A Thesis

Effective Topical Delivery of Ibuprofen through the Skin

by

Audree Porter

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Pharmaceutical Sciences

(Industrial Pharmacy)

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An Abstract of

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The ability to effectively deliver non-steroidal anti-inflammatory drugs (NSAIDs) topically and transdermally offers an increased localization of the drug to the site of pain and inflammation, while simultaneously reducing systemic absorption. This ultimately results in more effective treatment for localized pain and inflammation, while reducing the undesired side effects associated with NSAIDs.

In this work, effective topical delivery was studied, specifically to compare and contrast the effects of three separate penetration enhancers utilizing *in vitro* Franz cell testing methods. Ten formulations, using three different penetration enhancers (Kollicream OA, Kollicream IPM and Kollicream 3C) and two different active pharmaceutical ingredients (ibuprofen and sodium ibuprofen), were tested and results are given in this report. Ultimately, the penetration enhancers were found to impede delivery of active pharmaceutical drugs into the epidermis of the skin over an 8-hour period as compared to a standard blank cream run concurrently. Kollicream 3C was shown to have the best release profile over the initial first hour of the study, mimicking the initial

application of the pharmaceutical product. Kollicream OA was shown to have the best release profile over the entire 8-hour period.

Acknowledgements

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List of Abbreviations

API…….Active Pharmaceutical Ingredient

BCS…….Biopharmaceutical Classification System

CPE……...……..Chemical Penetration Enhancer

DDDS..... Dermal Drug Delivery System

Ibu……..….Ibuprofen

K$\frac{o}{w}$……octanol/water partition coefficient

MSDS……..Materials Safety Data Sheet

NaIbu……..Sodium Ibuprofen
NSAID……Non-Steroidal Anti-inflammatory Drug

O/W……Oil-In-Water
OTC……Over The Counter

PBS…….Phosphate Buffered Saline

TDDS ..... Transdermal Drug Delivery System

W/O……Water-In-Oil

List of Symbols

ε……...……epsilon; term designated for molar absorptivity
λ……..lambda; wavelength-dependent absorptivity coefficient

A…….....absorbance
b……...…….. path length from the sample cell to the detector
c……..…concentration of the analyte
kD……kilo Dalton, standard unit used for indicating atomic mass
I_0 ……...…..intensity of the light passing through the reference cell
I…….. intensity of the light when it passes through the sample cell
ℓ……..…..length of the solution that the light passes through (in cm)

Chapter 1

Introduction

1.1 The Skin: An Overview

Skin is the largest organ in the human body. It can be divided into three major layers: the epidermis, dermis and hypodermis. These layers combine to provide a protective barrier to the outer environment that the body is exposed to daily.

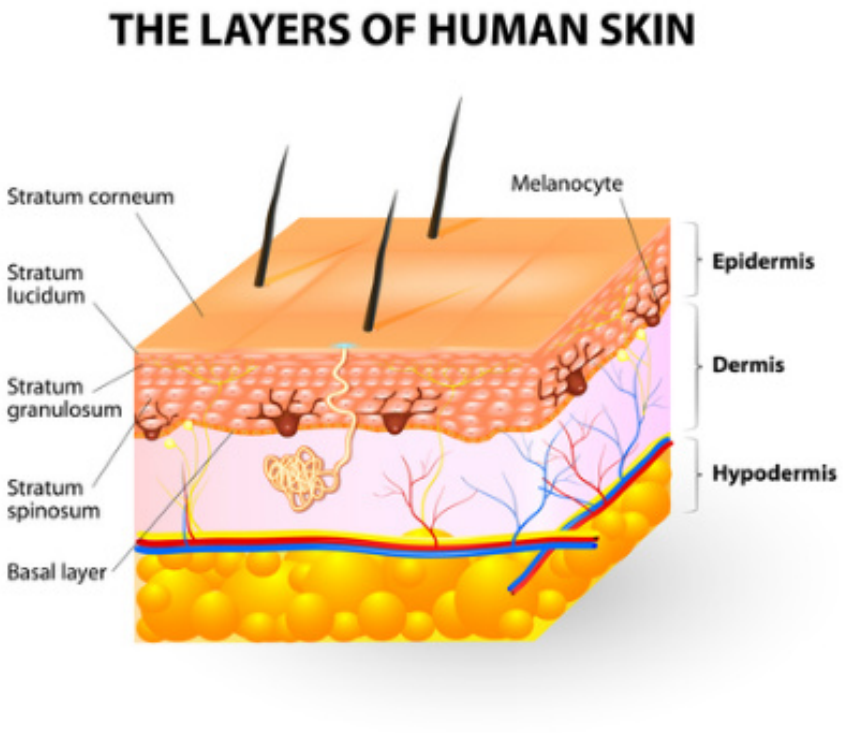

Figure 1. The layers of human skin. [1]

The epidermis is the outermost layer, thinly composed of five layers of its own: stratum germinativum, stratum spinosum, stratum granulosum, stratum licidum and stratum corneum. This latter layer of skin forms the barrier against particles that would otherwise enter your body as well as giving rise to melanin, which causes skin color and tone [2]. Topical and transdermal products are applied to this layer of skin where they may treat the skin topically or penetrate into the deeper layers and absorb into the blood.

The dermis is the middle layer of skin, holding blood vessels, nerves, hair follicles and sebaceous glands. This layer controls most of the proteins that keep skin healthy such as collagen and elastin; these two proteins keep the skin adaptable and flexible. This layer of skin also gives home to receptors, which are responsible for feeling pain and touch [2].

Finally the hypodermis is the layer deepest within the skin. It is the fatty layer, which is responsible for conserving body heat [2].

1.2 Topical Drug Delivery Systems

There are two different types of drug delivery systems: topical and transdermal. Transdermal drug delivery systems make use of external physical depots to deliver the active drug through the skin and into the systemic circulatory system. Topical drug delivery systems utilize creams and lotions to apply the active drug into the epidermis of skin but not usually into the circulatory system [3].

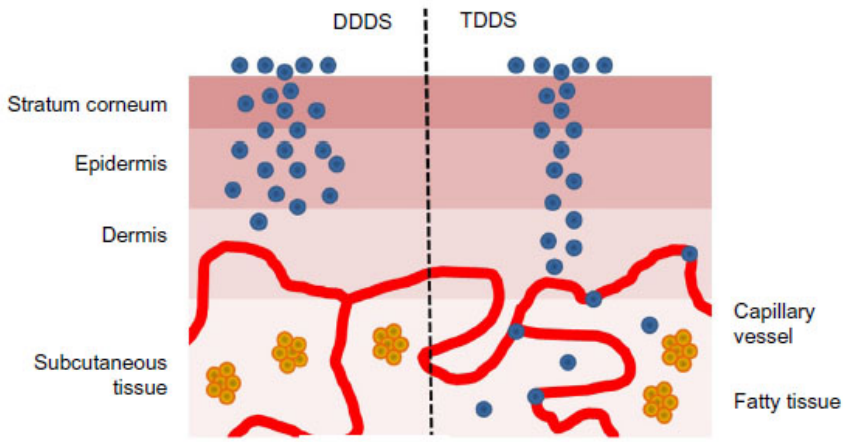

Figure 2. A visual representation of a DDDS, i.e., dermal drug delivery system, versus TDDS, i.e., transdermal drug delivery system] [4]

Topical drug delivery focuses on administering active ingredients directly onto the skin to treat various conditions, including local pain, topical infections, and cosmetic problems. Topical delivery systems can be divided into three conventional dosage forms, including liquid, semi-solid and solid systems. Liquid formulations include topical solutions, lotions, and suspensions. Semi-solids include creams, gels, pastes and ointments. Solid formulations could include powders and sticks [5].

Advantages associated with topical drug delivery include the bypass of first pass metabolism due to the medication being applied directly to the affected area and not absorbed into the skin. This lessens the chance for any metabolic transformations that would otherwise occur to the molecule while traveling to the target site. Topical medications are also easy for the patient to apply to the affected area as well as increasing patient compliance. Finally, by allowing the medication to be applied directly to the target site, systematic side effects are lessened, since the drug does not need to primarily circulate within the body.

1.3 Emulsions

Emulsions are defined by the FDA as "A dosage form consisting of a two-phase system comprised of at least two immiscible liquids, one of which is dispersed as droplets (internal or dispersed phase) within the other liquid (external or continuous phase), generally stabilized with one or more emulsifying agents." [6]

1.3.1 Composition of Emulsions

An emulsion in its most basic definition consists of an oil phase, a water phase and an emulsifier. This triphasic system results from one of the phases being dispersed through the vehicle; this is referred to as the internal or dispersed phase. The continuous, or external, phase describes the vehicle that surrounds the globules formed by the dispersed phase. Fundamentally, the emulsifier stabilizes this immiscible system. This component of the emulsion, referred to as surface-active agent (surfactant), is added to the system to thinly coat the globules of the dispersed phase and allow for stability in the immiscible system. Interfacial tension, or tension between the surfaces of the two liquids, is what keeps the system cohesive. When the two liquids are in contact with each other, the interface between the two liquids will be maintained at a minimum distance between the two, making it impossible to mix completely. However, when an emulsifier is added, it will orient itself so that the polar head group will face the polar phase and the nonpolar tail end will face the nonpolar phase of the emulsion. This lowers interfacial tension, and allows for dispersion of the two immiscible phases [7]. There are two basic types of emulsions; water-in-oil (W/O) and oil-in-water (O/W). In a W/O emulsion, the water

phase is dispersed into the oil continuous phase. Reversely, an O/W emulsion describes a system with the oleaginous phase dispersed into a continuous water phase.

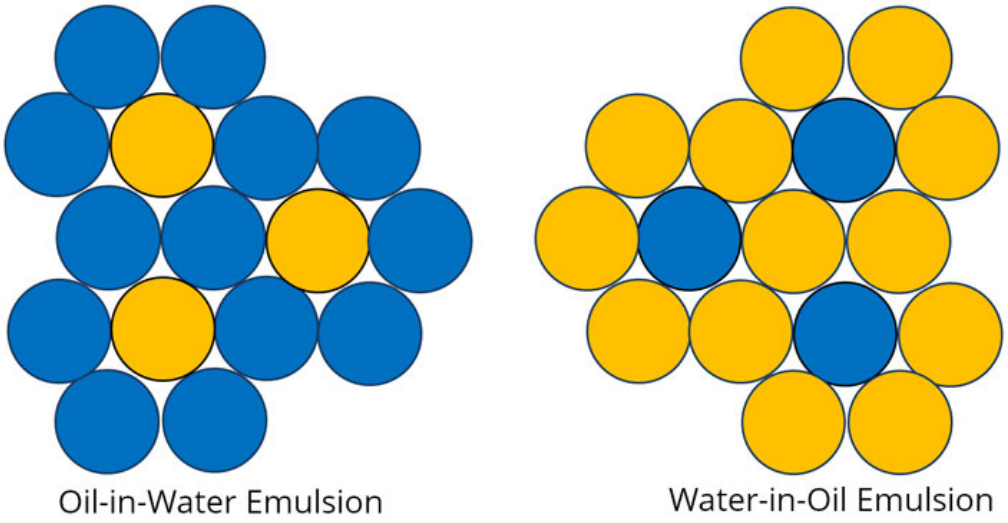

Figure 3. Oil-In-Water Emulsion versus Water-In-Oil Emulsion [8]

O/W emulsions are non-occlusive and more easily washed off. They have a pleasantly light skin-feel; since the oil phase is dispersed into the water phase, decreasing any "greasiness" in terms of skin feel. This can increase the patients' compliance when using the product.

W/O emulsions are perceived greasier and more occlusive and are primarily used when the skin needs to be hydrated. The thicker base allows for the hydration of the outer layer of the skin and prevention of evaporation of eccrine secretions. The internal water phase is dispersed throughout the external oleaginous phase, leading to a more water-resistant topical product.

1.3.2 Rheology and Stability of Emulsions

Emulsions are thermodynamically unstable due to the positive free energy at the interface of both phases. Thus, the need for external energy applied to the system to keep the system stable. Without this addition of energy, the phases pull apart from each other to reach the minimal amount of energy required [9]. Due to the thermodynamic instability of this drug delivery system, several different complications may arise in the form of physical changes; some of which are reversible and others are irreversible changes. Types of reversible physical instability include flocculation, creaming, and sedimentation; while irreversible changes include phase inversion, Ostwald ripening and coalescence (Figure 4).

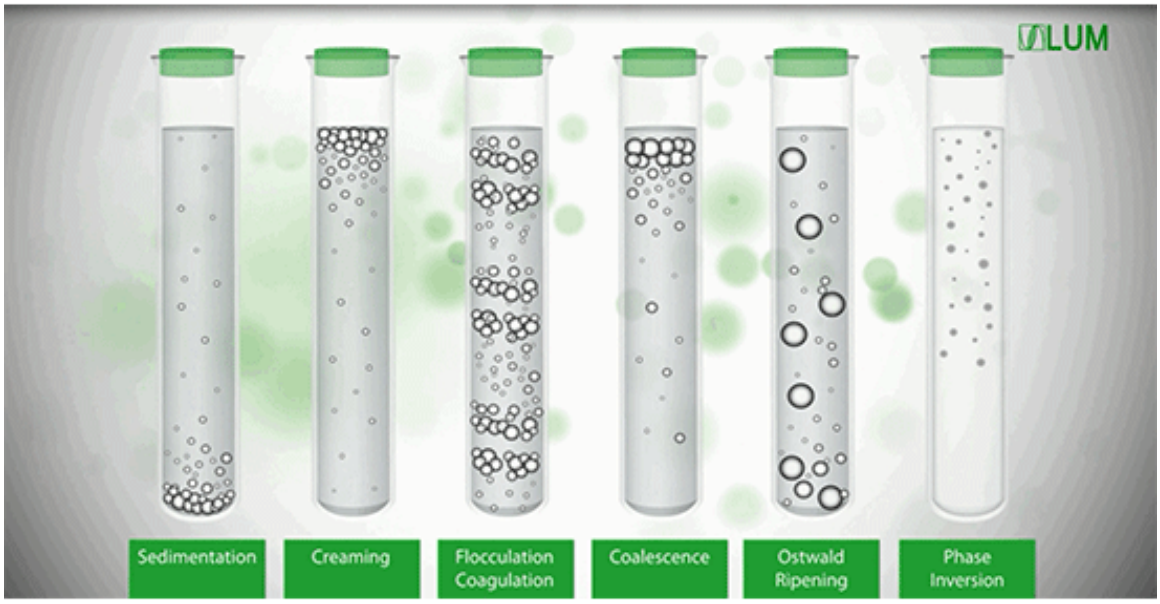

Figure 4. Reversible and irreversible changes in emulsions [10]

Flocculation is defined as the aggregation of globules into 3D structures without the emulsion breaking entirely. These globules still maintain their individual identity, but the weak attraction between the molecules allows for them to congregate [11]. These colonies may not be visible to the naked eye, but are the beginning of an unstable

emulsion. The extent of flocculation depends on the size distribution of the globules, the surface charge of the globules and the viscosity of the vehicle. To prevent flocculation, uniform size distribution and repulsive charge forces are ideal. Highly viscous vehicles also immobilize the particles and stop the congregation. Redispersing the emulsion with shaking may reverse this physical instability.

Physical creaming of the emulsion refers to the effect of gravity on the globules when the density of the droplets is less than that of the vehicle. The less dense droplets of the dispersed phase will float to the top of the emulsion, making the emulsion break into two phases: the "cream" on top and clear supernatant below [12]. *Sedimentation* is very similar, a gravity driven separation of phases, but in this case the dispersed phase has a higher density than the vehicle and it settles to the bottom of the emulsion.

Irreversible phase changes include phase inversion and Ostwald ripening. *Phase inversion* describes the process where the dispersed phase and the medium exchange over time. This means that an O/W emulsion could change into a W/O emulsion and vice versa. *Ostwald ripening* occurs in thermodynamically unstable systems, where the smaller molecules on the surface of larger molecules, dissolve into the solvent phase. This shrinks the particle size while increasing the number of dissolved particles. This cycle of particle size changes increases the mean particle size distribution (PSD), such as in an O/W emulsion where the oil phase may leach out of the aqueous phase and form larger globules. This can lead to phase inversion and the breaking of an emulsion [13]. *Coalescence* describes the phenomenon when the interfacial film between droplets is disrupted and allows multiple droplets to join together to create larger and larger droplets

until the two phases separate completely. Once the two phases have completely separated, the change is permanent and irreversible [14].

1.4 Local pain and inflammation

Pain, as defined by the International Association for the Study of Pain, is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [15]. Pain may be acute when there is an immediate injury, or become chronic if it lasts for up to 12 weeks.

Chronic pain symptoms include mild to severe pain, soreness, tightness, and stiffness, fatigue and depression. These conditions generally arise from areas such as the back, neck, knee, hip or wrist. Most commonly reported chronic pain stems from lower back pain (27% reported) followed by neck pain (15%) and facial ache (4%). It is estimated that up to 100 million Americans, and 1.5 billion worldwide, suffer from chronic pain daily [16].

The cost and burden of chronic pain on the United States health care system range from $560 billion to $630 billion as of 2010. It is estimated that approximately 20% of the American report some sort of pain during sleep that causes wakefulness [16]. Studies found that 59% of patients said that their chronic pain had an impact on their overall quality of life and 77% of patients felt depressed.

Inflammation occurs in tissue as a response to a stimulus, most commonly physical or bacterial. The body's response to the stimuli results in the expansion of blood vessels within the tissue, increasing the amount of blood reaching the affected area. This increase in blood allows for the circulation of more pathogen fighting cells to combat any

bacterial infections causing the inflammation. Symptoms of inflammation include redness, swelling, heat, pain and loss of function [17]. Unfortunately, inflammation may not always be a response to a stimulus but a symptom of chronic pain. For common diseases such as rheumatoid arthritis, psoriasis or ulcerative colitis, inflammation may occur and cause constant pain to the patient [17].

1.5 Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the leading pain relief drugs used today. Their uses range from analgesic to anti-pyretic and can also have anti-inflammatory benefits [18]. NSAIDs have been widely used in the United States since the 1950s, with the drugs being divided into six major classes, each having their own characteristics [19]. The six major classes include the acetic acids, COX-2 Inhibitors, fenamates, oxicam derivatives, propionic acids and salicylates [20]. This study primarily focused on two drugs, namely ibuprofen and sodium ibuprofen. These two drugs belong to the propionic acid class of NSAIDs.

Ibuprofen (Ibu), or isobutylphenylpropanoic acid, has a mechanism of action, which includes inhibiting cyclooxygenase systems (COX-1 and COX-2) from synthesizing prostaglandins [21]. Prostaglandins are responsible for the inflammation response that results within tissue in the body when infection or injury has occurred. COX-1 generates prostaglandins that regulate gastrointestinal, renal and vascular functions. COX-2 regulates prostaglandins that regulate inflammation, fever and pain [22].

Sodium ibuprofen (NaIbu) is the salt form of Ibu, working similarly in nature by inhibiting prostaglandin synthesis by inhibiting the COX mechanisms.

NSAIDs are used commonly in the United States, many are available over-the-counter (OTC) for patients to buy and use as they see necessary. The FDA list of OTC NSAIDs includes ingredients such as aspirin compounds, ibuprofen, naproxen and ketoprofen [23]. These are generally given as oral medications, such as capsules, tablets, pills, and liquid suspensions. Currently in the United States Ibu is only approved for systemic administration, and not for topical use. The FDA has not approved safety and efficacy claims, and side effects have not been studied [24]. The FDA has approved several topical NSAIDs such as Voltaren®, which contains diclofenac sodium as the active ingredient, but no products that contain Ibu as the active ingredient are fully approved as of yet.

Currently, in Canada and the European Union (EU), both the Health Products and Food Branch (HPFB) of Health Canada and the European Medicines Agency (EMA) have not approved any marketing authorizations for topical ibuprofen formulations [25]. There are two ways to get a drug approved in the EMU, a centralized procedure that results in approval throughout the entire EU or a national authorization process that results in the medicine being approved in individual countries throughout the EU. While there is no approval overreaching the entire EU, several individual countries have successfully introduced topical ibuprofen onto the market. One example, Algoflex Dolo 50 mg/g gel, is an ibuprofen gel approved for use in Hungary [26].

A study investigating the dissolution, plasma pharmacokinetics and safety of NaIbu versus Ibu should shed some light on the differences between the two compounds.

NaIbu dissolves significantly more rapidly at pH 1.2, 3.5 and 7.2 as compared to IBU. NaIBU reached the T_{max} significantly earlier than IBU, and showed a higher C_{max} than IBU. Finally, NaIBU had a significantly higher mean plasma concentration 10 minutes post dose than IBU [27].

1.6 Solubility and permeability of the two model active ingredients

The Biopharmaceutical Classification System (BCS) uses four different classes to define drugs based on their solubility and permeability (as shown in Figure 5). Class I has high permeability and high solubility. Class II has high permeability and low solubility. Class III has low permeability and high solubility. Class IV has low permeability and low solubility.

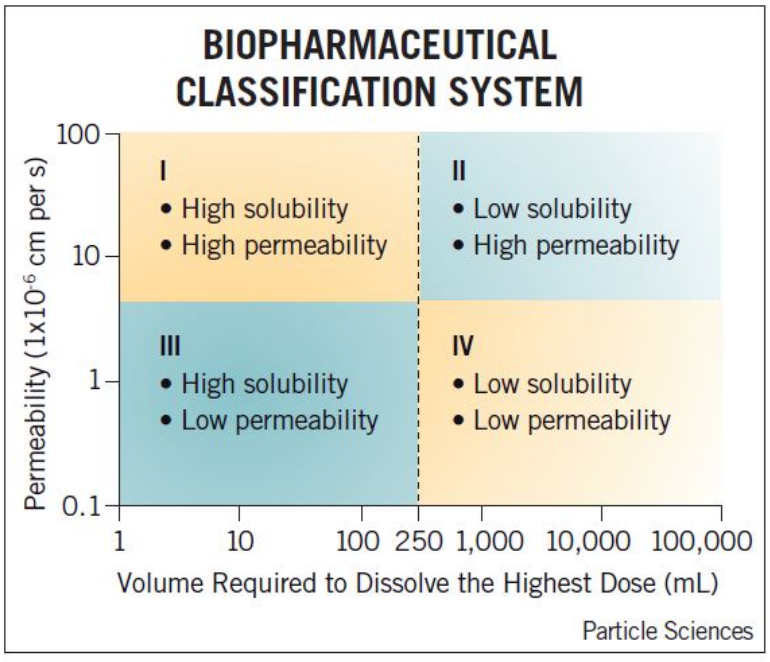

Figure 5. Biopharmaceutical Classification System of pharmaceutical active ingredients [28].

Permeability of a drug through the skin depends on several different considerations, including (1) lipophilicity/hydrophilicity, molecular charge, molecular weight, and ionization of the drug; (2) surface area, integrity and maturity of the skin; (3) as well as the formulation (i.e., vehicle). The larger the surface area of skin that the topical drug is applied to, the greater the absorption of the drug. The integrity of the skin is important in the permeation of drug, when the skin is well hydrated and has no abrasions; its barrier function is high. When the skin is mature, has small cuts or is dry, the drug may penetrate more quickly or deeply than on normal and healthy skin [29].

Molecular weight is defined as the mass of one mole of a substance [30]. The molecular weight of the drug is optimized for topical delivery at less than 500 daltons. The size of the substance entering the skin must be less than 500 daltons to be considered ideal characteristics for topical drug delivery [31].

Drugs that have an octanol/water partition coefficient ($K\frac{O}{W}$) that favors lipids have a better chance of permeating through the lipid bilayer of the skin. A partition coefficient shows the ratio of the concentration of the drug added to the immiscible system in both phases showing the difference in its solubility in each phase.

Intercellular lipids are also available between the cells of the skin, giving the drug a winding pathway to diffuse into the epidermis [32]. Molecular charge affects the ionization of the drug, which therefore affects its solubility. As the molecular charge increases, the bonds that strengthen the chemical entity become stronger, thus leading to increased difficulty in solubilizing the drug [33].

A drug is classified as highly soluble when the highest dose strength is soluble in less than 250 mL of water between the pH values of 1.0 and 7.5. A drug is highly

permeable when the absorption of the drug is <90% of the administered dose [34]. Ibu is considered a BCS II drug, with a low solubility at pH 1.2 and 4.5 and a high solubility at pH 6.8 [35]. The permeability of a drug is decided based on the intestinal permeability and the corresponding dose absorbed, >90% of the dose given means the drug is highly permeable [36].

Chemical penetration enhancers (CPEs) are chemicals identified to interact with the stratum corneum, thus increasing the permeation rate of drugs. They can also act as co-solvent for drugs. Most commonly, CPEs are surfactants, which have the ability to solubilize lipids on the stratum corneum, leaving openings for the drug to penetrate into [37]. Anionic surfactants, such as sodium lauryl sulfate (SLS), can cause irritation to the skin. This damage to the stratum corneum allows for the drug to permeate the skin with lessened resistance [38]. Cationic surfactants may also irritate the skin and allow for drug penetration, but have not been studied for efficacy [38]. Finally, non-ionic surfactants have been studied on both rat and murine skin to show increased permeation. Although these surfactants are much less irritating than the previously discussed surfactants, they have been shown to destructure the lipids of the skin and allow for drug penetration [38].

Alcohols can also be used as penetration enhancers, saturated and unsaturated long chain fatty alcohols have been shown to improve the amount of penetration into the skin. According to a study completed by Andega et al. (2001), maximum penetration occurred when the carbon chain length was 10. This penetration further increased when the unsaturated chain bonds increased from single to double bonds [39]. Oleyl alcohol has been used as a penetration enhancer following the cell envelope theory, which states

that this compound assists in disordering the lipid structure of the corneum stratum by enveloping, or covering the cell [40].

Amides, marketed as penetration enhancers, were introduced to research in the early 1980s [38]. Azone, comprised of a 12-carbon chain with a polar 7-carbon member ring attached. It was shown that the enhanced penetration came from the ability of the molecule to reduce diffusional resistance of the skin [38]. Diffusional resistance through the stratum corneum tends to be the rate-limiting step in percutaneous absorption and the entire diffusional resistance can be calculated by summing the individual resistance met in the stratum corneum, epidermis and papillary layer of the dermis [41].

Several esters, such as the benzoate ester octyl salicylate, have been shown to increase transdermal penetration. It has been shown in ATR-FTIR studies to change the conformational order of the lipid bilayer, allowing for the drug to permeate between the layers and though the stratum corneum [38]. Another ester commonly used as a penetration enhancer, fatty ester isopropyl myristate, has been thoroughly studied. Explanations of how this permeation occur ranges from increasing in the skins lipid fluidity to decreases in the width of the lipid bilayer to finally an increase in drug solubility of the skin [38].

Listed below in Table 1. are examples of products on the market that use patches applied topically to the skin to deliver drugs. These can be used intermittently to treat conditions such as motion sickness, to being used everyday for smoking cessation.

Table 1. Examples of common penetration enhancers used in marketed products [42]

Drug/Product Name	Indication	Company	Penetration enhancer
Scopolamine/Transderm-Scop	Motion sickness	Novartis Consumer Health	Light Mineral Oil
Nitroglycerin/Transderm-Nitro	Angina	Novartis Consumer Health	Ethylene-vinyl acetate copolymer
Nicotine/Nicoderm	Smoking Cessation	GlaxoSmithKline	Ethylene-vinyl acetate copolymer

Chapter 2

Materials & Methods

2.1 Materials

2.1.1 Active ingredients

Ibuprofen ($C_{13}H_{18}O_2$, Ibu) and Sodium Ibuprofen ($C_{13}H_{17}O_2$•Na, NaIbu) were used as model active pharmaceutical ingredients (APIs) in this study. Ibuprofen 50 and Sodium Ibuprofen (Lot No.: SB1W0030) were gifted by BASF (Ludwigshafen, Germany). Ibuprofen 50 (Ibu) is a white crystalline powder with a characteristic smell. Its melting point ranges between 75°C-77°C and it is not flammable. Its partitioning coefficient n-octanol/water is 3.87 at 25°C. Its solubility in water is 0.01139 g/l at 25°C.

Sodium Ibuprofen (NaIbu), marketed by BASF as Sodium Ibuprofen Dihydrate, is a white crystalline powder. NaIbu partitioning coefficient n-octanol/water is 0.35 at 25°C. NaIbu solubility in water is <100 g/l at 20°C.

2.1.2 Inactive ingredients

Kollicream® OA, Kollicream® IPM and Kollicream® 3C were used as a penetration enhancers. All three ingredients were gifted by BASF (Ludwigshafen, Germany). Kollicream® OA (Lot No 0012855172, USP/NF: Oleyl Alcohol) is a colorless

liquid with a fatty odor. Its partitioning coefficient n-octanol/water is 7.5-8 and its water solubility is <0.1 mg/l. Kollicream® IPM (USP/NF: Isopropyl Myristate, Lot No.: 0010084422) is a colorless, odorless liquid. Its partitioning coefficient n-octanol/water is measured as 7.71 and its water solubility is <50 μg/l. Kollicream® 3C (USP/NF: Cocoyl Caprylocaprate, Lot No.: 0009030982) is a slightly yellow colorless and odorless liquid. Its partitioning coefficient has not been measured and it is deemed insoluble by BASF.

Glycerol formal, 1,2,3-Propanetriol Glycerin, (Lot No 11290915) was ordered from Letco Medical (Decatur, Alabama). This was used in the aqueous phase in conjunction with distilled water (supplied by The University of Toledo, College of Pharmacy) for use in the formulation of the base of the cream.

Kolliwax® CSA 50 (USP/NF: Cetostearyl Alcohol, Lot No.: 0012982248) was gifted by BASF (Ludwigshafen, Germany). This was used as co-emulsifier and lipophilic thickener in the emulsion. It was presented as white solid pearls with a melting point of 49-56ºC

Kolliphor® CS 20 (USP/NF: Polyoxyl 20 Cetostearyl Ether, Lot No.: 0009584804) was gifted by BASF (Ludwigshafen, Germany). This product was used as a non-ionic emulsifier suitable for use in oil, lotions and creams made with hot process. It was presented as odorless, white pellets with a melting point of 39.0-41.0°C.

The preservative used, Euxyl® PE 9010, was supplied by Schülke (Norderstedt, Germany). This is a liquid cosmetic preservative using phenoxyethanol and ethylhexylglycerin, and is effective in preventing bacteria, yeasts, mold and fungi [43].

For the in vitro studies, a phosphate buffer solution composed of distilled water, NaOH and KPO_4 was made and the pH was adjusted to 7.4. All ingredients were supplied by The University of Toledo (Toledo, Ohio).

2.2 Methods

2.2.1 Formulation of the creams

Formulation of the creams began with four separate phases, each comprised of specific ingredients to be added in a specific order. Phase 1 was composed of distilled water and glycerol formal. Phase 2 was composed of Kolliwax CSA 50, Kolliphor CS 20 and one of the three Kollicreams used within the experiment: 3C, OA or IPM. Phase 3 refers to the preservative, i.e., Euxyl 9010. Phase 4 refers to the active ingredient used; either ibuprofen or sodium ibuprofen.

First, Phase 1 was mixed in a beaker with an overhead mixer at 300 rpm until mixed completely. Phase 4 was transferred into the beaker containing phase 1 and mixing continued at the same speed while increasing temperature to 75ºC. The decision to add the API into the aqueous phase was chosen in order to wet the powder before addition into the formulation; incorporation of the powder needs to be slow and with continued mixing. Simultaneously, phase 2 was added into a separate beaker and heated to 75ºC. When both beakers were at the same temperature, the beaker containing phase 2 was added to the beaker containing phases 1 and 4. This mixture was homogenized for five minutes using an overhead mixer at 550 rpm. After five minutes, the mixing speed was reduced to 200 rpm and the temperature was reduced to 40ºC. By reducing the mixing speed, the chance for air incorporation into the cream is lessened and an even application

of the cream on to the membrane is achievable. Now, phase 3 containing the preservative was added with mixing and allowed the product to cool to 30℃. Finally, it was let sit overnight.

Several variations of this formula were formulated during this study. Three formulations using ibuprofen as the active ingredients were made, each one using a different penetration enhancer (3C, OA or IPM). Three formulations using sodium ibuprofen were also formulated; each using an individual penetration enhancer. Finally, three formulations were made using no active ingredient but still maintaining the use of one of the three individual penetration enhancers. Table 2. describes the quantities of each ingredient within each cream.

Table 2. Table of Ingredients and Composition of Formulations

	1	2	3	4	5	6	7	8	9
Ingredients	Amount of each ingredient (%)								
Ibuprofen	5.0	-	-	5.0	-	-	5.0	-	-
Sodium Ibuprofen	-	5.0	-	-	5.0	-	-	5.0	-
Kollicream 3C	10.0	10.0	10.0	-	-	-	-	-	-
Kollicream IPM	-	-	-	10.0	10.0	10.0	-	-	-
Kollicream OA	-	-	-	-	-	-	10.0	10.0	10.0
Glycerol Formal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Kolliphor CS20	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Kolliwax CSA50	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Euxyl PE9010	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water	65.5	65.5	70.5	65.5	65.5	70.5	65.5	65.5	70.5

2.2.2 *In vitro* skin diffusion studies

Diffusion is defined as the process whereby particles of liquids, gases, or solids intermingle as the result of their spontaneous movement caused by thermal agitation and in dissolved substances move from a region of higher to one of lower concentration [44]. For a drug to diffuse into the skin of a subject, the solubilized drug must pass from high concentrations in the topical delivery system into the epidermis and dermis of the skin to affect the target area.

Franz diffusion cells are commonly used in the pharmaceutical industry to evaluate the *in vitro* release as well as penetration of various drugs. A Franz cell is a piece of glass equipment fitted together (Figure 6). It consists of a donor chamber where the drug is applied to, and a receptor chamber that is filled with a receptor medium. For penetration studies, a membrane that mimics the skin is placed and secured between the two chambers. Franz cells are available with or without jackets; the cells used in this study were jacketed Franz cells. Water from a heater/circulator continuously flows through the water jacket surrounding the receptor chamber keeping the temperature constant. A sampling port connected to the inner receptor chamber allows for withdrawing a quantity of fluid. This fluid represents the circulatory system of the patients' body, and is in direct contact with the membrane and drug applied to it. [45].

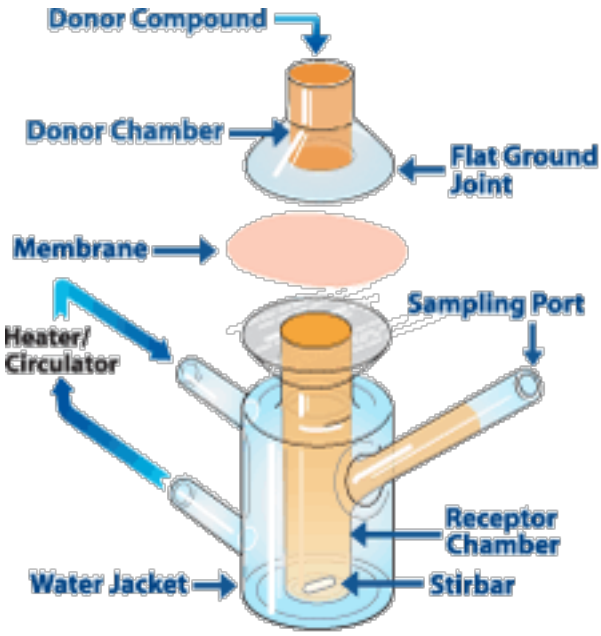

Figure 6. A jacketed Franz diffusion cell. [46]

A topical formulation is placed on top of the membrane, which is in contact with the receptor medium within the receptor chamber, such as a buffer solution that has a physiological pH, to study the diffusion of the drug. Different types of membranes can be used to study the penetration of active ingredients, including synthetic membranes, animal skin and human skin.

The membrane used in this study was pig ear skin. This skin was clean, shaved and excised before being placed between the donor chamber of the Franz cell and the top of the receptor chamber. The skin was collected from a local slaughterhouse. Shaving the skin removes the hair follicles that could absorb additional drug without yielding the relief the drug product intends. The thickness of pig ear skin has been found to be a good alternative to human skin according to a study done by Università degli Studi di Milano testing the flux of 7 benzoxazinones through both pig ear skin and human epidermis [47]. Another study using pig ear skin reported the range full thickness pig ear skin to be approximately 0.38 to 0.57 mm [48. The best way to ensure consistent thickness in each membrane is to use a dermatome, a piece of skin grafting equipment, set to a precise thickness to cut the pig ear skin [49].

A sample from the receptor phase is withdrawn with a syringe and may be tested to calculate the concentration of the drug present in the receptor phase. In the case of this experiment, 3 mL samples were withdrawn every 15 minutes for the first hour, every 30 minutes for the next three hours and finally every hour for the last four hours. PBS buffer was added after every withdrawal and the Franz cells were de-aerated. As the sample was replaced with PBS, dilution in the receptor phase occurred at each sampling. This dilution was taken into consideration when calculating the concentration of the drug.

2.2.3 Drug analysis

In this study ultraviolet-visible (UV-Vis) spectroscopy was used to analyze the amount of drug in the withdrawn samples. UV-Vis spectroscopy refers to the use of light absorption within the electromagnetic spectrum, specifically the visible light range, to determine the concentration of a solution based on a pre-calibrated curve. As shown in the chart below, the ultraviolet range incorporates 100 nm to approximately 390 nm while the visible light range incorporates frequencies between 390 to 700 nm. This range is the focus of UV-Vis spectroscopy analysis of drug concentration in samples tested.

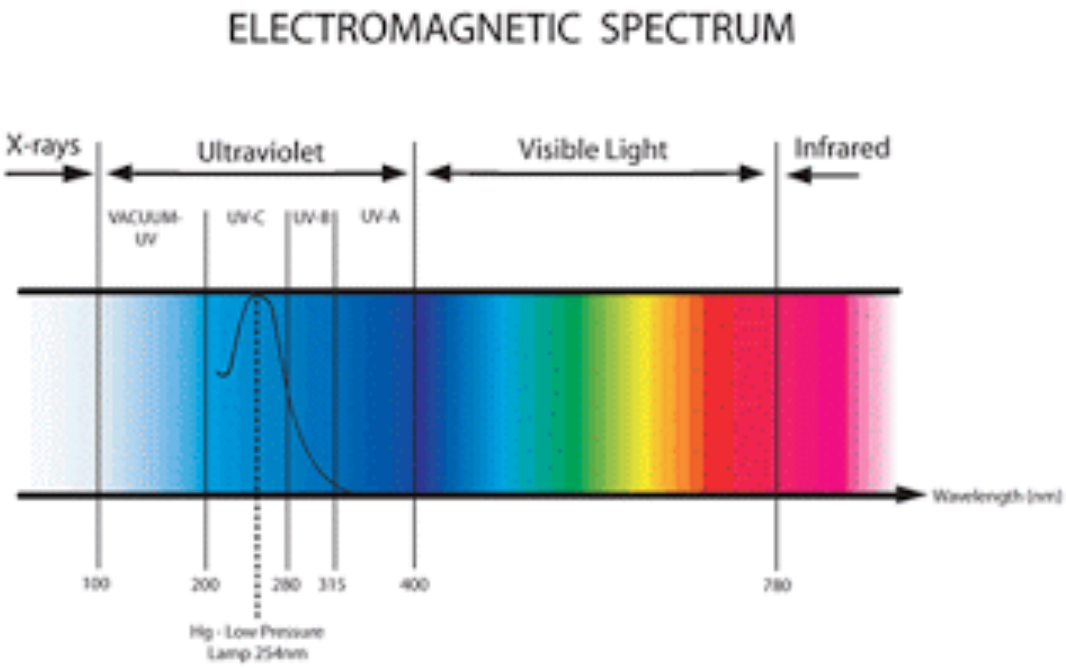

Figure 7. Electromagnetic Spectrum [50]

The Beer-Lambert theory states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length [51].

$$log_{10}\frac{I_0}{I} = \varepsilon * \ell * c \qquad \text{Equation 1}$$

where I_0 is the intensity of the light passing through the reference cell; "I" is the intensity of the light when it passes through the sample cell; epsilon, "ε" is the term designated for molar absorptivity. In general, absorbance can vary due to the concentration of the sample as well as the size of the container that it is being measured in, this term allows for a standard measurement of absorbance that can be compared between different substances. Length "ℓ" is the length of the solution that the light passes through in centimeters. Finally, concentration "c" is the concentration of the solution in $mol\ dm^{-3}$ [52].

When rewritten to solve for absorbance (A),

$$A = \lambda * b * c \qquad \text{Equation 2}$$

where lambda "λ" is the wavelength-dependent absorptivity coefficient; "b" is the path length from the sample cell to the detector and "c" is the concentration of the analyte. This formula allows for the linear relationship between absorption and concentration of the sample [53].

Samples taken via the sampling port of the Franz diffusion cell can be placed in a cuvette and analyzed with a UV-Vis spectrophotometer if the active ingredient absorbs light in the UV-Vis spectrum. The corresponding absorbance may be calculated using a calibration curve, which relates a known concentration of the specific active being investigated and its absorbance. This method allows for the determination of the amount

of drug that passed through the membrane in the Franz cell, which refers to the amount of drug that may theoretically absorb from a topical formulation.

The spectrophotometer was set to take measurements at 220 nm. It was allowed to warm up and equilibrate for 60 minutes before any readings were taken. Between each reading, the cuvette was rinsed with PBS buffer. In line with standard practices, if readings taken at 220 nm were above 1.0, the sample was diluted with PBS to give an absorbance less than 100%. Most readings should lie between 0.1 and 0.9 on the absorption y-axis when plotted against concentration on the x-axis.

Chapter 3

Results and Discussions

3.1 Results and Discussions

A calibration curve using phosphate buffered saline (PBS) as the medium was created using a UV-Vis spectroscopy. A series of dilutions prepared with PBS were analyzed at 220 nm. The calibration curves for Ibu and NaIbu are shown in Figures 8 and 9, respectively. The resulting equations (Eq.1 & Eq.2) were used in calculating the subsequent drug concentration values for formulations 1-9.

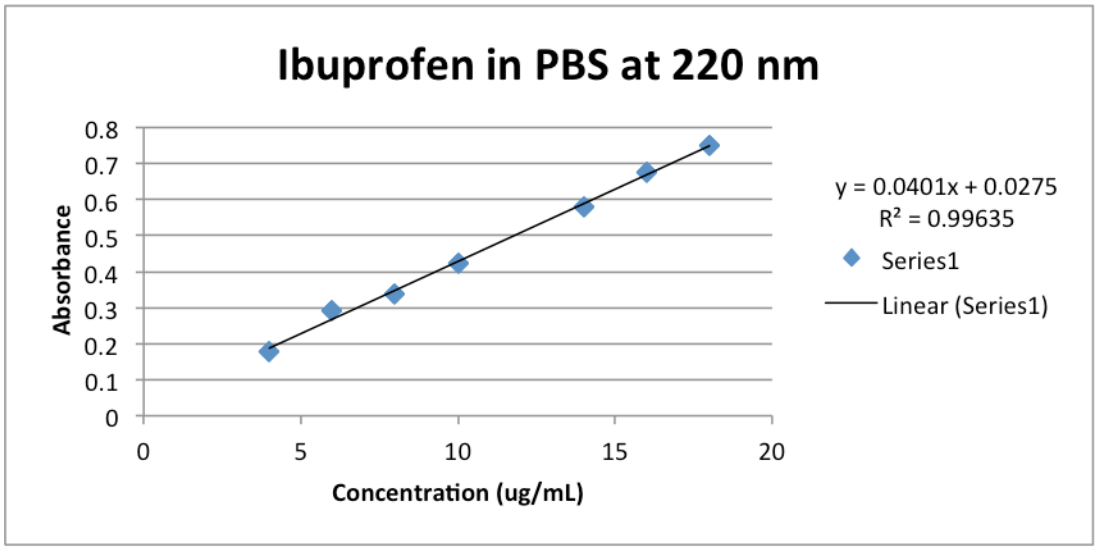

Figure 8. Graph depicting concentration versus absorbance of Ibuprofen in PBS

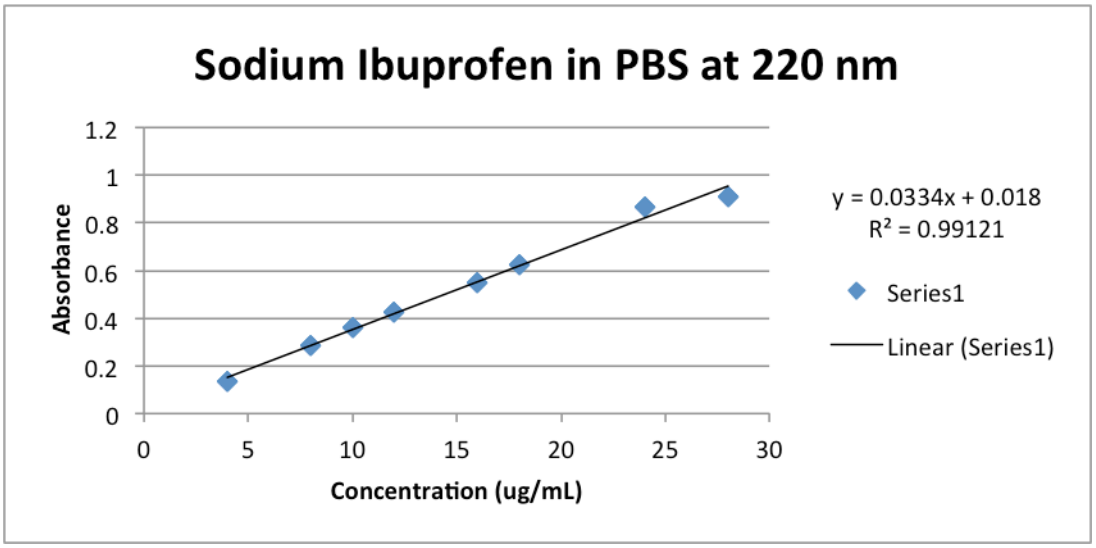

Figure 9. Graph depicting concentration versus absorbance of NaIbu in PBS

Results for this project are given in graphs depicting the cumulative amount of API released over time versus the blank cream using the same penetration enhancer.

When Kollicream 3C was used as the penetration enhancer with Ibu (Formulation 1), the cumulative amount of drug released after 8 hours was 3.6% of the total API found within the formulation, with a standard deviation of 0.39 (Figure 10). After 8 hours the blank cream released 5.77% of the total API within the formulation, with a standard deviation of ±0.30. These results suggest that the blank cream (formulated without any penetration enhancer) was releasing more of the API than the cream with the penetration enhancer. These results were not anticipated; theoretically the effect of the penetration enhancer would increase the potential of the skin to allow the API to penetrate, therefore the formulation with the penetration enhancer incorporated into the cream should yield a higher cumulative drug released. One possible explanation for this phenomenon could be that the solubility conditions within the cream were much more favorable than those of

the skin. Theoretically, penetration enhancers have a similar solubility as the API, but with an affinity for the skin. If the skin affinity is not strong enough, the API may prefer to retain in the cream instead of being delivered into the skin [53].

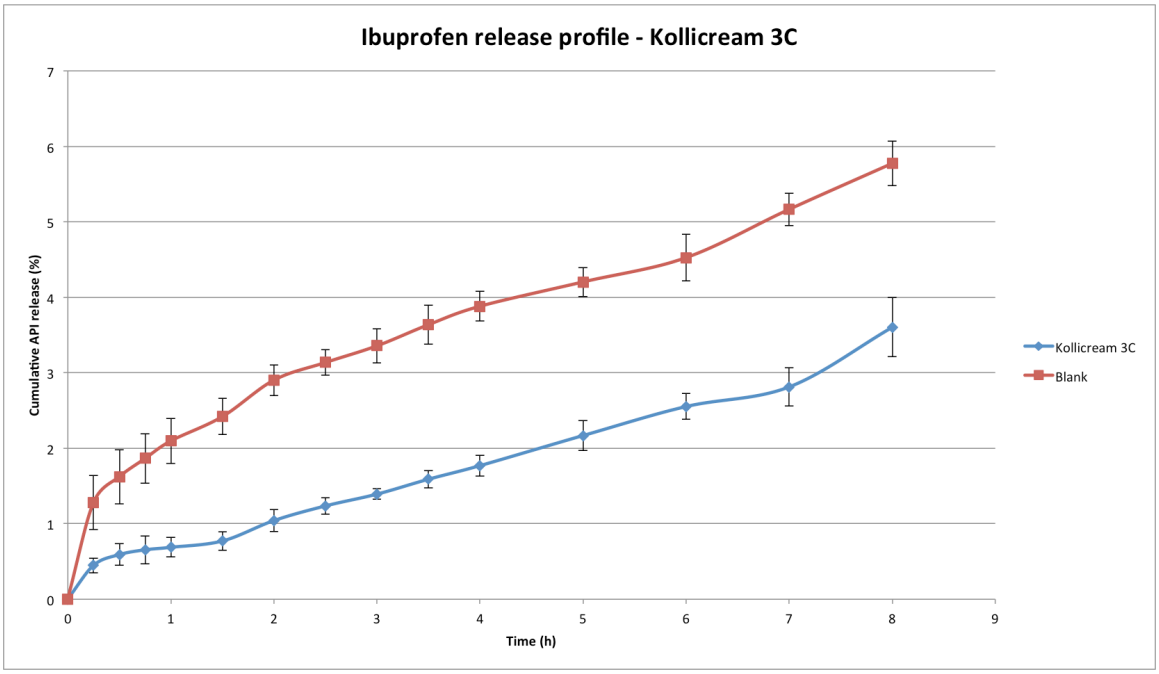

Figure 10. Graph depicting Formulation 1- release of Ibu versus a blank cream

In Formulation 2 Kollicream 3C was used with NaIbu, and the cumulative amount of drug released after 8 hours was 4.64% (with an SD of 0.29) (Figure 11). Once again it is shown that the blank cream is delivering more of the API into the epidermis of the skin as compared to the formulation with the penetration enhancer. However, more of the API (in this case, NaIbu) was delivered as compared to the previous study using the same penetration enhancer coupled with Ibu. These results can be expected because of the pharmacokinetic properties of the salt form of Ibu having a greater solubility. While according to BASF MSDS, Ibu is soluble in water up to 0.01139 g/l [54] but NaIbu is

soluble in water up to 100 g/l [55]. This difference may be the reason behind the increased release of API in formulation 2 compared to formulation 1.

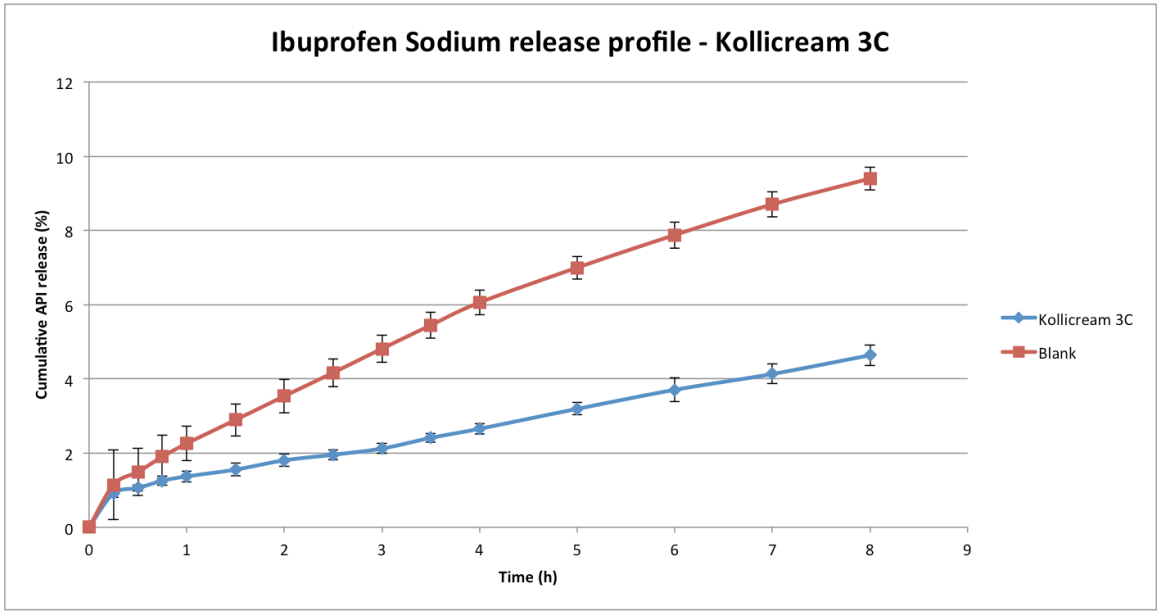

Figure 11. Graph depicting Formulation 2 - release of NaIbu versus a blank cream

Lastly, the Kollicream 3C sample without any API but still with the penetration enhancer (Formulation 3) was also tested over 8 hours. The cumulative release after 8 hours was 79.54% with a standard deviation of 3.76 (Figure 12).

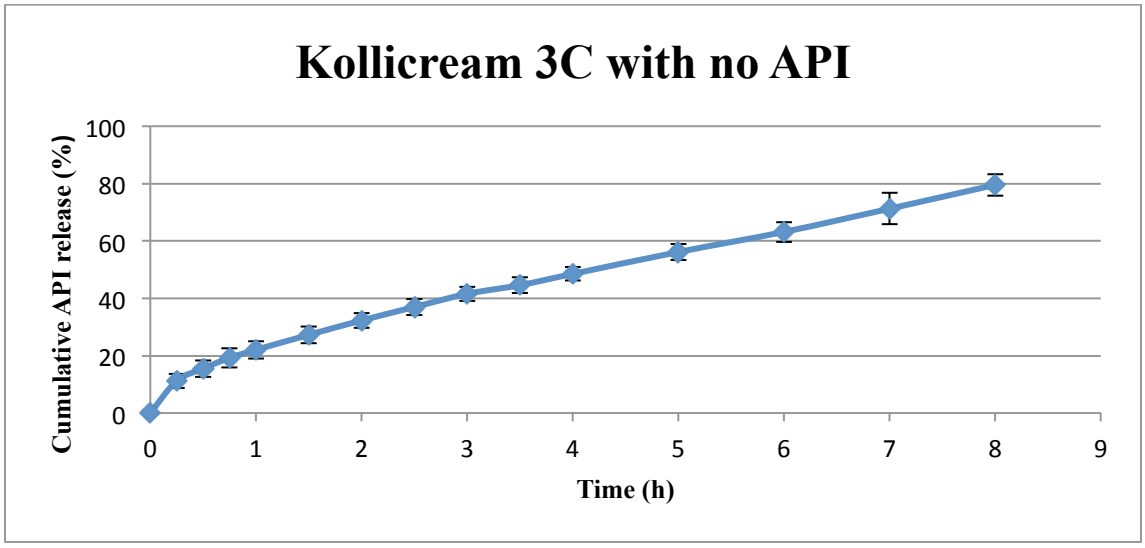

Figure 12. Graph depicting the cumulative amount of API released in Formulation 3

These results were unexpected in light of Formulation 3 not having any API added into the formulation. Without any API, no absorbance was originally expected at 220 nm. Absorbance values were still found using the spectrophotometer after 8 hours, reaching up to almost 80%. Therefore, it suggests that another ingredient within the base formulation is absorbing UV light at 220 nm. This information greatly skewed the potential results of this experiment due to the inability to exactly distinguish how much of the "cumulative API released" was actually the API or the unknown light absorbing ingredient. Once these results were seen, Formulation 10 (no API and no preservative) was added to the experiment. Results of Formulation 10 can be seen below in Figure. 16.

The next penetration enhancer tested was Kollicream IPM with Ibu (Formulation 4). This formulation resulted in a curve similar to Kollicream 3C with Ibu, with the amount of API released after 8 hours being 3.80% with a standard deviation of 0.06. Table 2. shows the amount of drug found at the corresponding withdrawn sample.

Table 3. Formulation 4- cumulative release results versus blank cream (n=4)

Kollicream IPM with Ibu (Formulation 4)			Blank Kollicream IPM	
Time (h)	Cumulative Released API (%)	Standard Deviation	Cumulative Released API (%)	Standard Deviation
0.00	0	0	0	0
0.25	0.77	0.28	1.28	0.36
0.50	0.86	0.18	1.62	0.36
0.75	1.07	0.18	1.87	0.33
1.00	1.27	0.16	2.10	0.30
1.50	1.47	0.15	2.42	0.24
2.00	1.70	0.12	2.90	0.20
2.50	1.84	0.12	3.14	0.17
3.00	2.10	0.08	3.36	0.23
3.50	2.32	0.14	3.64	0.26
4.00	2.54	0.10	3.88	0.19
5.00	2.85	0.08	4.20	0.19
6.00	3.23	0.32	4.53	0.31
7.00	3.55	0.13	5.17	0.22
8.00	3.80	0.06	5.77	0.30

Similarly, Kollicream IPM with NaIbu (Formulation 5) followed closely to the results given by Kollicream 3C with NaIbu. After 8 hours the cumulative amount of drug

released was 4.26% with a standard deviation of 0.31; while the corresponding blank cream released 9.40% after 8 hours. Finally, the formulation using Kollicream IPM with no API (Formulation 6) yielded a cumulative release after 8 hours of 59.35% with a standard deviation of 1.74. These results were once again unexpected, but are closely aligned with the results given using Kollicream 3C as a penetration enhancer. Therefore, possible explanations for these results are the same; whereas the solubility environment within the cream is so favorable that the affinity for the API to enter the skin is overcome in both Formulations 4 and 5. NaIbu has a higher solubility potential in Formulation 5 as compared to Ibu in Formulation 4. And while no API is added in Formulation 6, the unknown ingredient within the formulation that is absorbing light at 220 nm is present and giving readings up to almost 60% after 8 hours.

Lastly, the three final Kollicream OA formulations were tested. Kollicream OA with Ibu (Formulation 7) resulted in a cumulative release of API of 5.60% with a standard deviation of 0.28 (Figure 13).

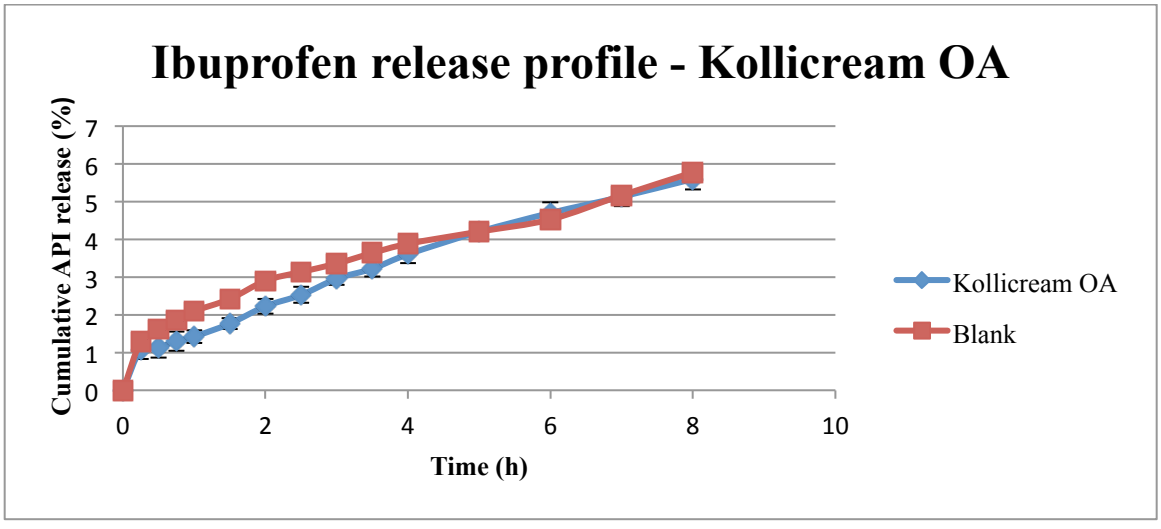

Figure 13. Graph depicting Formulation 7- release of Ibu versus blank cream

In this experiment, OA is shown to have the highest readings of cumulative API released over 8 hours when Ibu is used as the API. In hours 6,7 and 8 Formulation 7 and its comparative blank cream standard are closely correlated which previously had not happened. These results are the most positive, suggesting that Kollicream OA has a higher propensity for skin penetration as compared to Kollicream IPM and Kollicream 3C.

Next, Kollicream OA with NaIbu (Formulation 8) yielded a cumulative release of API after 8 hours of 5.95% (SD=0.32) (Figure 14). The blank cream yielded a cumulative release of API after 8 hours of 9.40% with a standard deviation of 0.31.

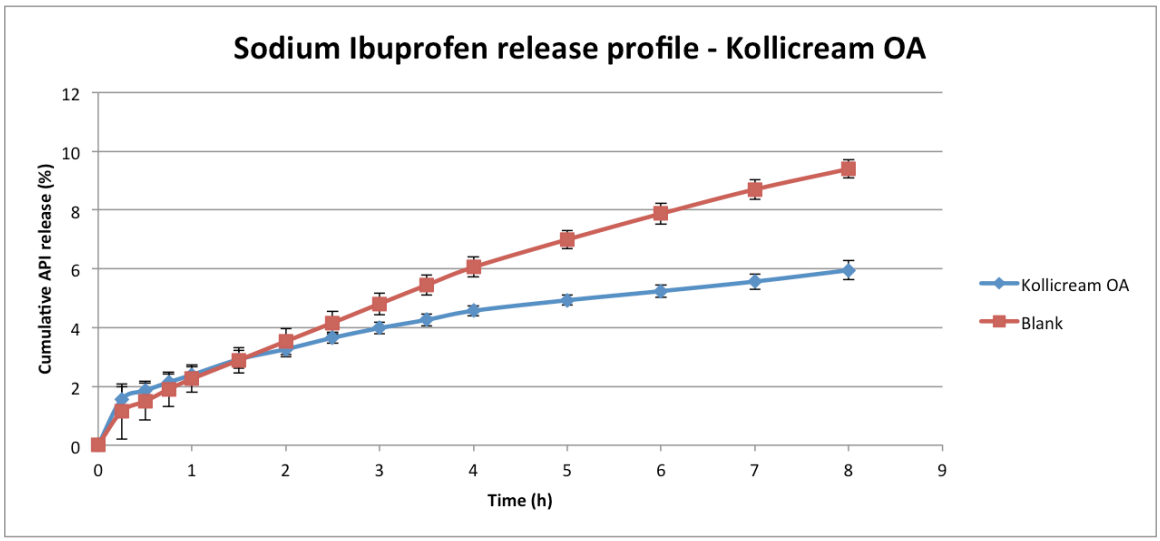

Figure 14. Graph depicting Formulation 8- release of NaIbu versus blank cream

Once again, the release of NaIbu was higher than the results given from Formulation 7 with Ibu as the API. However, the blank cream surpassed the results of Kollicream OA unlike the results given in Formulation 7.

Next, Kollicream OA with no API (Formulation 9) was tested and yielded a cumulative release of 105.78% with a standard deviation of 5.49 (Figure 15).

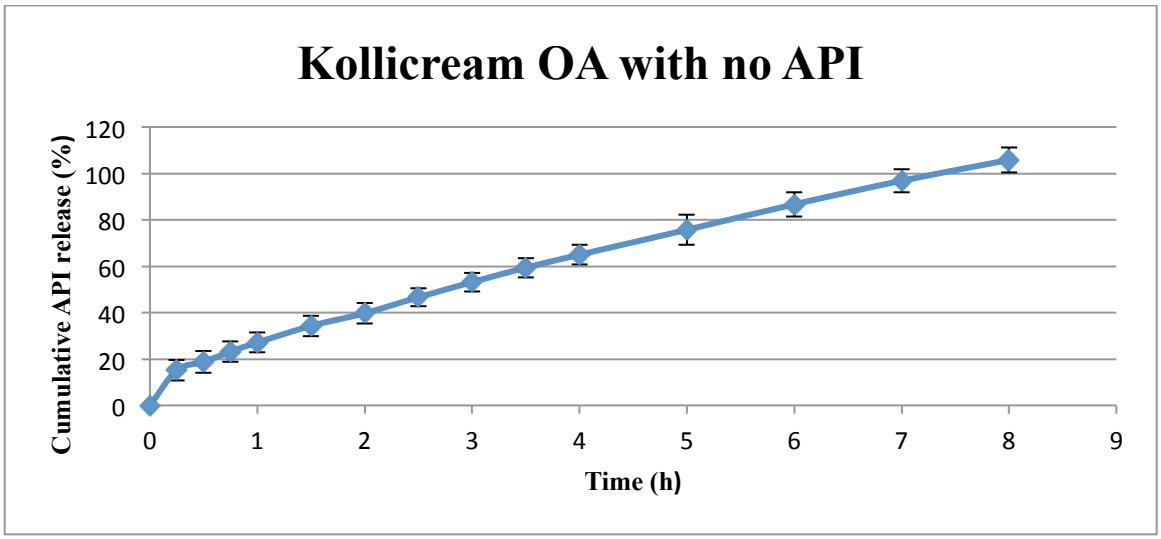

Figure 15. Graph depicting Formulation 9- cumulative release of API over time

These results are unexpected. Not only should the cumulative release of API not exceed 100%, the entire amount of API within the formulation has been administered to the skin, this high of a release greatly surpasses any of the blank creams previously tested. These results could have arisen if the membrane used in testing was damaged during the experiment. If the membrane was compromised, the formulation penetrating that membrane may have completely infiltrated the PBS buffer being tested within the Franz cell, giving an inaccurate reading.

Because the results given from the formulations with a penetration enhancer but without any API added had given readings suggesting an ingredient was being absorbed within the 220 nm wavelength, an additional formulation (Formulation 10) without any

API or penetration enhancer added was tested. This sample was formulated using the percentages listed below in Table 3.

Table 4. Ingredient and composition of formulation 10

No API No Penetration Enhancer (Formulation 10)	
Ingredients	Amount of ingredients (%)
Ibuprofen	-
Sodium Ibuprofen	-
Kollicream 3C	-
Kollicream IPM	-
Kollicream OA	-
Glycerol Formal	5.0
Kolliphor CS20	4.0
Kolliwax CSA50	10.0
Euxyl PE9010	0.5
Water	80.5

Formulation 10 was tested using UV-Vis and analyzed at 220 nm. The cumulative release percentage after 8 hours was 93.88 with a standard deviation of 5.50 (Figure 10).

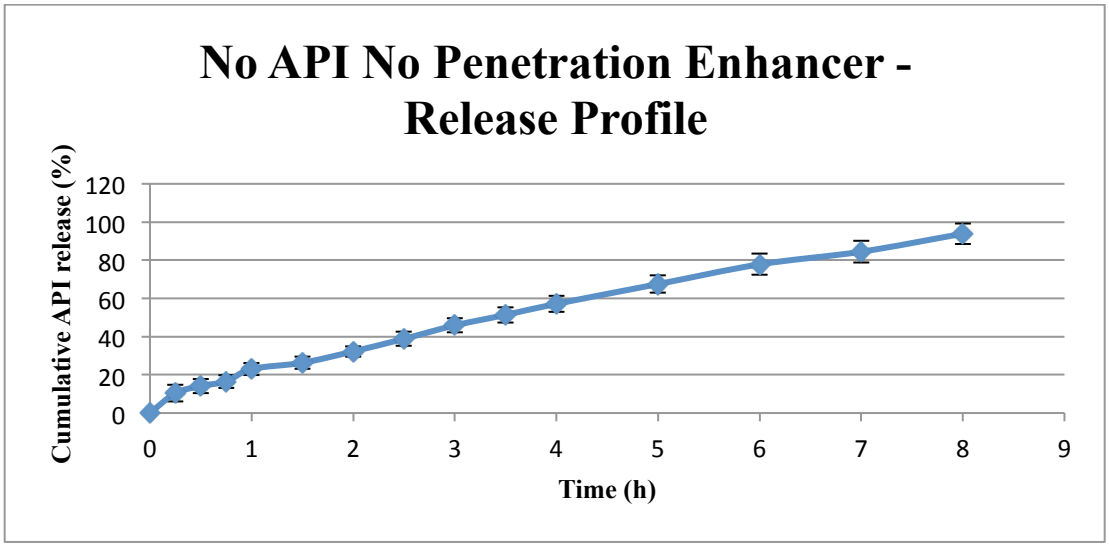

Figure 16. Formulation 10- cumulative release of API over

These results suggest that the penetration enhancer is not the ingredient responsible for the unknown light absorption shown in the results of Formulations 3,6 and 9. After viewing the ingredients using in each cream, taking out both the penetration enhancer and API, the results may suggest that the preservative used in this experiment (Euxyl® PE 9010) may be absorbing light within the 220 nm wavelength.

Chapter 4

Conclusion & Future Work

4.1 Conclusion

In this study we compared three separate penetration enhancers; Kollicream 3C, Kollicream IPM and Kollicream OA. One set of creams was formulation using Ibu as an API, the other set formulation NaIbu as an API. The last set of creams was formulation using no API whatsoever. The intention of this experiment was to see which penetration enhancer successfully increased the amount of API released into the membrane. Over the entire 8-hour study, Kollicream OA released the highest amount of API into the membrane when used with NaIbu (5.95% with a standard deviation of 0.32). When considering the highest amount of API released into the membrane over the first initial hour of application, Kollicream 3C yielded the highest results (1.37% with a standard deviation of 0.15).

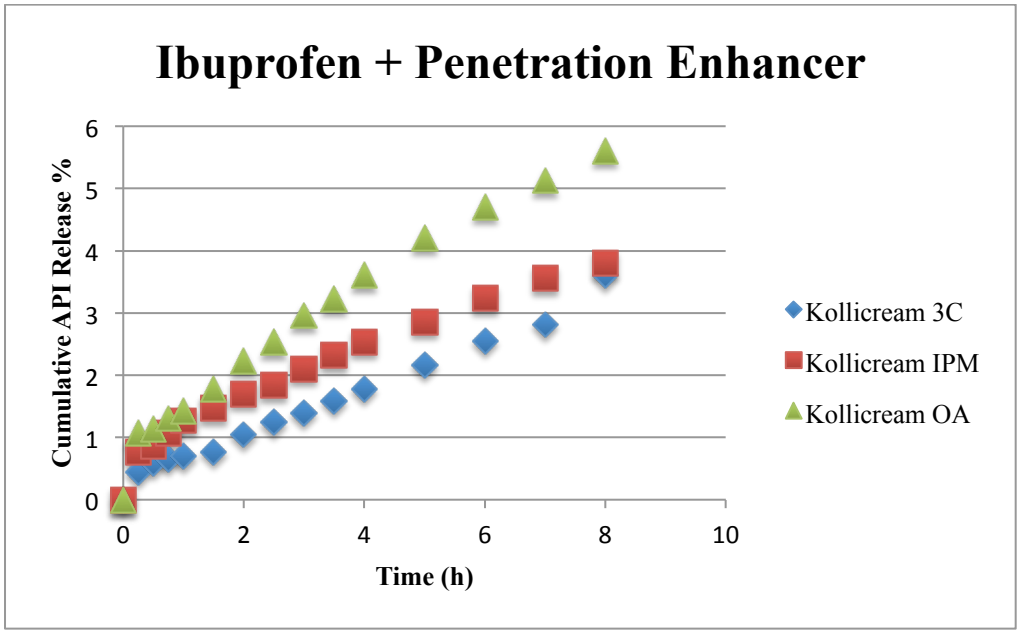

Figure 17. Comparison of Ibu with each penetration enhancer

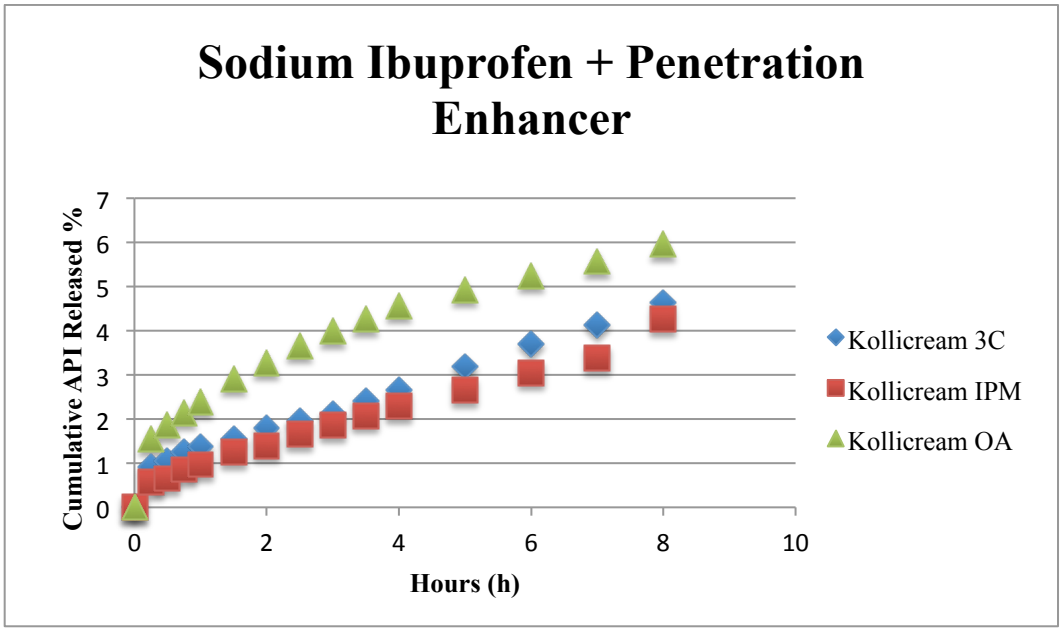

Figure 18. Comparison of NaIbu with each penetration enhancer

4.2 Future Work

To begin discerning which ingredient may be absorbing light at the same wavelength, a sample of each ingredient in PBS buffer or other suitable solvent that does not have absorption at 220 nm should be made and analyzed using a UV-Vis spectrophotometer at 220 nm to see if any absorbance is registered. If one or more ingredients absorbs at 220 nm, a different wavelength should be selected to analyze the overall formulation being tested in the experiment. This should give a clearer value of the actual concentration of the drug being found within the receptor phase.

Another consideration when analyzing the amount of drug delivered should be to take into account how much of the drug is still left on top of membrane, and within the membrane used. A system of rinsing and testing the rinses from the top of the membrane to see how much of the drug was left on top of the epidermis will allow for an understanding of how much drug was left on the skin without permeating into it. By separating the layers of the membrane (i.e., pig ear skin) further, testing to see how much of the drug was found within the different layers of the skin, but not yet into the receptor phase will show the rest of the drug concentration that is unaccounted for.

When evaluating the graphs of API versus a blank cream, there should be lag time in the case of drug-loaded formulations. In this study lag time was not found, this suggests that there may be some imperfections in the membranes used. A local slaughterhouse gifted the pig ears and natural cuts and scrapes on the skin may have played a role in the quick permeation of drug.

Because 3 mL samples were withdrawn at each time mark, and replaced with fresh buffer, some variations could have arisen due to a lack of equilibration period for

the fresh room temperature buffer to reach the correct temperature. Solubility of the drug may have been affected because of the temperature fluctuations.

Another suggestion for future work would be to test variations of the method of formulating the creams. Ibuprofen and sodium ibuprofen could be added to the oil phase as well, which may change the outcomes for the delivery of the drug. Adding the API to glycerol formal first, or perhaps adding it to the oil phase after it has been melted, may yield higher solubility of the API within the cream.

Finally, the study should be carried out over the entire time that the suggested product would work, 12 hours or 24 hours ideally.

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