amitriptyline compounded with Lipoderm cream®, Emollient cream®, and Mediflo® 30 PLO gel. The permeation of 1, 5 and 10% of the compound across synthetic human skin, Strat-M was used to estimate skin permeation. The compounding bases were selected based on their popularity and frequency of use for

compounding topical drugs in many pharmacies. The ingredients in Lipoderm® cream, Mediflo® 30 PLO gel, and Emollient cream® are listed in Table 14.

Table 14. Ingredients contained in Lipoderm® cream, Mediflo® 30 PLO gel, and Emollient cream® [66,67,90]

PCCA Lipoderm® Cream	Medisca Mediflo® 30 PLO gel	PCCA Emollient Cream®
1. Purified Water 2. Preliposomic Phosphatidylcholine 3. Isopropyl Myristate Cetearyl Alcohol 4. Ceteareth-20 5. Stearyl Alcohol 6. Cetyl Alcohol 7. Caprylic / Capric Triglycerides 8. Wheat Germ oil 9. Glycerin 10. Polydimethylsiloxane 11. Magnesium Aluminum Silicate 12. Xanthan Gum 13. Polyacrylamide 14. C13-C14 Isoparaffin 15. Laureth-7 16. Edetate Disodium 17. Butylated Hydroxytoluene 18. Phenoxyethanol 19. Methylchloroisothiazolinone/ Methylisothiazolinone	1. Benzalkonium chloride 2. Isopropyl palmitate 3. Lecithin soya granular 4. Poloxamer 407 5. Potassium sorbate 6. Purified water 7. Thimerisol	1. Purified Water 2. Base, Anhydrous 3. Sorbimacrogol Oleate 300 4. Butylated hydroxytoluene 5. Methylchloroisothiazolinone / Methylisothiazolinone

5.2 MATERIALS AND METHODS

5.2.1 Materials

Amitriptyline HCl (Lot CC006121), Lipoderm® Base (Lot 7048812), and Emollient cream® (Lot: 7302343) were kindly provided by PCCA (Houston, Texas, USA). Mediflo™ 30 PLO gel (Lot I243P/B) was purchased from Medisca Inc. (St-Laurent, QC, Canada). Ethoxy diglycol (Lot: 14164-5104-5321) was purchased from Galenova (Saint-Hyacinthe, QC, Canada). Monobasic sodium phosphate monohydrate

(Lot 46107638) and phosphoric acid (Lot 09813JE) were purchased from Sigma-Aldrich (Oakville, ON, Canada), while acetonitrile of analytical grade (Lot: 171203) was from Fisher Scientific (NJ, USA). Deionized water (18 Ω) was processed using a Barnstead Nanopure II filtering system. Phosphate buffered saline 10X (Lot: I2614) was bought from Sigma-Aldrich (Oakville, ON, Canada). Strat-M® membrane was acquired from EMD Millipore (Lot: K3BA7673; Billerica, MA, USA). Coarse Cellulose filter paper were purchased from Fisher Scientific (Ottawa, ON, Canada). Cellulose acetate membranes (Lot: 530CDC) with a pore size of 0.47 µm were obtained from Geotech Environmental Equipment Inc. (Denver, CO, USA). Tuffryn® membrane filters (Lot: 64535) with a pore size of 0.45 µm were obtained from Pall Corporation (Ann Arbor, Michigan, USA). Nylon 0.22µm membrane filters and Hamilton 18G, 4" blunt needles with a metal hub were purchased from Chromatographic Specialties Inc. (Brockville, ON, Canada).

5.2.2 Instrumentation

Six, 9mm clear jacketed Franz Cells with flat ground joint and 5 mL receptor volume were purchased from PermeGear, Inc. (Part number: 4G-01-00-09-05; Hellertown, PA, USA). The V-series stirrer (V6-CA-01) was purchased from PermeGear, Inc. (Hellertown, PA, USA). A Lauda Ecoline E100 heated water bath circulator (Lauda-Koenigshofen, Germany) was used to maintain the Franz cells at a specific temperature. A Varian-920 Liquid Chromatograph with a quaternary gradient pump, autosampler with a 50 µL sample loop, a UV-Vis detector and Galaxie chromatographic software were used for sample analysis. Chromatographic separation was performed with a Waters uBondapack 125 Å (3.9 mm x 300 mm; 10 µm) C18 column.

5.2.3 HPLC Method for the Quantification of Amitriptyline

Spectrophotometric methods described in the literature for amitriptyline detection involved complex derivatization procedures, had narrow detection ranges, low sensitivity and were costly [91,92,93]. Therefore, the United States Pharmacopoeia (USP) HPLC method for the analysis of the compound was explored [63]. Phosphate buffer was prepared by dissolving 11.04 g monobasic sodium phosphate in 900 mL of deionized water and adjusted to a pH of 2.5 ±0.5 with 70% phosphoric acid. The solution was then diluted with water to a final volume of 1000 mL and mixed. Mobile phase was prepared by combining phosphate buffer and acetonitrile in a 58:42 ratio v/v, vacuum-filtered through a 0.22µm nylon filter and degassed for 20 minutes. Chromatographic conditions are summarized in Table 15.

Table 15. Chromatographic conditions for the assay of amitriptyline.

Column	Waters µBondapack 125 Å (3.9 mm x 300 mm; 10 µm) C18 column
Mobile Phase	Acetonitrile /92 mM Monobasic sodium phosphate pH 2.5 (42:58)
Flow Rate	2 mL/min
Detection	UV-Vis at 254 nm
Injection	20 µL
Column Temperature	Ambient
Run time	5 minutes

5.2.4 Validation of the HPLC Quantification Method

The chromatographic method was previously validated in section 3.2.4 for specificity, linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) as per the International Conference on Harmonization (ICH) guidelines [52].

5.2.5 Preparation of Compounded Amitriptyline

Amitriptyline HCl was compounded with Lidoderm® Base, Emollient cream® and Mediflo™ 30 PLO gel. Three different concentrations (1, 5, and 10%) of amitriptyline was made by measuring a small amount of amitriptyline powder into a plastic weigh boat, wetting with ethoxy diglycol until a thick paste was formed and slowly incorporating the base using the principles of geometric dilution until the cream appeared uniform and smooth. Topical amitriptyline compounds were freshly prepared immediately prior to permeation or release studies.

5.2.6 *In Vitro* Drug Release and Permeation Studies

To characterize the release rate of amitriptyline from the different topical formulations, *in vitro* drug release studies were carried out in a Franz diffusion cell system with a diffusion area of 0.64 cm^2 and a receptor medium capacity of 5 mL. The system was maintained at a constant temperature of $32 \pm 0.5\ ^oC$ by attaching to a Lauda Ecoline E100 heated water bath circulator. A volume of 5 mL of PBS (pH 7.4) was added slowly using a pipette to each Franz cell through the receptor chamber orifice. Small magnetic stir bars were placed into the receptor chambers of each cell and the system was left to equilibrate for a minimum of 60 minutes.

To decide which membrane provides the least resistance to the diffusion of the active compound, cellulose filter paper, cellulose acetate and Tuffryn® membranes were soaked in PBS for 30 minutes. Each wetted membrane was placed on top of one receptor chamber with the Teflon® O-ring and donor chamber placed over the membrane and secured with a metal clamp. To the donor chamber, 0.5 mL of an amitriptyline 1000 µg/mL solution was added and both the donor chamber chimney as well as the sampling port were covered with parafilm. Receptor medium was stirred constantly at 650 RPM.

The 4" blunt needles were used to draw 0.5 mL samples from the sampling port over a 6-hour period (0, 0.5, 1, 2, 3, 5, 6 hours). After each sample was taken for later analysis, 0.5 mL of fresh PBS was added back into the receptor chamber to maintain both sink conditions as well as maintain PBS contact with the membrane. The amitriptyline concentration in each sample was quantified by HPLC and the cumulative drug release was calculated using equation 3. The mean percentage cumulative diffusion of amitriptyline through the three membranes, done in triplicate, at each time point were graphed and compared.

Cn = C'n + Vs/Vt (C'n-1+-----+C'1)……**Equation 3**

Where, Cn, C'n-1, C'1 = concentration at n, previous sample, and first sample, respectively. Vs, Vt = Volumes of sample in the donor and receiver compartments, respectively.

The membrane that demonstrated the least resistance to amitriptyline diffusion and was the cheapest to use was selected for all subsequent drug release experiments. The membrane and Franz cells were set up identically to what has already been described except instead of using an amitriptyline solution in the donor chamber, 100 $\pm$0.5mg of prepared topical amitriptyline was applied on the membrane using a glass rod. The mass of the glass rod was recorded before and after application of the topical to determine the exact quantity applied. One cell was reserved as a blank containing the base with ethoxy diglycol only. Both the sampling port opening as well as the donor chamber chimney was covered with parafilm. Samples (0.5 mL) were drawn at the 0.5, 1, 2, 3, 4, 6, 8, 12, and 24-hour mark. The same volume of receptor medium taken during each sample draw was replaced with fresh PBS. Cumulative drug release was calculated using equation 4 [31]**:**

$Q = (\mathbf{C_n V} + \sum_{i=1}^{n-1} \boldsymbol{Ci\ S})$……**Equation 4**

Where Cn is the concentration of drug determined at nth sampling interval; V is the volume of the Franz diffusion cell, $\sum_{i=1}^{n-1} Ci$ is the sum of concentrations of drug determined at sampling intervals 1 through n-1 and S is the surface area of the sample well.

Generated data were fitted into the first order, second-order, Higuchi, Korsmeyer-Peppas, and Hixon-Crowell kinetic models to determine the mechanism of drug release from each of the formulations. The kinetic model yielding a linear function with an R^2 closest to 1 is the most likely mechanism of drug release. The Korsmeyer-Peppas model could imply various mechanisms. Therefore, to characterize the mechanism, the slope of the linear regression equation was used.

Drug permeation studies were conducted in an identical manner to the drug release studies except for the membrane utilized and no occlusion of the receptor or sampling port occurred. Strat-M®, which is an *in vitro* membrane model that functionally simulates drug permeation through human skin, was placed on the receptor chamber and did not require hydration prior to use. Each topical formulation was tested in triplicate and flux and lag time were calculated. The steady-state flux was the slope divided by the diffusional area from the linear portion of the cumulative drug permeation graph, while lag time was the x-intercept. Additionally, after permeation experiments were completed, Strat-M® and the diffusion cell were rinsed with deionized water and used to determine overall drug recovery.

5.2.7 Statistical Analysis

Microsoft® Office Excel 2011 was used to determine *in vitro* release and permeation data. Differences between mean cumulative percent release or permeation at

each time point was determined using statistical two-way analysis of variance, ANOVA, with Tukey's multiple comparison post-hoc test using GraphPad Prism® software version 7.0 (GraphPad Software Inc., San Diego, CA, USA) with a 95% confidence interval.

5.3 RESULTS

5.3.1 Selection of Membrane for Drug Release Studies

There was no statistically significant difference in amitriptyline diffusion for cellulose filter paper vs cellulose acetate membrane and cellulose acetate vs Tuffryn® at all time points. However, at the 4, 5 and 6-hour time points, there was significantly more amitriptyline diffusion through the cellulose filter paper compared to Tuffryn® ($p<0.05$). Given that cellulose paper is the cheapest of all three membranes, it was selected for all subsequent drug release experiments. Data for amitriptyline release over a 6-hour period is depicted in Figure 29.

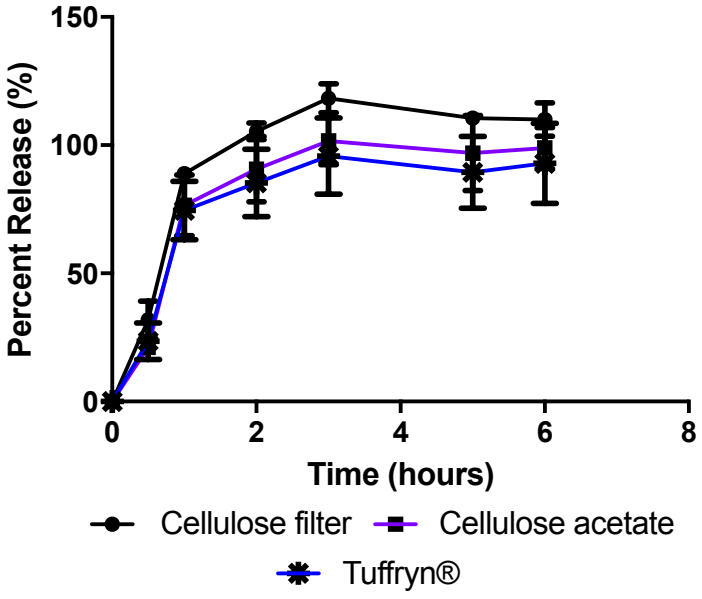

Figure 29. Amitriptyline diffusion into the receptor medium across a cellulose filter paper, cellulose acetate and Tuffryn®. Data represent mean ± SD, n = 3.

5.3.2 *In Vitro* Drug Release

Cumulative amitriptyline (10%) release from Lipoderm® cream, PLO Mediflo® gel, and Emollient cream® is shown in Figure 30. There was no significant difference in amitriptyline release from Lipoderm® cream and Emollient Cream® in the first 4 hours ($p<0.05$). However, after 4 hours, significantly more amitriptyline release from the Emollient cream® compared to Lipoderm® base ($p<0.05$) was observed. Drug release from PLO Mediflo® gel was significantly greater than the other two bases at all time points after 3 hours ($p<0.05$). Cumulative drug release after 24 hours was, 23.9±4.1%, 53.2±7.7%, and 41.8±3.1% for Lipoderm® base, PLO Mediflo® gel, and Emollient cream®, respectively. A large percentage of amitriptyline was retained in all three bases.

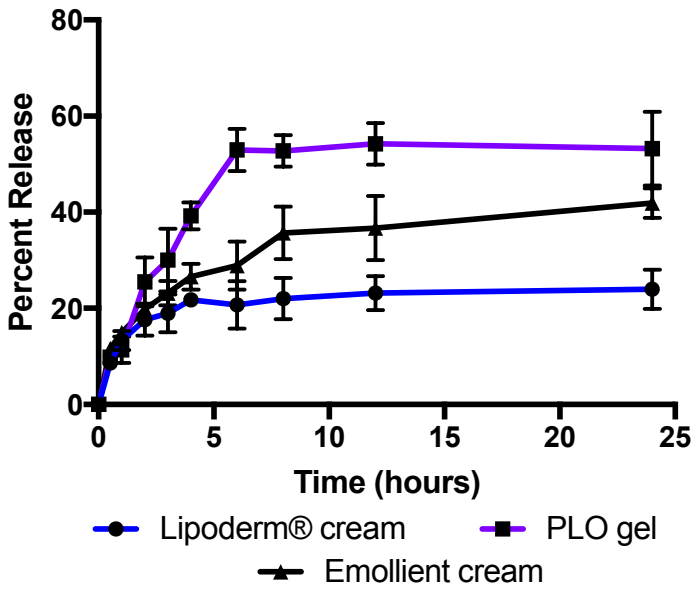

Figure 30. Cumulative amitriptyline (10%) release from Lipoderm® cream, PLO Mediflo® gel, and emollient cream. Data represent mean ± SD, n = 3.

Percent cumulative release of amitriptyline (5%) from all three bases followed a similar pattern to the 10% strength except the fact that no significant difference between

PLO Mediflo® gel and Emollient cream® was observed beyond 6 hours ($p>0.05$). However, there was no difference between all three bases in the first 2 hours ($p>0.05$). Like the 10% formulation, there was significantly less amitriptyline release from Lipoderm® cream at all time points after 4 hours ($p<0.05$). Cumulative drug release after 24 hours was, 23.56±4.1%, 39.7±2.8%, and 40.8±3.1% for Lipoderm® base, PLO Mediflo® gel, and Emollient cream®, respectively. Cumulative percent release of amitriptyline (5%) from the three described bases is illustrated in Figure 31.

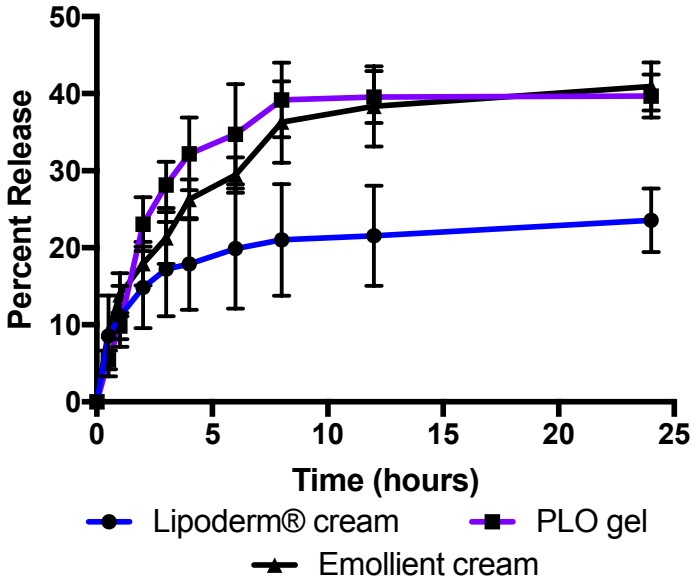

Figure 31. Cumulative drug release of amitriptyline (5%) from Lipoderm® cream, PLO Mediflo® gel, and emollient cream. Data represent mean ± SD, n = 3.

Figure 32 displays the significant differences in cumulative amitriptyline (1%) release from all three bases. After 2 hours, there is significantly more amitriptyline release from Mediflo® PLO gel compared to the other two bases ($p<0.05$). Also, there is significantly more amitriptyline release from Emollient cream® than Lipoderm® base ($p<0.05$). Cumulative drug release after 24 hours was, 24 ±2.2%, 64 ±13.8%, and

35.5±4.6% for Lipoderm® base, PLO Mediflo® gel, and Emollient cream®, respectively. There was consistently less amitriptyline release from Lipoderm® cream in all three compounded strengths (1,5, and 10%) compared to other bases.

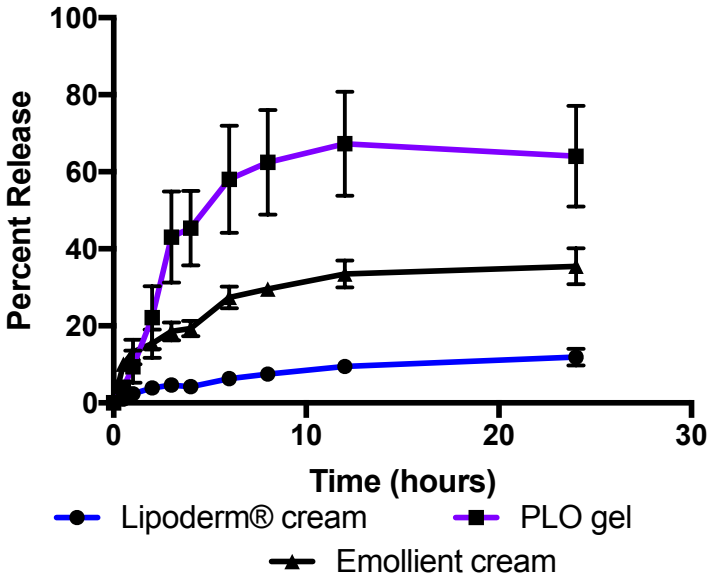

Figure 32. Cumulative drug release of amitriptyline (1%) from Lipoderm® cream, PLO Mediflo® gel, and emollient cream. Data represent mean ± SD, n = 3.

To ascertain drug release mechanisms from the bases, mean drug release was fitted to kinetic models. Based on this analysis, the R^2 for amitriptyline compounded with Lipoderm® cream (LIPO), PLO Mediflo™ gel (PLO) and emollient cream (EC) were highest for the Higuchi model, which implies that amitriptyline release concurred with the model. The R^2 values for the Korsmeyer-Peppas model were also relatively high, which may imply drug release by multiple mechanisms. The slope of the linear regression equations for the Korsmeyer-Peppas model were used to confirm if other release mechanisms were feasible. All slopes for amitriptyline (1, 5, and 10%) release from Lipoderm® base and Mediflo® PLO gel was between 0.5 and 1 which suggests anomalous

transport. However, amitriptyline (1, 5, and 10%) release from Emollient cream® fitted into the model was found to have slopes under 0.5, implying release by Fickian diffusion.

Table 16. Mean drug release modellling.

Amitriptyline % in vehicle	Higuchi (R^2)	Zero Order (R^2)	First Order (R^2)	Hixon-Crowell (R^2)	Korsmeyer-Peppas (R^2)
10%LB	0.9801	0.4059	0.4319	0.4232	0.8302
5%LB	0.9621	0.54043	0.5369	0.5260	0.8168
1%LB	0.9809	0.856	0.8687	0.8645	0.7179
10%EC	0.9843	0.6709	0.7371	0.7155	0.9521
5%EC	0.9944	0.6616	0.7141	0.6974	0.9558
1%EC	0.9771	0.6793	0.7252	0.7104	0.8732
10%PLO	0.9745	0.4724	0.5136	0.5005	0.7939
5%PLO	0.9452	0.4961	0.5337	0.5212	0.824
1%PLO	0.9819	0.543	0.6015	0.3441	0.844

AMIT = amitriptyline; PLO = Mediflo® PLO gel; LB = Lipoderm® Base; EC = Emollient cream®.

5.3.3 *In Vitro* Drug Permeation

Amitriptyline (10%) permeation through Strat-M® varied depending on the base used (Figure 33). Although the highest amitriptyline release was seen from Mediflo® PLO, it displayed significantly lower cumulative permeation at all time points compared to Lipoderm® and Emollient cream® ($p<0.05$).

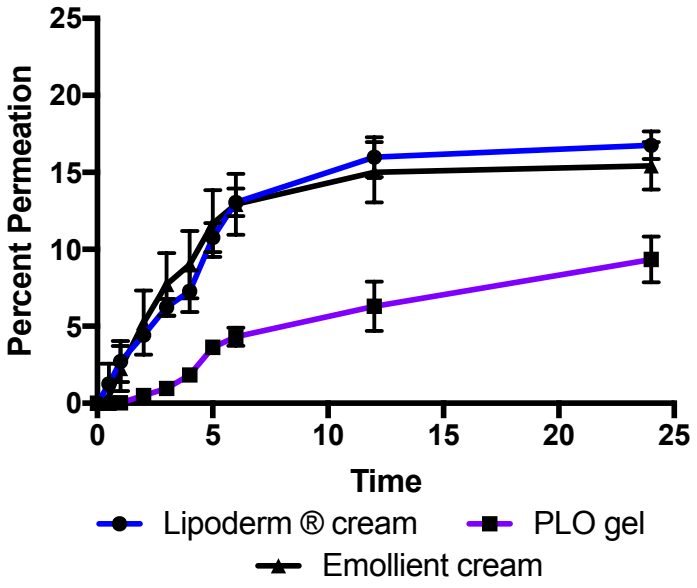

Figure 33. Cumulative amitriptyline (10%) permeation through Strat-M®. Data represent mean ± SD, n = 3.

Cumulative amitriptyline (5%) permeation through Strat-M® is illustrated in Figure 34. At a concentration of 5%, no significant difference in amitriptyline permeation at all time points was observed between Lipoderm® cream and Emollient cream® ($p>0.05$). Surprisingly, reducing the concentration of amitriptyline in PLO Mediflo® gel formulations from 10 to 5% resulted in no drug permeation.

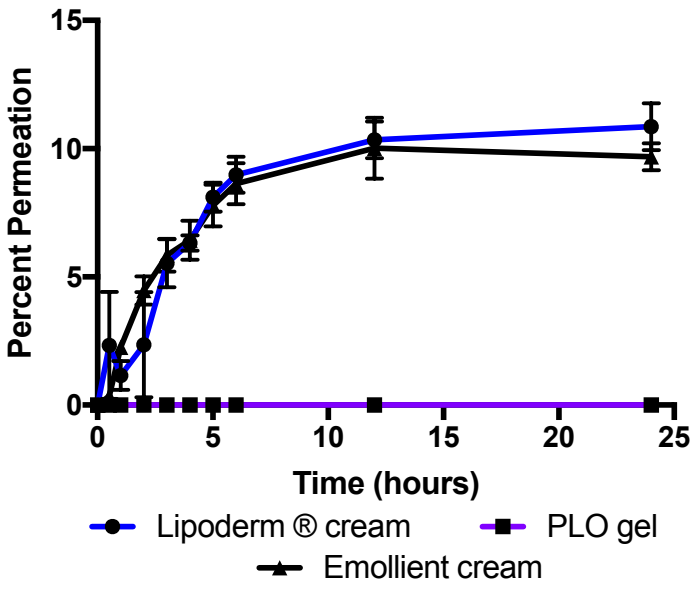

Figure 34. Cumulative amitriptyline (5%) permeation through Strat-M™. Data represent mean ± SD, n = 3.

Decreasing the amitriptyline concentration from 5 to 1% resulted in no permeation (Figure 35) apart from the 4 and 12-hour time points. Amitriptyline 1% in Lipoderm® cream displayed 0.43±0.04% permeation at 4 hours, with no further drug permeation beyond that point. However, at the same concentration in Emollient cream®, 0.37 ±0.24% amitriptyline permeated with no further drug permeation beyond this time point.

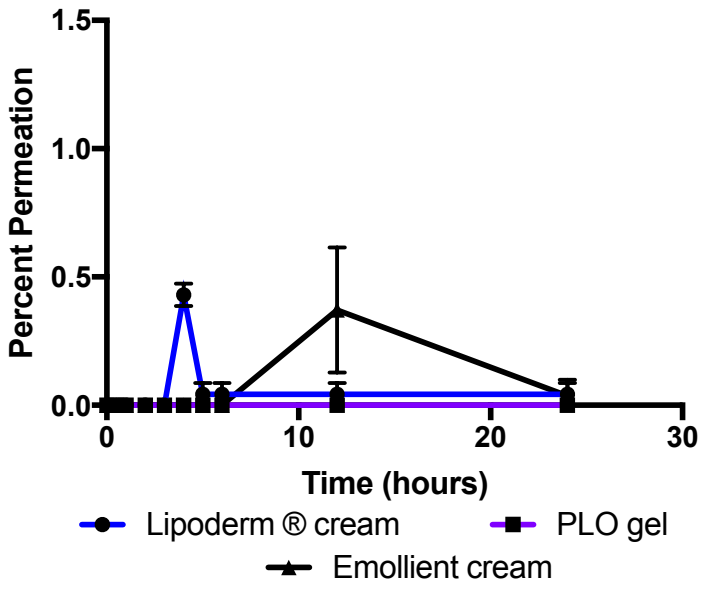

Figure 35. Cumulative drug permeation of amitriptyline 1% in Lipoderm® cream, PLO Mediflo® gel, and emollient cream through Strat-M®. Data represent mean ± SD, n = 3.

Total amitriptyline permeation through Strat-M® over a 24-hour period is summarized in Figure 36. At a 10% strength, 16.8 ±0.9%, 9.3 ±1.5%, and 15.4±1.5%, permeated through Strat-M® when compounded in Lipoderm® cream, Mediflo® PLO gel, and Emollient cream®, respectfully. There was significantly less permeation with Mediflo® PLO gel ($p<0.05$), but no difference between Lipoderm® cream, and Emollient cream® ($p>0.05$). At a 5% strength, 10.9±0.9%, 0% and 9.7±0.5% permeated through Strat-M® when compounded in Lipoderm® cream, Mediflo® PLO gel, and Emollient cream®, respectfully. There was significantly less permeation with Mediflo® PLO gel ($p<0.05$), but no difference between Lipoderm® cream, and Emollient cream® ($p>0.05$). It was found that decreasing the strength from 10% to 5% and 5% to 1% resulted in significant differences in total percent permeation ($p<0.05$). The total amitriptyline recovery from Strat-M, the receptor medium and equipment was within acceptable limits (90-110%).

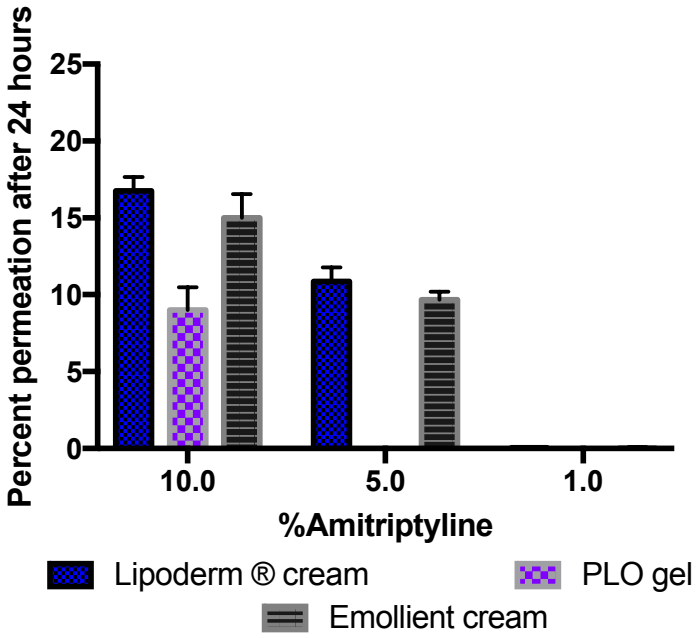

Figure 36. Total percent amitriptyline (10, 5, and 1%) permeation through Strat-M™ over a 24-hour period. Data represent mean± SD, n = 3.

The time it takes for amitriptyline permeation across Strat-M® to start is the lag time which varies based on several factors such as drug concentration and formulation. In our study, 5 and 10% amitriptyline compounded with Lipoderm® base permeated the skin rapidly with flux being almost double in the higher concentration (Table 17). For amitriptyline 5 and 10% in Emollient cream®, permeation started after approximately 45 and 7 minutes, respectively. There was an overall strong correlation between amitriptyline concentration, lag time, and flux. Higher concentrations were associated with significantly reduced lag times and increased flux. The highest lag time and flux were observed when Mediflo® PLO gel was used as a base.

Table 17. Lag time and flux of amitriptyline through Strat-M®.

Amitriptyline % in vehicle	Lag time (minutes)	Flux (mg/hr/cm^2)
10%Lipoderm	0	0.2601
5%Lipoderm	0	0.1458
10%Emollient	6.8	0.3878
5%Emollient	46.3	0.1459
10%PLO	47.6	0.0913

5.4 DISCUSSION

In vitro drug release testing (IVRT) and subsequent permeation testing of semi-solid preparations serves as a valuable tool in formulation development, quality control procedures, monitoring changes in formula and procedures, and predicting *in vivo* performance of a product [94]. Vehicle selection and composition plays a very important role in drug release and permeation behavior, which ultimately hinders or enhances clinical response [31]. This information is important to gather and understand prior to undertaking animal and later expensive human clinical trials. There are several patient case reports, as well as controlled trials utilizing topical amitriptyline for treating peripheral neuropathic pain, however, there is shockingly an absence of vehicle description as well as release and permeation data. In trials that did show evidence for efficacy, higher concentrations (>5%) were used while those that used lower strengths or described the base as being PLO had lack of benefit [21,20,87]. These results may partially be explained by the data generated in our *in vitro* studies.

In addition to aiding in formulation development, IVRT also serves to compare performance of different non-commercial compounded medications with various bases and excipients [31]. The data are fitted by well-established kinetic models to predict and understand mechanism of drug liberation from the formulation [32]. Amitriptyline release from all formulations most closely followed the Higuchi kinetic model, which suggests

that amitriptyline release was controlled by its rate of diffusion from the upper areas of the vehicles towards the surface exposed to the membrane [95]. However, there was also a relatively high R^2 observed with the Korsmeyer-Peppas model, which implies other release mechanisms may also play a role [35]. The slopes of the linear regression equation for the Korsmeyer-Peppas model was used to determine what other contributing mechanisms existed. Amitriptyline (1, 5, and 10%) release from Emollient cream® was found to have slopes under 0.5 implying release by Fickian diffusion. On the other hand, all slopes for amitriptyline (1, 5, and 10%) release from Lipoderm® base and Mediflo® PLO gel was between 0.5 and 1. The mechanism, in this case, follows non-Fickian or anomalous transport meaning that vehicle swelling and/or erosion contributed to drug release [35].

For drug release to occur, the affinity for the active drug must be somewhat greater to the membrane than to the vehicle of the formulation [96]. Where roughly half of amitriptyline was not released, this could suggest equal affinity to both the membrane as well as the vehicle. The vehicle that allowed for the greatest mean drug release overall between all concentrations was Mediflo™ PLO gel. Theoretically, more active drug is available to permeate through the stratum corneum, which is the biggest barrier to drug absorption, and into the dermis, where peripheral nerve endings are located [97]. Interestingly, drug permeation and flux was the least in this formulation.

Drug permeation studies were conducted using Strat-M®, which is a synthetic membrane that mimics the morphology of the human skin [41,40]. It has a high *in vitro-in vivo* correlation (IVIVC) for a variety of active pharmaceutical ingredients and offers lower sample variability compared to human skin [41,40,38]. Additionally, it is easy to acquire and does not require any ethics approval. It was found that when amitriptyline

was compounded in Mediflo® PLO gel at strengths of 1 and 5%, no drug permeation occurred. It is possible that Mediflo® PLO gel allows for amitriptyline retention in the *stratum corneum* and/or viable epidermis, hence no detection in the receptor fluid. This may also be the case for amitriptyline 1 % in Emollient cream® and Lipoderm® base where a very small quantity of drug was detected at the 12 and 4 hour, respectively. Nevertheless, the use of pig or human skin, where the different layers could be dissected and analysed, would be required to confirm this.

A low lag time and high flux are important characteristics for a topical analgesic to have because with typical patient use, the cream may be rubbed or washed off in a short period of time. As shown in Table 17, amitriptyline (5, and 10%) in Lipoderm® base and Emollient cream® have a high flux and low lag time. The lack of clinical efficacy in formulations containing less than 5% amitriptyline makes sense given poor permeation at that concentration. Overall, amitriptyline 5 or 10% compounded in Lipoderm® cream or emollient cream offer the greatest drug permeation through Strat-M® as well as low lag times.

The *in vitro* data generated through the permeation experiments reflects a conservative or the most absorption that could potentially occur due to several factors that differ from application on human subjects. The first factor is the fact that after application of a topical cream, it is often rubbed or washed off after a relatively short period of time while the applied dose in the Franz diffusion system remains. Additionally, desquamation of the skin may contribute to drug loss, however, at 24 hours this effect is likely to not have a significant impact as only one layer of the *stratum corneum* is lost per day [98].

5.5 CONCLUSIONS

Topical amitriptyline has been described in the literature as having mixed clinical efficacy. However, a few case reports utilizing higher concentrations of the compound found clinical benefit. Many of these studies did not describe the components in the amitriptyline formulations. *In vitro* drug release and permeation conducted revealed that roughly half of the active drug is retained in all three formulations studied after 24 hours as well as almost no permeation through the Strat-M® occurred when amitriptyline is present in concentrations less than 5%. Data from our studies may help compounding pharmacists and prescribers with selecting appropriate amitriptyline concentrations and vehicles for patient use.

CHAPTER 6- GENERAL DISCUSSION AND CONCLUSIONS

6.1 THESIS SUMMARY

Neuropathic pain is a condition that tends to be chronic in duration and resistant to many analgesics. Amitriptyline and gabapentin are two oral medications currently recommended as first line treatment options for such patients. These medications are associated with several side effects that, in some cases, are not tolerable. Additionally, there is some evidence that these two medications target peripheral ion channels located on peripheral nerve fibers in the integumentary system. There are several case reports showing clinical efficacy of topical gabapentin and amitriptyline formulations. Unfortunately, there is a lack of data on the drug release characteristics, permeation, and stability of these compounds.

In this thesis, analytical techniques were explored and validated to be able to detect amitriptyline and gabapentin with sufficient specificity, linearity, accuracy, and precision. Stability of the two drugs in three different commonly used bases were investigated at three different temperatures over a 90-day period. Potency, pH and organoleptic properties were recorded. Currently, USP standards assigns a beyond use dating of 30 days for semi-solid formulations. It was found that preparations of gabapentin 10% in Lipoderm® base were stable in Ecolojars® for 28 days at room temperature. However, gabapentin 10% was not stable in both Versabase® gel as well as Emollient cream®. Compounded preparations of amitriptyline 10% in Lipoderm® base and Mediflo® 30 PLO, respectively were stable in Ecolojars® for 90 days at room temperature. Physical changes were observed at high (40^{o}C) and low temperatures (4^{o}C), hence these compounds should be stored at room temperature.

In vitro drug release and permeation studies conducted for topical gabapentin revealed complete release of the active drug within the first 6 hours of application in all bases following the Higuchi model. No permeation through Strat-M® occurred when gabapentin was present at concentrations of 1%. Additionally, permeation of gabapentin using Versabase® gel and Lipoderm® base were not significantly different. However, Emollient cream® resulted in a significantly lower total gabapentin permeation, which may be due to a lack of sufficient permeation enhancers in the base. Although Versabase® gel and Emollient cream® displayed some gabapentin permeability, it is important to consider that gabapentin showed poor stability in these formulations as seen in Chapter 2. Hence, it is not a practical base for this drug.

Topical amitriptyline has been described in the literature as having mixed clinical efficacy. *In vitro* drug release and permeation studies conducted revealed that roughly half of the active drug was retained in all three formulations studied after 24 hours. Furthermore, at concentrations lower than 5%, no permeation through Strat-M® occurred. When amitriptyline was compounded in Mediflo® PLO gel, concentrations below 10% resulted in no detectable permeation. This may explain why trials conducted with low amitriptyline concentrations were unsuccessful. Amitriptyline (5 and 10%) in Lipoderm® base and Emollient cream® had the highest permeability and flux. However, lack of amitriptyline stability observed in Emollient cream® beyond 14 days eliminates it as a useful base for this drug. This information may help compounding pharmacists and prescribers with selecting appropriate amitriptyline concentrations as well as vehicles for patient use.

6.2 CHALLENGES AND PROSPECTS OF COMPOUNDED TOPICAL PAIN FORMULATIONS

The area of topical analgesic compounding has grown in popularity in the past few years with a large array of bases and combinations of drugs used in practice. Unfortunately, many compounding pharmacists do not have a sufficient understanding of factors that may impact product performance and stability. With the lack of literature information in this area, a trial and error approach is often adopted. This can be time consuming, wasteful, and expensive for both the pharmacy and the patient. Therefore, generating biopharmaceutical and pharmacokinetic data in this area is of immense importance for patient safety.

6.3 SIGNIFICANCE OF CURRENT WORK AND FUTURE RESEARCH OPPORTUNITIES

The information generated in this thesis provides additional information for compounding pharmacists to make a professional judgment regarding acceptable dosing range, topical product performance and beyond use dating. Where little to no information existed on the stability of the compounds studied, it is a challenge to answer patient questions regarding storage conditions other than recommending room temperature for 30 days. Future research could involve using human skin to predict *in vivo* drug permeation and further move on to human trials with serum concentration measurements. Another future project may be to develop a novel formulation to extend stability of gabapentin beyond 28 days.

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