Pharmacokinetic Modeling for In Vitro/In Vivo Transdermal Absorption

by

Yann-Huei Phillip Lee

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
December 16, 2017

Keywords: Pharmacokinetic Modeling, Transdermal,
Population Pharmacokinetics, In vitro, In vivo

Approved by

Dr. Jayachandra Babu Ramapuram, Chair, Professor of Pharmaceutics
Dr. William Ravis, Co-Chair, Professor of Pharmaceutics
Dr. Daniel Parsons, Professor of Pharmaceutics
Dr. Robert Arnold, Associate Professor of Pharmaceutics
Dr. Robert Judd, Professor of Pharmacology

Abstract

The objective was to determine if a linear pharmacokinetic model could be developed to describe in vitro and in vivo transdermal absorption across normal and microporated skin. Such a model would enable formulators to predict the effects of rate of drug delivery on pharmacokinetics and assist with dosage form design. An important concern for transdermal formulations is skin reactions, which can occur due to components of the transdermal product or from pre-existing skin conditions. The reactions can be reduced by the modification of the formulation or delivery method. Beta-blockers, such as propranolol, acebutolol, atenolol, and sotalol, are explored by transdermal passive diffusion, iontophoresis and microporation. Transdermal delivery offers the advantage of avoiding hepatic first pass metabolism. Though pharmacokinetic modeling of transdermal delivery is evident, such models have not been applied to microneedle and iontophoresis enhancement techniques.

The pharmacokinetics of two aminoglycoside antibiotics in a topical cream were determined from a series of plasma drawn on day 1 and 20, shortly before application of the cream and in the minutes and hours immediately afterwards, as well as single samples periodically in between. The data set contained 573 observations, with greater than 1100 dosing events. Pharmacokinetic results were determined from traditional noncompartment analysis. Although the pharmacokinetics and safety of these antibiotics separately were well known, it had not been determined to what extent they would be absorbed into systemic circulation when applied to the open skin wounds of cutaneous leishmaniasis lesions. From the results, one

aminoglycoside showed a wide range of variation, and many of the results were below the lower limit of quantification. No pharmacokinetic analysis was performed with the second aminoglycoside as most plasma concentrations were below the limits of detection. The sources and correlations of variability in drug concentrations among the target patient population can be used to determine population pharmacokinetics. Certain patient features, such as body weight, excretory and metabolic functions, and the presence of other therapies, can regularly alter dose-concentration relationships. The collection of relevant pharmacokinetic information in patients who are representative of the target population can help develop a quantitative estimation of the magnitude of the unexplained variability in the patient population.

In addition, the skin permeation of various cobalamins across dermatomed and microporated human skin was studied to identify cobalamins that provide better skin absorption. Preliminary data for linear pharmacokinetic modeling was gathered to describe the in vitro absorption of four cobalamin derivatives: cyanocobalamin, hydroxocobalamin, methylcobalamin, and adenosylcobalamin. Correlation between in vitro absorption through microporated skin and the properties of the cobalamins was examined. No measureable levels of any of the B12 vitamins were detected in the passive diffusion experiments.

Acknowledgments

I would like to thank Dr. Jayachandra Babu Ramapuram and Dr. William Ravis for their constant guidance, support, and for the opportunity to continue furthering my education. Over the years, they have been my mentors and have continuously pushed me to continue my research and to better myself both in and outside the laboratories. Their sharing of their own experiences have offered me greater insight to myself and towards my future.

I would also like to express my gratitude to my committee members, Dr. Daniel Parsons, Dr. Robert Arnold, and Dr. Robert Judd, for their efforts and input for my research and dissertation. I would like to extend my special thanks to Dr. Satyanarayana Pondugula for serving as the outside reader of my dissertation. I also would like to thank all the faculty members, staff and fellow students in the Department of Drug Discovery and Development for their support, companionship, and help.

I would definitely like to thank my family members, for helping me become who I am. They have always been part of my inspiration to what I do and I could not have achieved any of my current accomplishments. Their successes have driven my own, and I hope to live up to all their expectations.

Table of Contents

List of Tables

List of Figures

1. Introduction to Pharmacokinetic Modeling for In Vitro/In Vivo Transdermal Absorption

1.1 Abstract

Linear models can be developed to describe pharmacokinetic processes in *in vitro* and *in vivo* transdermal absorption. Such a model would enable formulators to predict the effects of rate of drug delivery on drug pharmacokinetics and assist with dosage form design. An important concern for transdermal formulations is skin reactions, which can occur due to components of the transdermal patch or from pre-existing skin conditions. The reactions can be reduced by the modification of the formulation or delivery method.

While the skin has been used throughout history as a site for topical administration, transdermal drug delivery is a more recent development as a route of administration where active ingredients are delivered across the skin for systemic distribution. The skin functions mainly to protect the body from external injuries and to contain bodily fluids. However, some drugs are capable of penetrating the skin in sufficient amounts to produce a systemic action. The transdermal route is notable in that it can accommodate drugs with a short elimination half-life, or undergo extensive first pass metabolism, which would otherwise require frequent dosing.

The basic premise of pharmacokinetics is that there is a relationship between the pharmacologic or toxic response to a drug and the concentration of the drug in the blood plasma. However, for some drugs there is no simple relationship between such effects and the concentration in plasma. Nevertheless, pharmacokinetics plays a role by providing a quantitative relationship between dose and effect and the framework with which to interpret measurements of the concentrations of drugs. In most cases, the concentration of drug in the systemic circulation is related to the concentration of drug at its sites of action. Empirical data can therefore be used to model pharmacokinetic parameters and be used in dose prediction.

1.2 Background

1.2.1 The Skin

The skin functions mainly to protect the body from external injuries and to contain bodily fluids. There are two important layers to the human skin, the epidermis and the dermis. The epidermis contains no blood vessels, but the deepest cells are supplied with diffused oxygen from the surrounding air and by capillaries extending to the outer layers of the dermis. [1] The epidermis is divided into several layers where cells are formed through mitosis at the innermost layers. From the deepest to superficial, the epidermis consists of stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (Fig. 1.1). [2] The cells migrate outwards, changing shape and composition to fill with keratin and form the top layer of the epidermis, the stratum corneum. It is a physical barrier formed from the attachment of the keratinocytes, and a chemical barrier due to the highly organized lipids, acids, hydrolytic enzymes and antimicrobial peptides. [3]

The stratum corneum is the outermost layer of the epidermis, consisting of dead cells, and is the primary layer of concern for transdermal studies. The stratum corneum is composed of 15 to 20 layers of flattened cells with no nuclei or cell organelles. Their cytoplasm shows filamentous keratin and are embedded in a lipid matrix composed of ceramides, cholesterol, and fatty acids. The stratum corneum functions as a barrier to protect the underlying tissue from several things, including infection, dehydration, chemicals and mechanical stress. [4]

Past the epidermis is the dermis, consisting of dense irregular connective tissue. It is divided into two layers, the papillary region adjacent to the epidermis and a thicker area further below called reticular dermis. [5] The dermis is approximately one millimeter thick, 100 times the thickness of the stratum corneum. The dermis contains capillaries that distribute drugs into the systemic circulation. [6] The dermis contains two vascular networks that run parallel to the skin surface, which are connected by vertically communicating blood vessels (Fig 1.2). The function of the blood vessels within the dermis is to supply nutrition, regulate temperature, modulate inflammation, and help heal wounds. These blood vessels also serve as the route of administration for transdermal drugs for systemic distribution. [7]

Figure 1.1. Layers of the epidermis, with the stratum corneum being the outermost layer. [8]

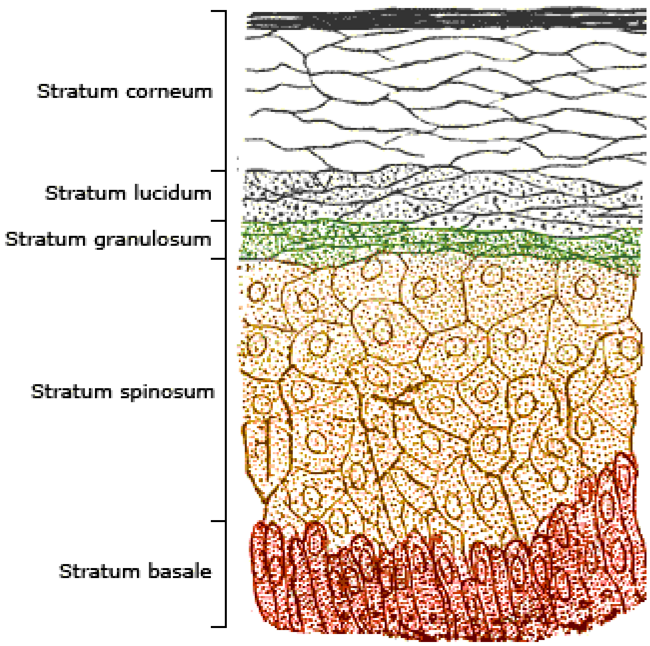
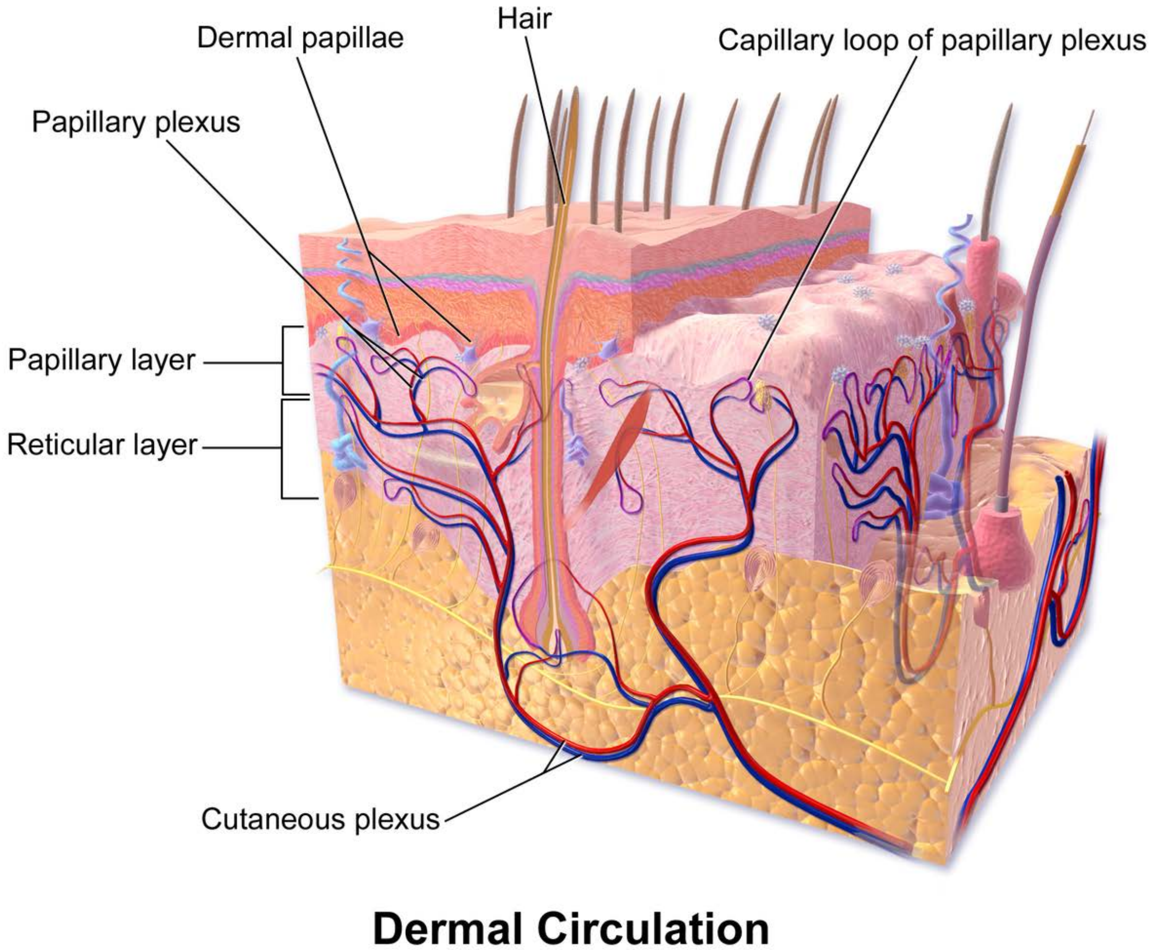


Figure 1.2. Dermal circulation. [9]



1.2.2 Transdermal delivery

Transdermal delivery is the transport of chemicals from the outer surface of the skin both into the skin and into circulation. Along with inhalation, ingestion and injection, absorption through the skin is a route of exposure for toxic substances as well as a route of administration for medication. [10] Various factors can affect transdermal drug delivery, the main ones being drug concentration, contact time, drug solubility and physical barrier integrity of the skin and body site of where applied. Skin absorption can occur from exposure in occupational, environmental, or consumer interaction with chemicals, cosmetics, or pharmaceutical products. Some potent chemicals can cause detrimental systemic effects even when only a small quantity is absorbed. In order to assess if a chemical can be a risk of either causing dermatitis or other more systemic effects and how that risk may be reduced one must know the extent to which it is absorbed, thus dermal exposure is a key aspect of human health risk assessment. [10, 11]

A chemical must pass through the epidermis, glands, or hair follicles to be absorbed through the skin into systemic circulation. Sweat glands and hair follicles make up about 0.1 to 1.0 percent of the total skin surface. [10] While small amounts of chemicals may enter the body through the glands or hair follicles, they are primarily absorbed through the cell layers of the epidermis. Chemicals must pass through the five cell layers of epidermis before entering the dermis where they can enter the bloodstream or lymph system and circulate to other areas of the body. The stratum corneum is the outer layer of the epidermis and the rate-limiting barrier in absorption, and varies in thickness from approximately ten to several hundred micrometers, depending on the region of the body (Figure 1.3). [12] The layers of dead, flattened keratinocytes are surrounded by a lipid matrix, which act together as a brick-and-mortar system that is difficult to penetrate. The stratum corneum is primarily composed of cholesterol, cholesterol esters and ceramides. [13] Lipid-soluble chemicals make it through the layer and into the circulation faster, but nearly all molecules penetrate it to some minimal degree. Below the stratum corneum lies the viable epidermis which is about ten times as thick as the stratum corneum. Diffusion is much faster in the viable epidermis due to the hydration in the living cells. (Figure 1.4)

The transport of drugs across skin into systemic circulation occurs by 1) transcellular pathway and 2) intercellular pathway. In addition there is another minor pathway called trans-appendageal pathway (transport across hair follicles and sweat ducts (Figure 1.5). [12] The more direct route is the transcellular pathway, with the drugs crossing the skin by directly passing through both the phospholipid membranes and the cytoplasm of the dead keratinocytes that constitute the stratum corneum. While the transcellular route is the shortest path, drugs encounter significant resistance to permeation, due to the drugs having to cross the lipophilic membrane of each cell, the hydrophilic cellular contents, and then the phospholipid bilayer of the cell membrane again. This is then repeated multiple times while permeating through the full thickness of the stratum corneum. In the intercellular route, drugs molecules have to pass through the small spaces between the stratum corneum cells. Although the thickness of the stratum corneum is approximately 20 µm, the actual diffusion path of most molecules crossing the skin is several times that, on the order of 400 µm. [14]

Transdermal drug delivery offers many benefits over traditional solid and liquid oral dosage forms. The first benefit of using transdermal patches is the delivery of the drug directly to the bloodstream while bypassing the liver's metabolic activity via oral administration. [15] Another benefit of using patches is that medication is administered gradually and constantly, instead of in a large, single dose. Patches use the skin's natural properties in order to achieve a constant permeation of the drug and result in steadier blood levels compared to other delivery routes. Also, their use allows the medication to bypass the acidic environment found in the digestive system, as well as avoiding first pass metabolism. Because the medication bypasses the gastrointestinal tract, patches may also reduce the risk of side effects associated with oral delivery of solid and liquid dosage forms. There are also user related advantages over hypodermic injections, which are painful to the patient, generate dangerous medical waste and pose the risk of disease transmission due to the reuse of needle, which is more common in developing countries. [15] In contrast, transdermal delivery formulations are non-invasive and can be self-administered, and can provide release for long periods of time. They also improve patient compliance and the systems are generally inexpensive.

Currently, successful transdermal drug products share a few similar properties. They have molecular weights that are up to several hundred Daltons, exhibit lipophilic octanol-water partition coefficients and the product only provides doses in amounts of several milligrams per day or less. The first generation of transdermal delivery systems is responsible for most of the transdermal patches in clinical use. Recent advances in transdermal patch technology have enabled an influx of first-generation transdermal patches reaching the market, though this surge will slow as drugs with suitable properties are limited. Usually, for these drugs, transdermal delivery should be preferable to oral delivery due to low oral bioavailability, the need for less frequent dosing, or steady delivery profiles. A variation on the first-generation delivery systems for transdermal patches involves no patch backing. Instead, the drug is applied from a metered topical formulation to the skin that drive small lipophilic drugs into the stratum corneum, while also acting as the drug reservoir for extended release into the viable epidermis. [16]

The components of a transdermal drug release system are the liners, adherents, drug reservoirs, and drug release membrane that play a role in the release of the drug through the skin.

(Figure 1.6) The polymer used in the transdermal patch should be stable, non-reactive with the drug, easily manufactured and fabricated into desired product, and inexpensive. Properties of polymers such as molecular weight, glass transition temperature, and chemical functionality should be such that the specific drug diffuses and releases into systemic circulation properly. Mechanical characteristics of the polymer should not deteriorate excessively when large amount of active agents are incorporated into the patch. The transdermal patch should provide consistent and effective delivery of a drug throughout the product's life. The reservoir type transdermal system has a rate controlling membrane which provides a constant, predetermined drug release from the patch. In this system, the drug is physically blended with polymeric powder, or blended in a gel matrix and is then molded into a medicated disc with a defined surface area and controlled thickness. [16]

The adhesive plays an intimate contact between the delivery system and the skin. It is related to drug delivery and therapeutic effect. It carries the drug which can either be dispersed or dissolved in the matrix or the compartment containing drug (solution or suspension) is separated from the adhesive layer by a diffusion controlling membrane, the drug permeates through this adhesive membrane to reach the skin. Quality of bond between patch and skin holds importance as it directly reflects consistency of drug delivered. Application of transdermal devices to the skin can be achieved by using a pressure sensitive adhesive. The patch is covered during storage by a protective liner that is discarded before application. Since the liner is in intimate contact with the patch, the liner should be chemically inert. Examples of transdermal patch backings are polyester film, polyethylene film and polyolefin film, and aluminum vapor coated layer. Other important concerns are the leaching of additives and the diffusion of drug formulation through the backing.

However, an overemphasis on the chemical resistance may lead to stiffness and high resistance to moisture vapor and air, which can cause the patch to lift and may possibly irritate the skin. [17]

The second generation of transdermal delivery systems recognizes that skin permeation enhancement is needed so that adequate dose of a given drug can be delivered across the skin into the systemic circulation. The ideal permeation enhancer should reversibly disrupt stratum corneum structure, and provide a driving force for transport of drugs into the skin. Many methods including conventional chemical enhancers, iontophoresis and non-cavitational ultrasound, were able to successfully introduce small molecule delivery for localized, dermatological, cosmetic and some systemic applications, but has made little impact on delivery of macromolecules. [16] Chemical penetration enhancers in general disrupt the ordered bilayer structures of the intracellular lipids in the stratum corneum by either inserting amphiphilic molecules to disorganize the molecular packing of lipids or by extracting the lipids to create temporary defects in the layers so that the drug molecules can penetrate the skin. One challenge of this approach is that increased permeation enhancement, even of small molecules, typically correlates with increased skin irritation. Some of these enhancers that increase skin permeability without irritation have been used to successfully deliver small molecules, but do not solve the problem of delivering hydrophilic compounds or macromolecules. Overall, chemical enhancers can increase skin permeability and provide an added driving force for transport by increasing drug partitioning into the skin. Chemical enhancers such as liposomes, dendrimers and microemulsions have also been used in dosage forms to increase skin permeability, drug solubilization in the formulation, and drug partitioning into the skin. The large molecular size of these chemical enhancers are unable to permeate into the skin and helps localize the effects to the stratum corneum. These approaches have found success for enhanced topical delivery of some small molecules. [18]

Penetration enhancers are compounds which improve skin permeability by altering the skin as a barrier to the flux of the interested penetrant. [17] Desirable properties for penetration enhancers acting within the skin should be non-irritating, non-sensitizing, and non-phototoxic. They should work quickly with the effect duration and mechanism being both predictable and reproducible, without pharmacological activity within the body. The enhancer should allow therapeutic agents into the body while preventing the loss of material from the body. It should show barrier properties which must return both rapidly and fully when removed from the skin, show compatibility with formulation and system components, be odorless, tasteless, colorless, and cosmetically acceptable, and have a desired solubility parameter that approximates that of the skin. The biochemical order of the intercellular lipid matrices of the stratum corneum or the keratinized environment of the corneocytes is altered to allow the penetration of compounds at a suitable rate to the desired site of activity. The three main mechanisms of enhancement are the interactions with the intercellular lipids, the interactions with the intracellular keratin, and the penetration of high amounts of enhancers or so-called co-solvents into the stratum corneum with a resulting improved dissolving capacity of the barrier for drugs and co-enhancers. [17]

Transdermal iontophoresis is the method where the movements of ions across the layers of the skin is enhanced using an externally applied potential difference. [17] In iontophoresis, cationic therapeutic agents are placed under an anode, or anionic therapeutic agents under a cathode. When a low voltage and low current density is applied, ions are repelled into and through the skin. Cationic drugs are driven into the skin by the anode, which also extracts anion from the tissue underneath it. At the cathode, anionic buffer ions are driven into the skin and cations from the tissues are extracted (Figure 1.7). It is also possible to include an additional charged drug or a mixture of drugs in the electrode to be delivered simultaneously to enhance the desired effect or to increase skin permeation. Transdermal iontophoresis has been used for both local and systemic drug delivery. Applications include the delivery of anesthetics to a specific location, steroids and retinoids to treat acne scarring, and the administration of pilocarpine to diagnosis cystic fibrosis. Other applications of transdermal iontophoresis include the administration of anti-inflammatory drugs into subcutaneous tissues and joints. [19]

The third generation of transdermal delivery systems can make a significant impact on drug delivery due to its mechanism of action targeting the stratum corneum. This targeting enables a greater disruption of the stratum corneum while still protecting deeper skin layers. These advances were made possible by the emergence of technologies to localize effects to the stratum corneum along with the recognition that the safety achieved by localization should make these more aggressive approaches more medically acceptable. [16]

Microneedles have been shown to deliver macromolecules, including therapeutic proteins and vaccines, across the skin in human clinical trials. [17] Injection provides a low-cost, rapid and direct way to deliver almost any type of molecule into the body. However, hypodermic needles cannot be easily used by patients themselves and are therefore utilized primarily in the clinic or at home by patients who have received special training on correct injection method, safe needle disposal, and other issues. Compliance is further limited by pain and needle-phobia experienced by many patients. By shrinking the needle to micron dimensions, its delivery capabilities are preserved while improving patient compliance and safety. In general, microneedles can be grouped as solid microneedles, drug-coated microneedles, dissolving microneedles, and hollow microneedles. Each of these microneedle designs enables drug delivery by different mechanisms. (Figure 1.8) Solid microneedles can be used as a skin pretreatment. After inserting and removing the microneedles to form micron-scale pores in the skin surface, a drug formulation can be applied to the skin for slow diffusion of drug through the pores and into the body. Microneedles can also be coated with a drug typically using a water-soluble formulation. After insertion of microneedles into the skin, the drug coating is dissolved off the microneedles and into the skin, after which the microneedles are removed. Alternatively, microneedles can be made completely out of a water-soluble or biodegradable polymer that encapsulates the drug within the microneedle matrix. In this way, the microneedles completely dissolve or degrade in the skin, thereby releasing the encapsulated drug payload and leaving behind no sharps waste. Finally, hollow microneedles can be used for infusion of liquid formulations into the skin or, alternatively, for diffusion into the skin through the needle bore. [20]

Figure 1.3. Regional differences in skin thickness. [21]

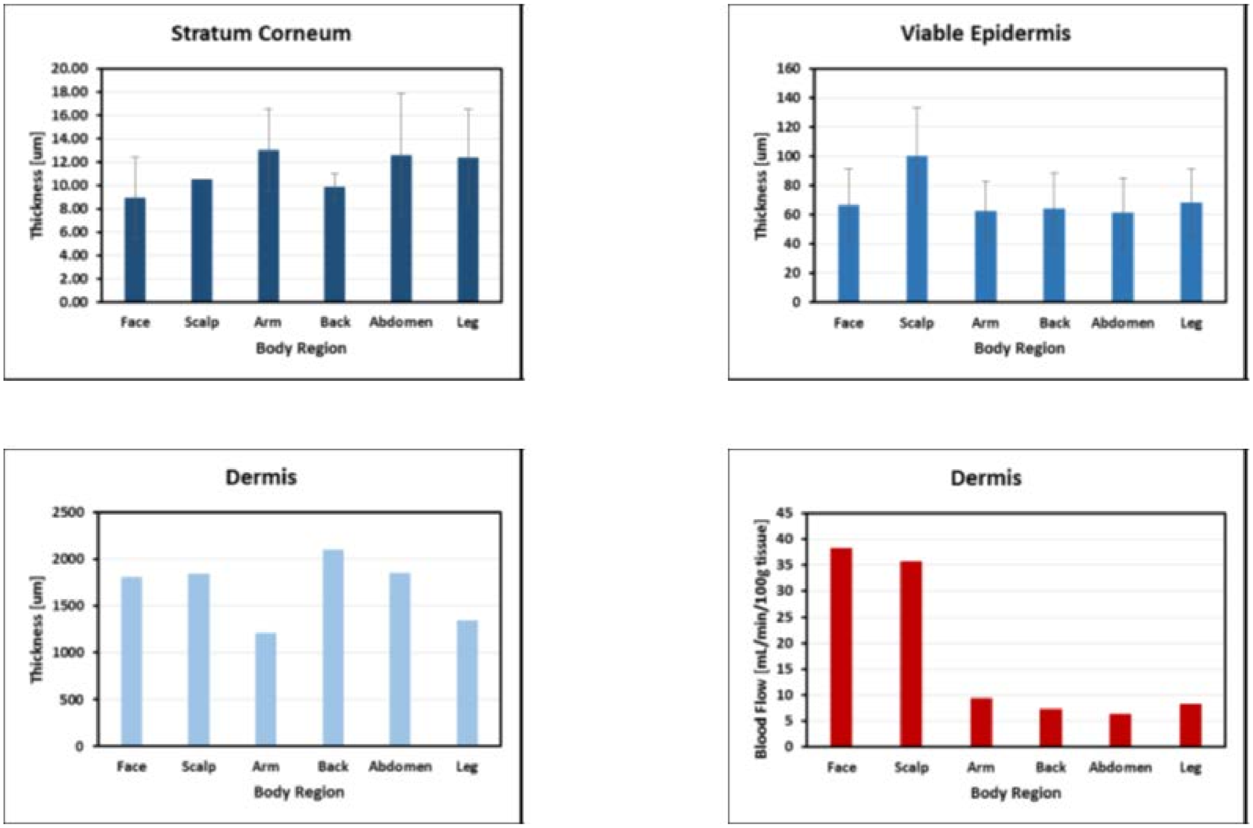


Figure 1.4. The skin represented as 2 layers. These are the dead stratum corneum and the viable tissue consisting of the rest of the epidermis as well as the dermis layer of skin. Diffusion coefficients Ds and Dv with diffusion path lengths ls and lv, with the partition coefficient K, govern the transport of chemical through the skin. [22]

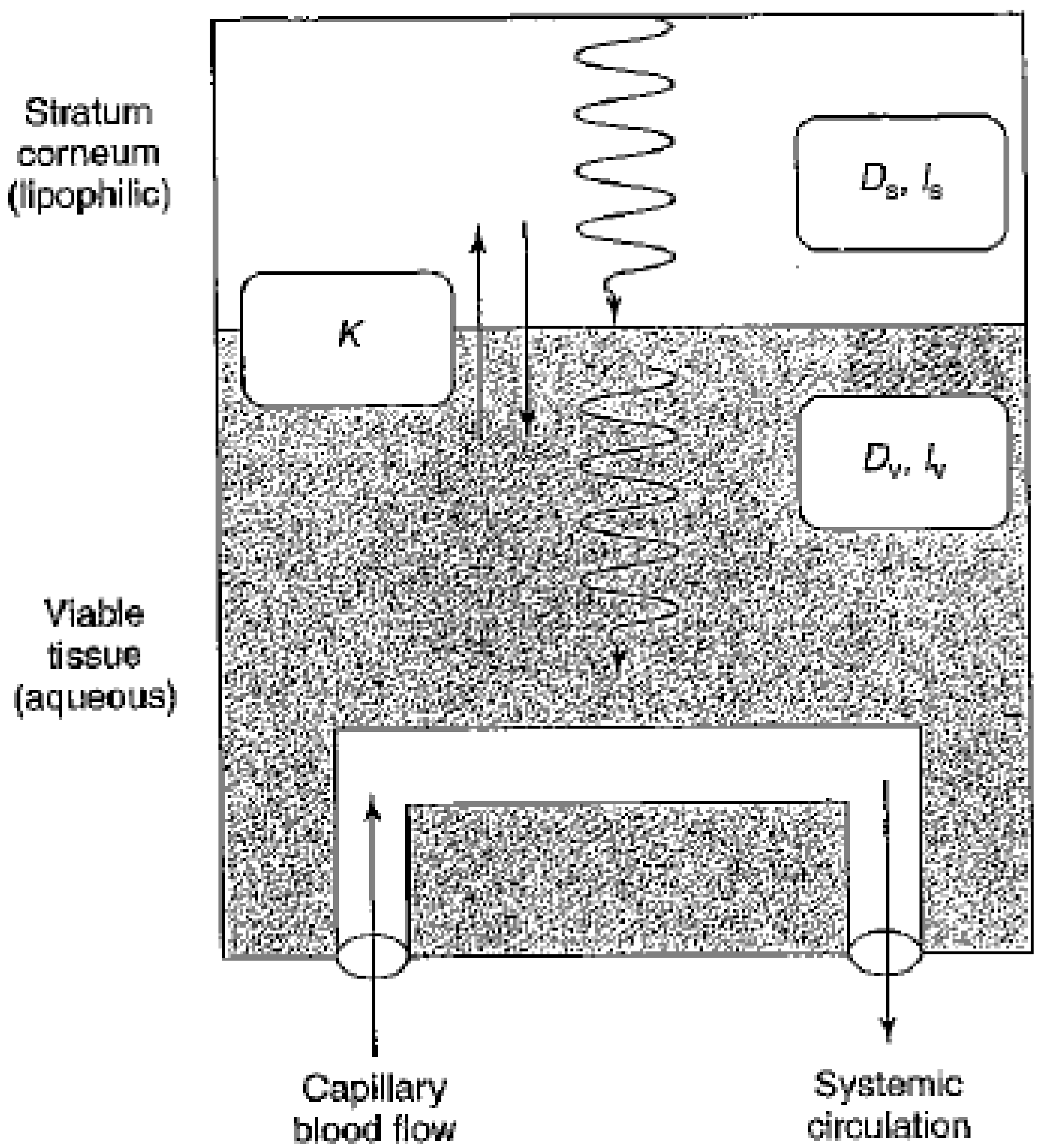


Figure 1.5. Brick and mortar model for the stratum corneum. Example intercellular routes and transcellular routes displayed along with tissue makeup of the layers. [23]

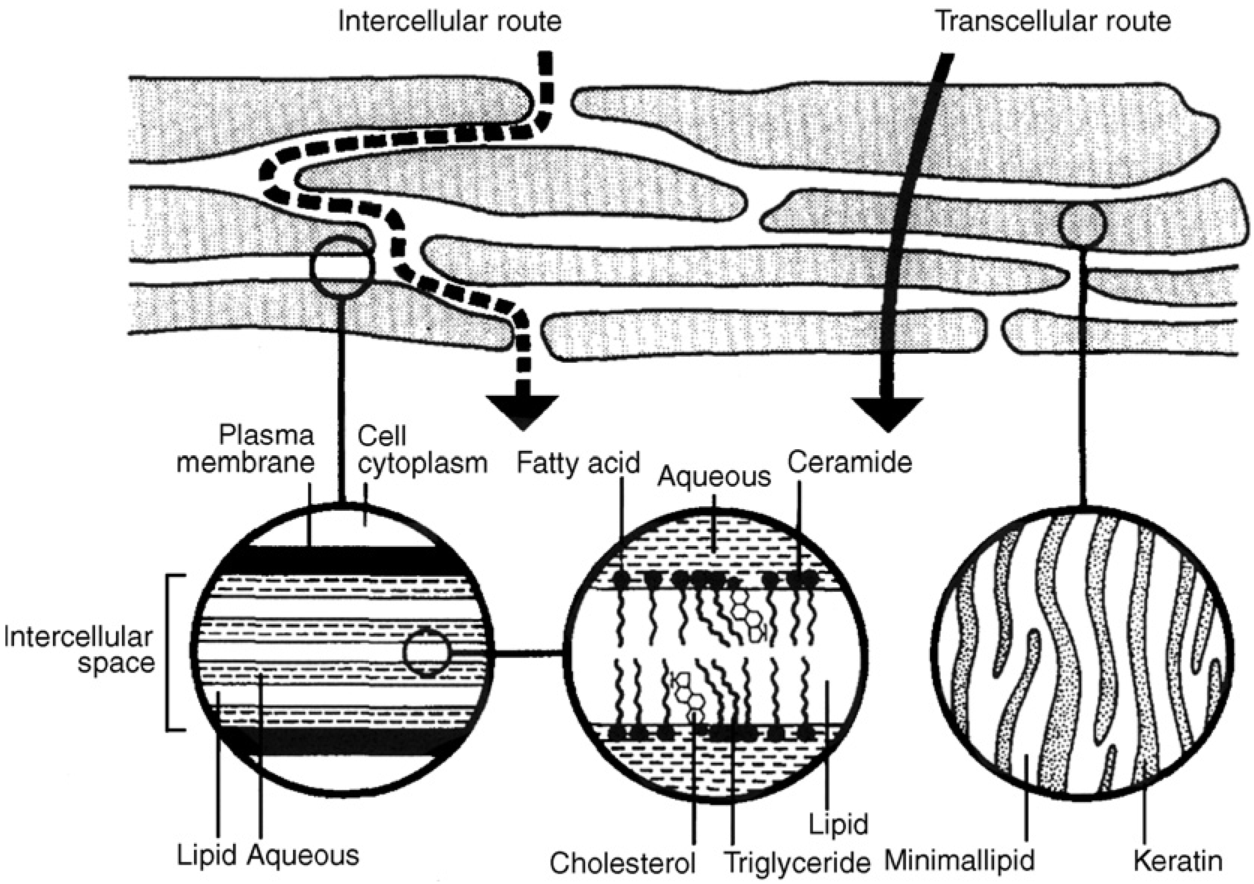


Figure 1.6. Types of transdermal patches: (A): micro reservoir controlled (B): membrane permeation controlled (C): adhesive dispersion controlled (D): matrix diffusion controlled. [24]

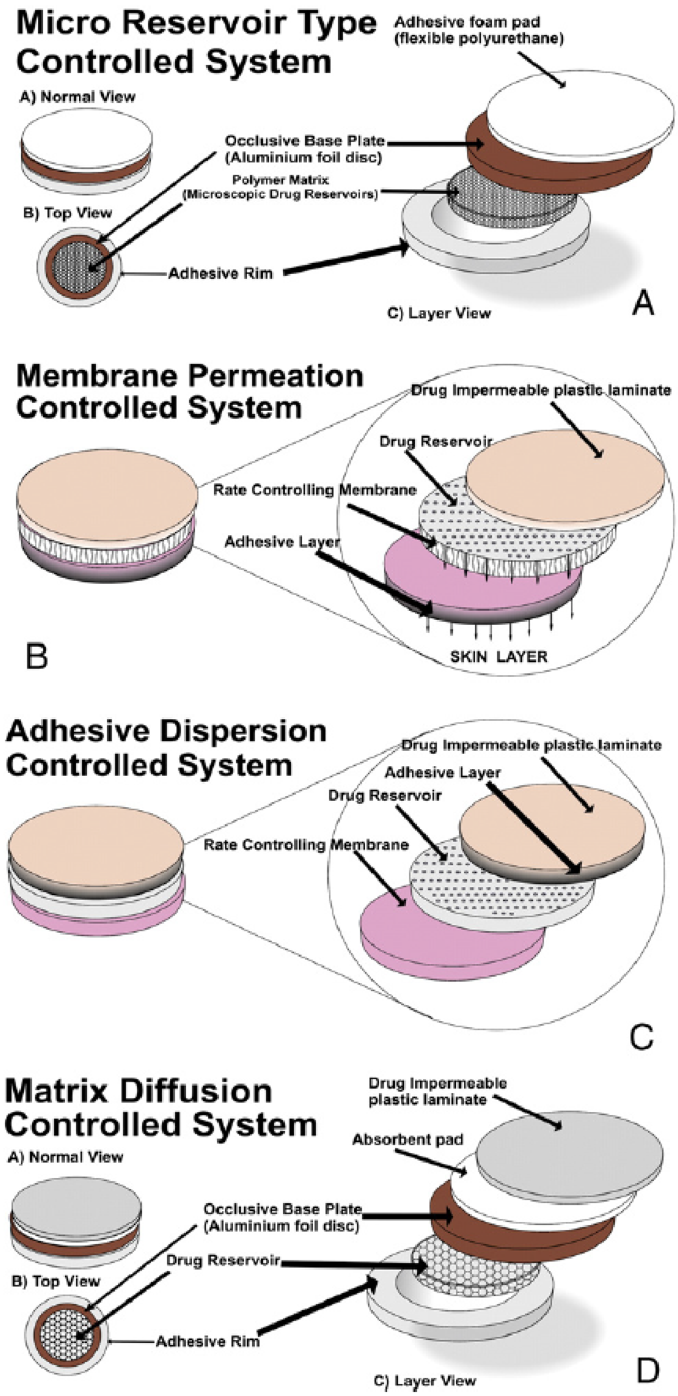


Figure 1.7. Iontophoresis patch. [25]

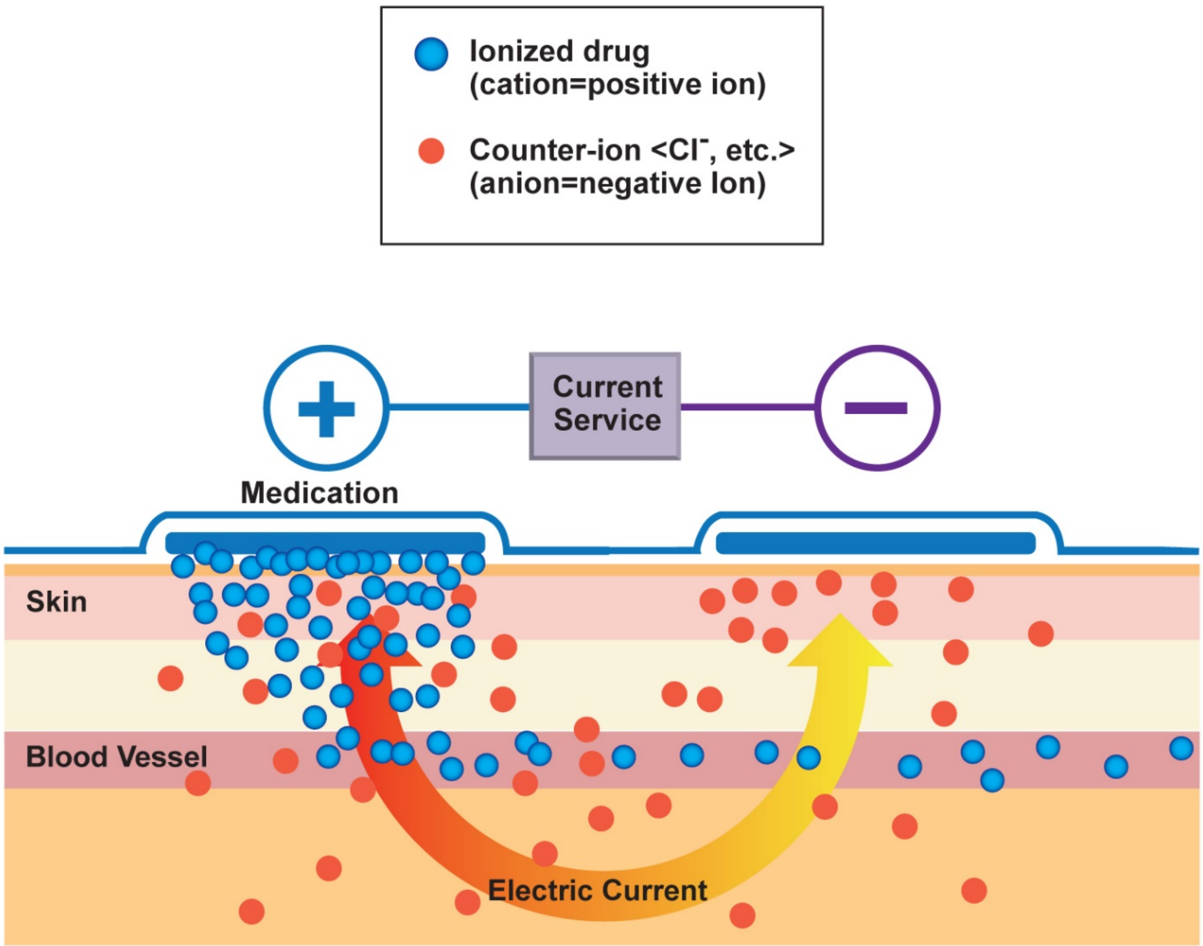
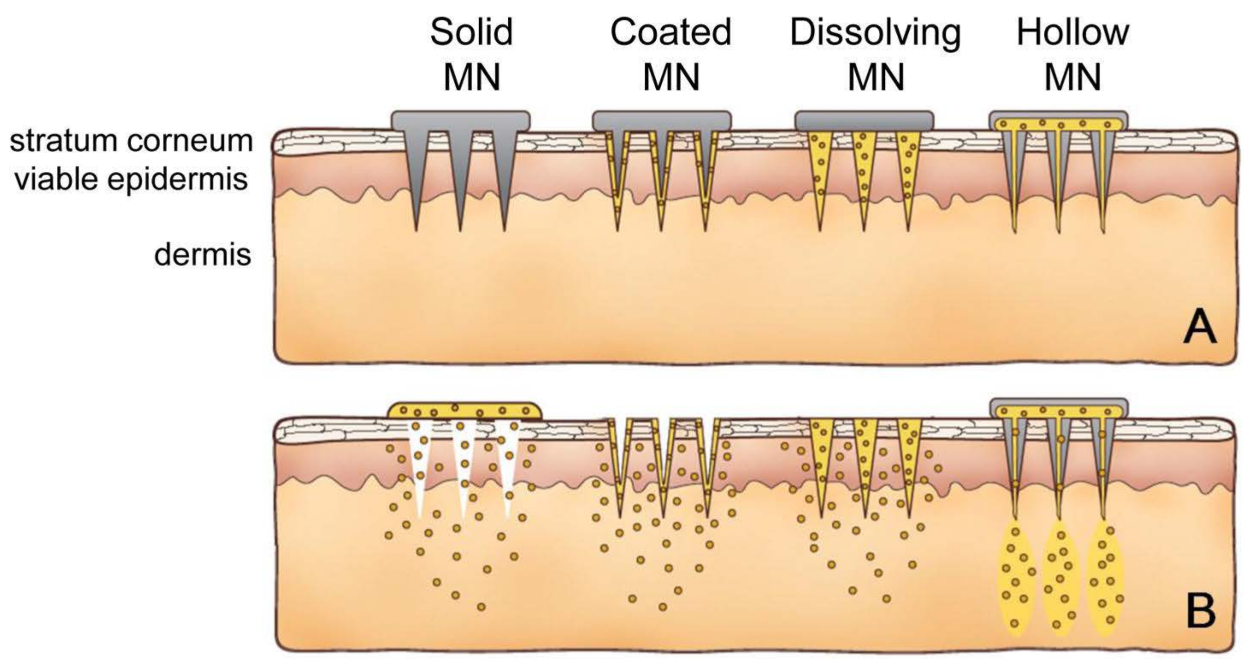


Figure 1.8. Drug delivery to the skin using microneedles. Microneedles are first applied to the skin (A) and then used for drug delivery (B). Solid microneedle can be used as a pretreatment, after which drug can diffuse through residual holes in skin from a topical formulation. With drug-coated microneedles into the skin, the drug coating dissolves off the microneedles in the aqueous environment of the skin. Drug-loaded microneedles are made of water-soluble or biodegradable materials, with the encapsulated drug released in the skin upon microneedle dissolution. Hollow microneedles are used to inject liquid formulations into the skin. [26]



1.2.3 Pharmacokinetic Modeling

1.2.3.1 Empirical Models

A fundamental hypothesis of pharmacokinetics is that a relationship exists between the pharmacologic or toxic response to a drug and the concentration of the drug in the blood (plasma). However, for some drugs there is no clear or simple relationship between pharmacologic effects and concentration in plasma. Nevertheless, pharmacokinetics plays a role in a dose-efficacy scheme by providing a quantitative relationship between dose and effect and the framework with which to interpret measurements of concentrations of drugs in biological fluids. In most cases, the concentration of drug in the systemic circulation is related to the concentration of drug at its sites of action.

The amount of chemical that is absorbed through the skin and finding the rate at which the drugs penetrate the skin is important for assessing the efficacy of the permeation enhancement of the transdermal formulation. Techniques such as static diffusion cells (Franz cells) have been used for direct measurement of the drug concentration *in vitro*. The Franz cell apparatus consists of two chambers separated by a membrane. As there are differences in chemical absorption by different species, the measurements in laboratory animals may not reflect human absorption, therefore testing on human skin is preferable. The test product is applied to the membrane via the top chamber of the Franz cell, while the bottom chamber contains fluid from which samples are taken at regular intervals to determine the amount of product that has permeated the membrane. For measuring the amount of drug *in vivo*, the drug may be directly applied to the skin followed by blood and urine measurements at set time points to assess the amount of chemical in the body. The concentration in the blood or urine at particular time points can be plotted to show an area under the curve and the extent and duration of absorption and distribution.

Computational tools are increasingly being used for pharmacokinetic modeling. Some are designed for general modeling applications (MatLab) or for simple applications similar to Excel Spreadsheet. [27] These tools have evolved from programming languages, such as Fortran [28], and progress to simulation languages, such as advanced continuous simulation language [29] and MatLab Simulink [30]. However, the majority are designed specifically for modeling pharmacokinetics [31] as well as pharmacodynamics [32]. They may be further grouped into the software used for a specific parameter such as absorption rate (GastroPlus and iDEA) and metabolism, as well as for the complete process within pharmacokinetics and pharmacodynamics (PK-Sim). Each modeling technology has its own strengths and weaknesses and may be employed for different purposes. The major advantage of the general modeling tools is their flexibility, but they require the user to possess a mathematical background and time-consuming programming skills.

The foundations of predictive modeling of transdermal and topical delivery were laid in the 1940s to 1970s. During this period, it became understood that partitioning and solubility were important factors that determined a drug's skin penetration. In the early 1940s Rothman identified the importance of the physicochemical properties of the chemical, such as the solubility and the critical influence of the delivery method on permeation. In the mid 1950s, Hadgraft and Somers found that solutes with a partition coefficient between 1 and 3 are associated with optimum skin permeation. In 1960, Higuchi derived mathematical models to describe the percutaneous absorption as a passive diffusion process. Since then, a large number of models have been developed to account for various aspects of skin permeation. These models vary widely in scope, ranging from simple models that only consider the stratum corneum, or sometimes the entire skin, as a single compartment to those that explicitly consider the structural complexity of the skin (Figure 1.9). [33]

Mathematics is widely used for the quantitative description of drug absorption, distribution, metabolism and excretion (ADME). Some typical pharmacokinetic parameters are defined by mathematical equations. More complex mathematical manipulations called mathematical models have been used to describe pharmacokinetics. [34] There are many classifications of pharmacokinetic models. Two general modeling approaches, compartment based [35] and noncompartment-based [36], take advantage of the quantitative structure-pharmacokinetic relationships that are described by empirical mathematical algorithms. They can be used to estimate the activity of a compound based on its chemical structure in a numeric format. Compartmental models estimate the concentration-time graph using kinetic models. Noncompartment models estimate the amount of a drug by determining the area under the curve of a concentration-time graph. These are often more versatile in that they do not assume any specific model and can produce accurate results, which are also useful for bioequivalence studies. Many functional models have been developed in order to simplify the estimation of pharmacokinetic variables. The simplest model would be to consider an organism as a single homogenous compartment. This single compartment model assumes that the blood plasma concentrations of the drug are an accurate reflection of the drug's concentration in other fluids or tissues and that the elimination of the drug is directly proportional to the drug's concentration in the organism. However, due to the reality of the body not being homogenous through its many organs, a multi-compartmental model can be used for greater accuracy.

Two main approaches have emerged for the prediction of drug permeation, empirical models and mechanistic models. Empirical models are derived from a retrospective analysis of experimental results, and correlate skin permeation data with drug properties. Mechanistic models are derived from first principles, with permeation being predicted and modelled based on skin compartments and diffusion pathways. [37]

With empirical models, the fundamental equation to describe skin transport under steady-state conditions can be given by Fick's first law. The amount of solute or drug, Q, crossing the skin membrane with area, A, over a period of time, ΔT, with the constant concentration gradient across the two interior surfaces of the skin, ΔCs, the diffusion coefficient in the skin membrane, D, and the path length, h, as follows:

Eqn 1.1

$$Q = \frac{DA\Delta T\Delta Cs}{h}$$

The assumptions of which are that the stratum corneum, as the primary barrier within the skin, is a homogenous membrane that does not vary with time or position. Steady state can only be reached after the lag time for solute diffusion, which can be estimated from the x intercept of the linear portion of the plot of the drug permeation as a function of time. The equation can then be expressed in terms of steady state skin flux Jss, defined as:

Eqn 1.2

$$Jss = \frac{Q}{A\Delta T} = \frac{D\Delta Cs}{h}$$

However, the actual structural heterogeneity of the stratum corneum and the likely presence of slow reversible binding phenomena does not always accurately represent the diffusion process in the stratum corneum. Therefore, efforts have been put forth to account for the structural complexity of skin. Due to the presence of both intracellular and transcellular pathways through the brick and mortar representation of the stratum corneum (Figure 1.10), the steady state permeability should be expressed as:

Eqn 1.3

$$k_p = \frac{D_{SC} K_{SC}}{h_{SC}}$$

where k_p is the permeation coefficient, D_{SC} is the average diffusion coefficient in the stratum corneum, K_{SC} is the average partition coefficient in the stratum corneum and the vehicle, and h_{sc} is the average diffusion path length through the stratum corneum. [33] Taking into account different factors involved in drug permeation through the skin, multiple empirical models have been developed over the years. [38]

Modeling approaches must be combined with the knowledge of the potency of the drug at the target site so that therapeutically relevant candidates can be progressed to final formulations. Mathematical models can also provide a way to calculate the likelihood of systemic exposure upon skin contact with toxic chemicals. However, the current level of advancement in modeling chemical transport through skin has not found broad application due to several reasons. Many of the models are not being easily accessible, and many existing predictive models are limited to single chemicals in simple solvents such as water, while complex mixtures are the reality in most industrial and environmental settings.

Mechanistic models predict permeation based on knowledge of skin compartments and diffusion pathways. There are three basic mechanistic models for skin permeation, related to exposure time, which allows the estimation of unsteady state exposure. (Figure 1.11) The first regards the stratum corneum as a single finite layer, the second has the stratum corneum being sufficiently thick to be considered as a semi-infinite membrane, and the third with the stratum corneum and viable epidermis as a finite two layered membrane, taking into consideration the partition coefficient, diffusion rate, and diffusion path length through both layers. For short, unsteady state exposure times, the drug will not have reached the viable epidermis to any significant extent and the first and second models are applicable. The third model is important for longer exposure times, when steady state may be reached, and the overall flux may be affected by the hydrophilic epidermis. This occurs with highly lipophilic drugs which will partition slowly from the SC and eventually become rate-limited by the viable epidermis layer.

1.2.3.2 Population Pharmacokinetics

Pharmacokinetic studies have usually been carried out in small numbers of people, often healthy, adult volunteers, with the average pharmacokinetic data used for study, with minimal variation between the subjects. Furthermore, these studies are usually focused on a single variable such as kidney function, which makes it difficult to study interaction with other factors. In population pharmacokinetics, samples are collected from actual patients taking the drug. Population pharmacokinetics (popPK) is the study of the variation in the concentrations of the drug throughout the target patient population. Certain patient features such as body weight, excretory and metabolic functions, and other therapies, can alter the relationship between the dose and concentration. Population pharmacokinetics tries to identify the measurable factors in the dose-concentration relationship and the significance of changes in these factors so that if it results in clinically significant differences in the therapeutic index, the dosage can be appropriately adjusted.

Using the popPK approach in drug development offers the possibility of gaining information on pharmacokinetics, from relatively sparse data obtained from study subjects, as well as from relatively dense data or even a combination of both sparse and dense data. This approach allows the analysis of data from a variety of unbalanced designs as well as from studies that are normally excluded, such as drug-concentration data obtained from pediatric and elderly patients, or data obtained during the evaluation of the relationships between dose or concentration and efficacy or safety.

In contrast to traditional pharmacokinetics, the popPK approach encompasses several key features. Relevant pharmacokinetic information is collected from patients who are representative of the target population to be treated with the drug. Variability during drug development and evaluation is identified and measured. The variability is explained by quantifying factors of demographic, pathophysiological, environmental, or drug-related origin that may influence the pharmacokinetic behavior of a drug. The magnitude of the variability in the patient population is then able to be quantitatively estimated.

In situations with sparse data, a nonlinear mixed effect modeling approach can be taken. The traditional two-stage approach where the pharmacokinetic parameters are first estimated from empirical data and then used to calculate statistics on the original sample, can only be achieved with a data rich study. The nonlinear method considers the population study sample, rather than the individual, as a unit for the estimation of the parameters. It consists of directly estimating the parameters of the population from the full set of individual concentration values, while still maintaining and accounting for the individuality of each subject.

Figure 1.9. Detailed, multi compartment model of transdermal absorption. [39]

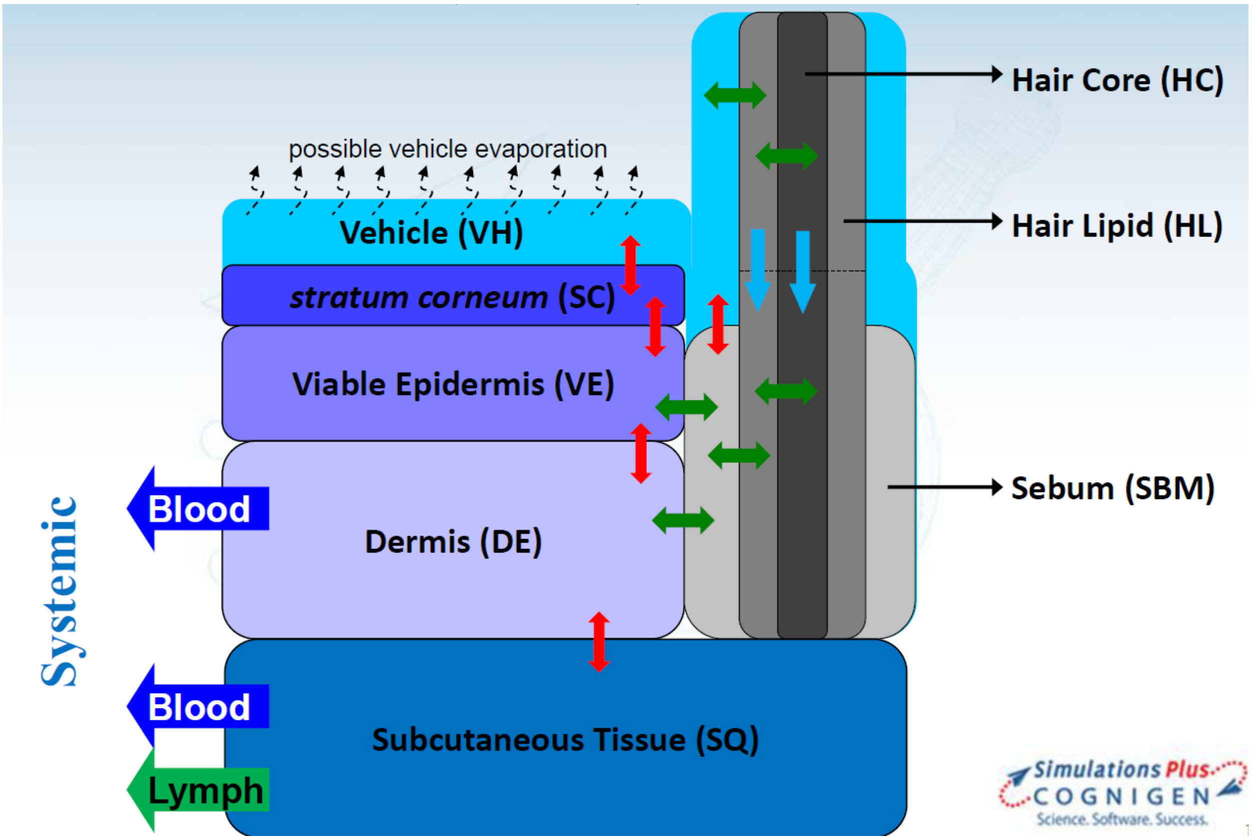


Figure 1.10. Schematic diagram for a brick-and-mortar model of the stratum corneum. Key model parameters are the corneocyte aspect ratio, $\alpha = d/t$; the lipid/corneocyte thickness ratio, $\beta = g/t$ with g being the thickness of the lipid layer between corneocytes; and the offset ratio, $\omega = d_l/d_s$. [40]

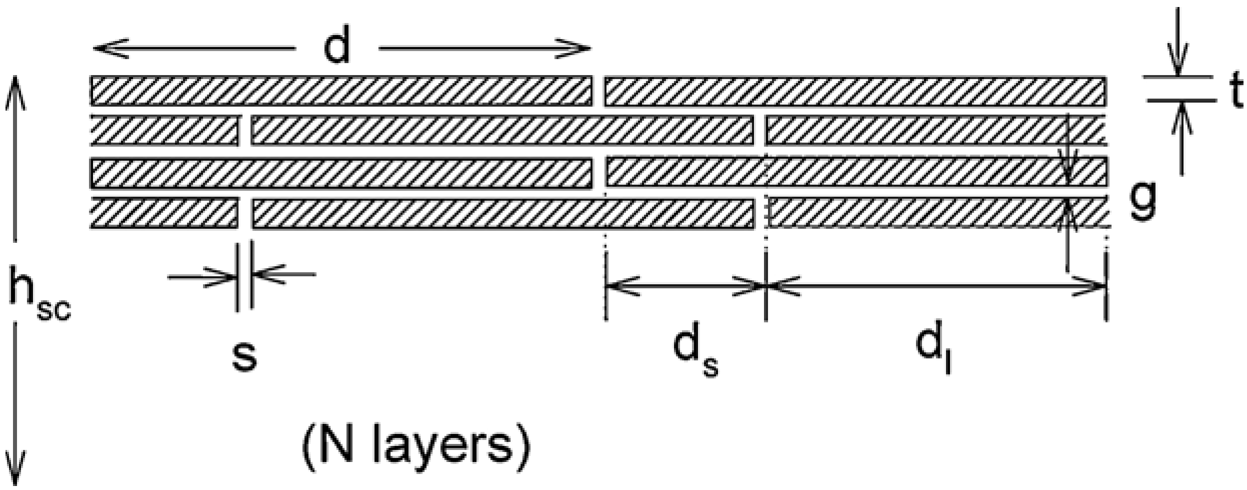
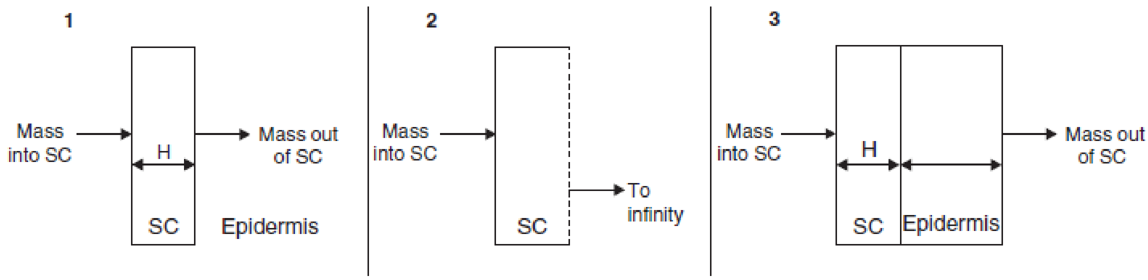


Figure 1.11. Three mechanistic models: 1) Single finite membrane; 2) semi-infinite membrane; and 3) finite two-membrane composite, with H as the stratum corneum (SC) thickness. [41]



1.3 Drug Modeling Studies

Three studies were performed to investigate transdermal pharmacokinetic modeling. The first study was performed with four different beta blockers. The amount of drug permeated through the skin was presumably varied due to differences in the drugs' physicochemical properties. These differences were studied by modeling the results from in vitro skin samples where the drugs permeated the skin passively, with skin permeation enhancement methods. These included microporation of the skin to simulate a transdermal microneedle patch, iontophoresis through the skin sample to represent iontophoretic transdermal patches, and a combination of both enhancement methods.

The second study was a population pharmacokinetic analysis of a clinical study for two aminoglycoside antibiotics, used in treating skin lesions caused by cutaneous leishmaniasis. The analysis was performed with a nonlinear mixed effect model to take into account the sparse data from the study, as well as effects due to the skin damaged from the lesions, and removal of excess medication in between dosage application. Different skin models of varying complexity were tested in order to determine a more accurate model for the study.

The third study was performed using cobalamin (vitamin B12) in crystalline cyanocobalamin and three other B12 vitamers. Transdermal absorption of B12 has recently been studied as an alternate route for patients with low absorption of the vitamin due to the lack of the intrinsic factor. Because of its large molecular weight and hydrophilic nature, the passive and chemically-enhanced delivery of cyanocobalamin is unlikely able to deliver doses within the therapeutic window. This study also took into account the different physicochemical properties of the four drugs and compared their transdermal permeability through passive diffusion, microporated skin, and iontophoretic enhancement.

1.3.1 Drugs in the Studies

1.3.1.1 Beta Blockers

Beta blockers are medications used to treat a multitude of conditions, primarily cardiac arrhythmias and preventing heart attacks after an initial one. [42] They are also widely used to treat hypertension, but are no longer the first choice for initial treatment. [43] Beta blockers are competitive antagonists that block the receptor sites for epinephrine and norepinephrine on beta receptors of the nervous system. [44] Some block activation of all types of β-adrenergic receptors and others are selective for one of the three known types of beta receptors, designated β1, β2 and β3 receptors. β1 receptors are located in the heart and kidneys, β2 receptors are in the lungs, gastrointestinal tract, liver, uterus, vascular smooth muscle, and skeletal muscle, and β3 receptors are located in the fat cells. [45] The receptors are found on cells of the heart muscles, smooth muscles, airways, arteries, kidneys, and other tissues that are part of the sympathetic nervous system and lead to stress responses.

Although beta blockers were once contraindicated in congestive heart failure, they have since shown their efficacy at reducing morbidity and mortality in studies in the late 1990s. [46] They are known primarily for their reductive effect on heart rate, causing a decrease in renin secretion, which reduces the heart's demand for oxygen by decreasing extracellular volume and increasing the oxygen carrying capacity of blood. Heart failure has been seen to involve increased catecholamine activity on the heart, which is responsible for several effects, including increased oxygen demand, propagation of inflammatory mediators, and abnormal cardiac tissue remodeling, all of which decrease the efficiency of cardiac contraction.

There are many adverse drug reactions associated with the use of beta blockers, which include nausea, diarrhea, bronchospasm, dyspnea, cold extremities, bradycardia, hypotension, heart failure, heart block, fatigue, dizziness, alopecia, abnormal vision, hallucinations, insomnia, nightmares, sexual dysfunction, erectile dysfunction and alteration of metabolism. Lipophilic beta blockers, such as propranolol, have high penetration through the blood–brain barrier, and are more likely than less lipophilic beta blockers to cause sleep disturbances. [47] Adverse effects associated with β2-adrenergic receptor antagonists are less common with β1-selective agents, but the receptor selectivity decreases at higher doses. As beta blockers inhibit renin release, the release of aldosterone is also decreased, causing hyponatremia and hyperkalemia. Hypoglycemia can occur with beta blockers because the targeted β2-adrenoceptors normally stimulate glycogen breakdown in the liver and pancreatic release of glucagon, which increases glucose in the plasma. β1-blockers have fewer side effects in diabetic patients, but the rapid heart rate that serves as a warning sign for insulin-induced low blood sugar may be masked, resulting in hypoglycemia unawareness. A 2007 study revealed that diuretics and beta blockers used for hypertension can increase a patient's risk of developing diabetes mellitus. [48] Beta blockers must not be used in the treatment of selective alpha-adrenergic agonist overdose. The blockade of only beta receptors increases blood pressure, reduces coronary blood flow, left ventricular function, and cardiac output and tissue perfusion by means of leaving the alpha-adrenergic system stimulation unopposed. [49]

Glucagon is used in the treatment of beta blocker overdoses, increasing the strength of heart contractions, intracellular cAMP, while decreasing renal vascular resistance. [50] People experiencing bronchospasm due to nonselective beta blockers may be treated with anticholinergic drugs, such as ipratropium, which are safer than beta agonists in patients with cardiovascular diseases. Other antidotes for beta-blocker poisoning include salbutamol and isoprenaline.

Beta blockers are not officially approved for anti-anxiety use by the U.S. Food and Drug Administration. [51] However, many controlled trials in the past 25 years indicate that beta blockers are effective in anxiety disorders, though the precise mechanism of action is not known.

1.3.1.1.1 Propranolol

Propranolol is a beta blocker used to treat high blood pressure, several types of irregular heart rate issues, thyrotoxicosis, capillary hemangiomas, performance anxiety, and essential tremors. It is used to prevent migraines, and to prevent further heart problems in patients with angina or who have previously suffered heart attacks. The oral formulation comes in short acting and long acting versions. Beta blockers were downgraded to fourth-line from being a once first line treatment for hypertension, as newer drugs have been developed that outperform them, particularly in the elderly, and there is growing evidence that there is an unacceptable risk of provoking type 2 diabetes. [52] Propranolol has also been investigated as a potential treatment for PTSD. [53] Those who were given propranolol immediately after experiencing trauma experienced fewer stress-related symptoms and lower rates of PTSD than control groups who did not receive the drug. [54] Due to memories and their emotional content reconsolidating in the hours after they are recalled/re-experienced, propranolol can also diminish the emotional impact of already formed memories and is being studied in the treatment of various phobias. [55]

Propranolol is rapidly absorbed, with peak plasma levels a few hours after oral administration, with increased bioavailability when taken with food. [56] Despite being complete absorbed, propranolol has a variable bioavailability due to extensive first-pass metabolism. The main metabolite 4-hydroxypropranolol, with a longer half-life (5.2–7.5 hours) than the parent compound (3–4 hours), is also pharmacologically active. Propranolol is highly lipophilic and can achieve high concentrations in the brain. The duration of action of a single oral dose may be up to 12 hours, which is longer than its half-life. It is classified as a non-cardioselective sympatholytic beta blocker that crosses the blood–brain barrier, blocking epinephrine and norepinephrine at both $\beta 1$- and $\beta 2$-adrenergic receptors, as well as having sodium channel blocking effects. In addition to blocking adrenergic receptors, propranolol can also weakly inhibit norepinephrine transporters. [57]

Originally, propranolol was marketed in 1965 under the brand name Inderal and manufactured by ICI Pharmaceuticals. Propranolol is now also marketed under brand names Avlocardyl, Deralin, Dociton, Inderalici, InnoPran XL, Sumial, Anaprilin, and Bedranol SR.

1.3.1.1.2 Acebutolol

Acebutolol is a lipophilic beta blocker that can act both as an agonist and antagonist at the receptors. Due to its lipophilicity, it easily crosses the blood brain barrier. The drug works in hypertensive patients although it may be more efficient in patients with high or normal renin plasma concentrations than those with lower concentrations.

Acebutolol is easily absorbed from the GI tract, but undergoes significant first-pass-metabolism, leading to a bioavailability of only 35% to 50%. With oral dosing, peak plasma levels of acebutolol are reached in 2 to 2.5 hours, and has a half-life of 3 to 4 hours. It undergoes hepatic metabolism resulting in acetolol which is then converted into diacetolol. The incidence of both anti-nuclear antibodies and symptomatic disease under acebutolol is higher than under propranolol. Only a small percent, 5 to 6% of all patients treated, have to discontinue acebutolol due to adverse effects. When possible, ending the treatment should be done gradually in order to avoid withdrawal and myocardial infarction.

1.3.1.1.3 Atenolol

Atenolol was developed as a replacement for propranolol for hypertension treatment. It works by slowing the heart rate and reducing its workload. Atenolol is not lipophilic, and does not readily pass through the blood brain barrier, which leads to a decrease of side effects in the central nervous system. [58] Atenolol is effective at reducing blood pressure, but recent studies indicate that it does not reduce the morbidity or mortality caused by hypertension, and may even increase mortality in some subgroups. [59]

Symptoms of atenolol overdose are due to excessive blocking of the β1 and β2-receptors. These include bradycardia, severe hypotension, acute heart failure, hypoglycemia and bronchospastic reactions. Activated charcoal is useful for absorbing the drug when treating overdoses. Blood or plasma concentrations of atenolol can be used to confirm a diagnosis of poisoning in hospitalized patients.

1.3.1.1.4 Sotalol

Sotalol is a beta blocker used to maintain normal heart rhythm in people with ventricular arrhythmias or atrial fibrillation, though due to the risk of serious side effects, should be reserved for life-threatening occurrences. Sotalol binds non-selectively to both β1- and β2-adrenergic receptors, preventing the activation of the receptors. [60] Sotalol also acts on potassium channels and causes a delay in the relaxation of the ventricles. [61] By blocking these potassium channels, sotalol causes an increase in the time between contractions, which helps to correct arrhythmias by reducing premature or abnormal contraction of the ventricles while also prolonging the frequency of ventricular contraction to help treat tachycardia.

Sotalol is eliminated by the kidneys, so should not be used by people with low creatinine clearance or poor kidney performance. It is also excreted in breast milk, so mothers should not take sotalol while breastfeeding. Because sotalol prolongs the ventricle contraction interval, the FDA recommends against using it in conjunction with other drugs that prolong it. Studies have found serious side effects to be more common in patients taking digoxin, possibly due to pre-existing heart failure in those patients. As with other beta blockers, it may interact with calcium channel blockers, catecholamine-depleting drugs, insulin or antidiabetic drugs, beta2-adrenergic agonists, and Clonidine. [61]

1.3.1.2 Cutaneous Leishmaniasis Drugs

Paromomycin is an antibiotic used to treat a number of infections including leishmaniasis, a disease caused by parasitic protozoa spread by sandflies, with the cutaneous form presenting with skin ulcers. Risk factors include poverty, malnutrition, deforestation, and urbanization. Common side effects when taken orally include gastrointestinal issues such as loss of appetite, vomiting, abdominal pain, and diarrhea. When applied to the skin, paromomycin side effects include itchiness, redness, and blisters. Paromomycin is in the aminoglycoside family of medications and causes bacterial death by protein synthesis inhibition, binding to ribosomal RNA. [62] Paromomycin was demonstrated to be effective against cutaneous leishmaniasis in clinical studies in the USSR in the 1960s. [63] A multistep model has been proposed to explain the antibacterial effect of aminoglycosides, which involves antibiotic uptake, mistranslation by chain-elongating ribosomes, membrane damage, and the subsequent ribosomal blockade which prevents further protein synthesis. [64]

Gentamicin is an antibiotic that is active against a wide range of bacterial infections. Like paromomycin, gentamicin is an aminoglycoside, which can cause nephrotoxicity and inner ear damage with higher doses in approximately 10% of those who take the medication. [65] In the clinical study that was analyzed for population pharmacokinetics, gentamicin was used in combination with paromomycin in one of the treatment formulations for cutaneous leishmaniasis.

1.3.1.3 Vitamin B12

Vitamin B12, also known as cobalamin, has a key role in the normal functioning of the brain and nervous system via the synthesis of myelin. [66] B12 is involved in the metabolism of every cell of the human body, affecting DNA synthesis, and fatty acid and amino acid metabolism. As medication, it is used to treat B12 deficiency and cyanide poisoning. The synthetic form of the vitamin B12 molecule is cyanocobalamin. Cyanocobalamin is usually given after surgery to remove part or all of the stomach or intestine, to ensure adequate levels of vitamin B12 in the body. Cyanocobalamin is one of the most widely manufactured vitamers of B12, due to it not being susceptible to oxidation in its crystalline form. Its stability leads it to be used in many food additives and multivitamins.

Hydroxocobalamin is also a commonly used form of B12. It is produced by bacteria, after which it can be further converted to cyanocobalamin with a charcoal filtration step where the hydroxo ligand is replaced by cyanide naturally found in activated charcoal. This affinity for cyanide allows it to be used to treat cyanide poisoning. It is also more easily converted to the active forms of B12 than cyanocobalamin, and has a longer retention time in the body, which allows it to be used with patients suffering from intrinsic metabolic diseases. [67]

Adenosylcobalamin and methylcobalamin are the two active forms of B12 that occur naturally within the body. Along with the other B12 vitamers, transdermal B12 dosing is a more effective alternative for patients with a poor ability to absorb it orally.

1.4 Purpose

With the development of mathematical models and the use of modeling software to compute pharmacokinetic parameters, the efficacy of formulations and dosage design can be optimized. Data taken from *in vitro* experiments with static diffusion cells, or *in vivo* experiments with laboratory animal surrogates or clinical trials with patients, can be used to develop transdermal formulations. The advent of new transdermal delivery technologies like microneedle and iontophoretic patches allow for greater transdermal delivery of a wider multitude of drugs. Previously, lipophilic drugs would be less suitable for transdermal delivery as the drug molecules would either be bound in the lipophilic membranes of the cell layers of the skin along the intracellular route, or restricted from diffusion into the bloodstream by the hydrophilic cellular contents along the transcellular route. Further modeling would result in greater efficacy in overcoming the obstacles between topical application and systemic distribution, allowing more drugs to share in the advantages of transdermal drug delivery, avoiding significant first pass metabolism of the oral delivery route, and better patient compliance and ease of dosing as compared to intravenous delivery.

The assessment of transdermal absorption of drug molecules is a very important step in the evaluation of any transdermal drug delivery system. A key goal in the design and optimization of dermal or transdermal dosage forms lies in understanding the factors that determine a good *in vivo* performance. Skin permeation of molecules is a multifactorial phenomenon depending on diverse types of physical, chemical and biological interactions. A large portion of these interactions is nonlinear, making mathematical modeling of percutaneous absorption difficult. In vitro skin permeation studies are frequently performed for screening of molecules and drug carrier systems aimed at optimizing dermal or transdermal delivery. Therefore, one of the main objectives of in vitro permeation studies is the prediction of in vivo absorption.

Both diffusion and compartmental models have been presented to describe the pharmacokinetics of passive transdermal drug delivery. These models have been further refined based on cutaneous physiology. There are limited reports regarding the pharmacokinetics of transdermal delivery when modified by skin penetration enhancers such as microneedle patches or iontophoresis. While these methods have been shown experimentally to increase drug permeation, there has been little to no modeling with microneedles or iontophoresis, in general or with specific drugs. As one of the factors in determining the diffusion rate constants through the skin is the distance the drug molecule must traverse as described in Fick's law, methods that shorten or change the distance should represent a significant influence on the diffusion rate constant. This study seeks to model this influence by studying microneedles, which provide a physical alteration to the stratum corneum to provide a new path for the drug to reach systemic distribution. Microneedle influence was compared with passive diffusion, which is the most basic of transdermal patches, as well as compared separately and in combination with iontophoresis to measure the influence in an active delivery system. Another focal point is to look at damaged skin, which also changes the skin's layer profile, in the form of cutaneous leishmaniasis, with a population pharmacokinetic modeling approach.

1.5 References

1. Stücker M, Struk A, Altmeyer P, et al. The cutaneous uptake of atmospheric oxygen contributes significantly to the oxygen supply of human dermis and epidermis. J Physiol. 2002(538), 985-994.
2. Marks JG and Miller J. Lookingbill and Marks' Principles of Dermatology. Elsevier Inc , 2006, 10-25.
3. Proksch, E, Brandner, JM, and Jensen, JM. The skin: an indispensable barrier. Exp Dermatol. 2008(12), 1063–72.
4. Ovaere P, Lippens S, Vandenabeele P, et al. The emerging roles of serine protease cascades in the epidermis. Trends Biochem Sci. 2009(9), 453–463.
5. James W, Berger T, Elston D. Andrews' Diseases of the Skin: Clinical Dermatology. Saunders. 2005, 11–12.
6. Morganti P, Ruocco E, Wolf R, and Ruocco V. Percutaneous absorption and delivery systems. Clin Dermatol. 2001(19), 489-501.
7. McCarley KD and Bunge AL. Review of pharmacokinetic models of dermal absorption. J Pharmaceut Sci. 2001(90), 1699–1719.
8. Gray H. Anatomy of the Human Body. Lea and Febiger. 1918, 941. Reproduced with permission
9. Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436.
10. Eaton DL and Klaassen CD. Principles of Toxicology. In Cassarett & Doull's Toxicology, The Basic Science of Poisons. 5th ed. McGraw-Hill, 1996.

11. Baynes RE and Hodgson E. Absorption and Distribution of Toxicants. In A Textbook of Modern Toxicology. 3rd ed. John Wiley & Sons, Inc. 2004.
12. Flynn, GL. Cutaneous and transdermal delivery: Processes and systems of delivery. In Modern Pharmaceutics. Banker, G.S & Rhodes. 1996, 239-299.
13. Feingold KR. The outer frontier: the importance of lipid metabolism in the skin. J Lipid Res. 2009(50), 417-422.
14. Hadgraft, J. Modulation of the barrier function of the skin. Skin Pharmacol Appl Skin Physiol. 2001(14): 72-81.
15. Miller MA, Pisani E. The cost of unsafe injections. Bull World Health Organ 1999(77),808–811.
16. Williams AC and Barry BW. Penetration enhancers. Adv Drug Deliv Rev 2004(56), 603–618.
17. 24. Alexander A, Dwivedi S, Giri TK, et al. Approaches for breaking the barriers of drug permeation through transdermal drug delivery. J Control Release. 2012(164), 26-40.
18. Touitou E, and Godin B. Enhancement in Drug Delivery. CRC Press. 2007, 255-278.
19. Rawat S, Vengurlekar S, Rakesh B, et al. Transdermal delivery by iontophoresis. Indian J Pharm Sci. 2008(70), 5–10.
20. Kim HM, Lim YY, An JH, et al. Transdermal drug delivery using disk microneedle rollers in a hairless rat model. Int J Dermatol. 2012(51), 859-863.
21. GastroPlus™ Introductory Workshop February. 2015. Reproduced with permission from Cognigen
22. Guy RH and Hadgraft. Physicochemical aspects of percutaneous penetration and its enhancement. J. Pharm Res. 1988(5), 753-758. Modified and used with permission.

23. El Maghraby GM, Barry BW, and Williams AC. Liposomes and skin: From drug delivery to model membranes. Eur J Pharm Sci. 2008(34), 203-222. Reproduced with permission.

24. Alexander A, Dwivedi S, Giri TK, et al. Approaches for breaking the barriers of drug permeation through transdermal drug delivery. J Control Release. 2012(164), 26-40. Modified and used with permission.

25. http://electronicsmaker.com/microcontrollers-drive-home-drug-delivery-2 Reproduced with permission from electronicsmaker.com

26. Kim YC, Park JH, and Prausnitz MR. Microneedles for drug and vaccine delivery. Adv Drug Deliv Rev. 2012(14), 1547–1568. Reproduced with permission

27. Haddad S, Pelekis M, Krishnan K. A methodology for solving physiologically based pharmacokinetic models without the use of simulation softwares. Toxicol Lett. 1996(85), 113-26.

28. Lewis JC, Luecke RH, Wosilait W. Microcomputer program for interactions in drug elimination in the rat. Comput Methods Programs Biomed 1988(26), 137-43.

29. Thomas RS, Lytle WE, Keefe TJ, et al. Incorporating Monte Carlo simulation into physiologically based pharmacokinetic models using advanced continuous simulation language (ACSL): a computational method. Fund Appl Toxicol. 1996(104), 858-865.

30. Wada DR, Stanski DR, Ebling WF. A PC-based graphical simulation for physiological pharmacokinetic models. Comput Meth Prog Bio. 1995(46), 245-255.

31. Levitt DG. PKQuest: a general physiologically based pharmacokinetic model. Introduction and application to propranolol. BMC Clin Pharmacol. 2002(15), 5.

32. Aarons L. Software for population pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 1999(36), 255-264.

33. Mitragotri S, Anissimov YG, Bunge AL, et al. Mathematical models of skin permeability: An overview. Int J Pharm. 2001(418), 115-129.
34. Bonate PL. A brief introduction to Monte Carlo simulation. Clin Pharmacokinet 2001(40), 15-22.
35. Holz M, and Fahr A. Compartment modeling. Adv Drug Deliv Rev. 2001(48), 249-264.
36. Veng-Pedersen P. Noncompartmentally-based pharmacokinetic modeling. Adv Drug Deliv Rev. 2001(48), 265-300.
37. Russel LM and Guy RH. Measurement and prediction of the rate and extent of drug delivery into and through the skin. Expert Opin. Drug Deliv. 2009(4), 355-369.
38. Lian G, Chen L, Han L. An evaluation of mathematical models for predicting skin permeability. J Pharm Sci. 2008(97), 584-598.
39. GastroPlus™ Introductory Workshop February. 2015. Reproduced with permission from Cognigen
40. Mitragotri S, Anissimov YG, Bunge AL, et al. Mathematical models of skin permeability: An overview. Int J Pharm. 2001(418), 115-129. Reproduced with permission from Mitragotri, 1991
41. Russel LM and Guy RH. Measurement and prediction of the rate and extent of drug delivery into and through the skin. Expert Opin. Drug Deliv. 2009(4), 355-369. Reproduced with permission.
42. Freemantle N, Cleland J, Young P, Mason J, Harrison J. β Blockade after myocardial infarction: systematic review and meta regression analysis. Bmj Brit Med J. 1999(318), 1730-1737.

43. James PA, Oparil S, Carter BL, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014(311), 507–20.
44. Frishman WH, Cheng-Lai A, Nawarskas J. Current Cardiovascular Drugs. Springer Science & Business Media. 2005, 152.
45. Clément K, Vaisse C, Manning BS, et al. Genetic variation in the beta 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. New Engl J Med. 1995(333), 352–354.
46. Hjalmarson A, Goldstein S, Fagerberg B, et al. Effects of controlled-release metoprolol on total mortality, hospitalizations, and well-being in patients with heart failure: the Metoprolol CR/XL Randomized Intervention Trial in congestive heart failure (MERIT-HF). MERIT-HF Study Group. JAMA. 2000(283), 1295–1302.
47. Cruickshank JM. Beta-blockers and heart failure. Indian Heart J. 2010(62), 101–10.
48. Elliott WJ, Meyer PM. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. Lancet. 2007(9369): 201–207.
49. Richards JR, Derlet RW, Albertson TE. "Treatment & Management". Methamphetamine Toxicity. Medscape. WebMD. 2016.
50. Weinstein RS, Cole S, Knaster HB, et al. Beta blocker overdose with propranolol and with atenolol. Ann Emerg Med. 1985(14), 161–163.
51. Schneier FR."Clinical practice. Social anxiety disorder. N. Engl. J. Med. 2006(355), 1029–1036.
52. Ladva S. NICE and BHS launch updated hypertension guideline. National Institute for Health and Clinical Excellence. 2006.

53. Brunet A, Orr SP, Tremblay J, et al. Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. J Psychiat Res. 2008(42), 503–6.

54. Vaiva G, Ducrocq F, Jezekiel K, et al. Immediate treatment with propranolol decreases post-traumatic stress disorder two months after trauma. Biol Psychiat. 2003(54), 947–949.

55. Steenen SA, van Wijk AJ, van der Heijden GJ, et al. Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis. J Psychopharmacol. 2015(30), 128–139.

56. Rang HP. Rang & Dale's pharmacology. Edinburgh: Churchill Livingstone. 2011, 106.

57. Davids E, Lesch KP. The 5-HT1A receptor: a new effective principle in psychopharmacologic therapy? Fortschr Neurol Psychiatr. 1996(64), 460–472.

58. Agon P, Goethals P, Van Haver D, et al. Permeability of the blood–brain barrier for atenolol studied by positron emission tomography. J Pharm Pharmacol. 1991(43), 597–600.

59. Testa G, Cacciatore F, Della-Morte D, et al. Atenolol use is associated with long-term mortality in community-dwelling older adults with hypertension. Geriatr Gerontol Int. 2014(14), 153–158.

60. Bertrix, L, Timour-Chah Q, Lang J, et al. Protection against ventricular and atrial fibrillation by sotalol. Cardiovasc Res. 1986(20), 358-363.

61. Edvardsson N, Hirsch I, Emanuelsson H, et al. Sotalol-induced delayed ventricular repolarization in man. Eur Heart J. 1980(1), 335-343.

62. Vicens Q, Westhof E. Crystal Structure of Paromomycin Docked into the Eubacterial Ribosomal Decoding A Site. Structure. 2001(9), 647–658.

63. Neal RA, Murphy AG, Olliaro P, et al. Aminosidine ointments for the treatment of experimental cutaneous leishmaniasis. T Roy Soc Trop Med H. 1994(88), 223–225.

64. Fernández MM, Malchiodi EL, and Algranati ID. Differential effects of paromomycin on ribosomes of Leishmania mexicana and mammalian cells. Antimicrob Agents Ch. 2011(55), 86-93.
65. Lopez-Novoa JM, Quiros Y, Vicente L, et al. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int. 2011(79), 33–45.
66. Miller A, Korem M, Almog R, et al. Vitamin B12, demyelination, remyelination and repair in multiple sclerosis. J Neurol Sci. 2005(233), 93–97.
67. Shepherd G, Velez LI. Role of hydroxocobalamin in acute cyanide poisoning. Ann Pharmacother. 2008(42), 661–669.

2. Pharmacokinetic Modeling of Transdermal Beta-blockers

2.1 Abstract

Propranolol, acebutolol, atenolol, and sotalol are beta-blockers used for the treatment of hypertension. Recently, alternate methods, such as transdermal and microneedle routes, have been investigated for the delivery of these beta-blockers. These delivery systems possess several advantages over the conventional oral forms of the drugs such as avoidance of hepatic first pass metabolism and prolonged delivery. An important concern for transdermal formulations is skin reactions. The most common side effects include mild skin rash, itching, redness and irritation. These skin reactions can occur due to components of the transdermal patch and can be reduced by the modification of the formulation or delivery method. Another limitation is the lag time to the onset of action that is typically longer with transdermal systems.

The transdermal permeability of these drugs was investigated in in vitro conditions using a Franz diffusion cell apparatus, with passive diffusion (PD) through the skin as a control. The influence of skin perforated by microneedles (MN) and iontophoresis (ITP) on permeation enhancement was examined. Data from these investigations were then modeled using WinNonLin software. A simple computational model was used to calculate the rate constants into the skin (Kp for passive diffusion and Km for microneedles) and into the collection volume (Kd). For iontophoresis, an additional rate constant during the application of iontophoresis (Ki) was included.

With few exceptions, regression of the transdermal permeability study data sets converged to provide rate constant values. Each beta blocker showed significant increases in transdermal permeation compared to passive diffusion with the application of microneedles, iontophoresis, or both in combination. From the calculated rate constants, a simple model was developed to predict

plasma concentration, taking into consideration pharmacokinetic parameters to account for the lag time between application of the formulation and diffusion through the skin.

2.2 Introduction

Beta blockers are used to treat cardiac arrhythmias and prevent subsequent heart attacks. [1, 2] They are also used to treat hypertension, but are no longer the first choice for initial treatment. Beta blockers are competitive antagonists that block the beta receptor sites for epinephrine and norepinephrine. Some block activation of all β-adrenergic receptors and others are selective for either β1, β2 or β3 receptors. β1 receptors are located in the heart and kidneys, β2 receptors are in the lungs, gastrointestinal tract, liver, uterus, vascular smooth muscle, and skeletal muscle, and β3 receptors are located in fat cells. The receptors are found on cells of the heart muscles, smooth muscles, airways, arteries, kidneys, and other tissues that are part of the sympathetic nervous system and lead to stress responses. [3]

The use of β-blockers in noncardiac surgery was associated with a decrease in nonfatal myocardial infarction, but with greater occurrence of nonfatal stroke, bradycardia, and hypotension. Preoperative β-blocker use was not associated with a significant decrease in mortality or hospital complications such as strokes or prolonged ventilation. [4] Clinical guidelines do recommend the use of preoperative β-blockers to reduce atrial fibrillation and can be considered a performance measure for patients undergoing surgery for coronary artery bypass grafting. [5] Long term treatment can decrease the initial blood lactate concentration in patients with severe sepsis or septic shock. [6]

Propranolol has been approved by the FDA to treat essential tremors, with anecdotal evidence that it is more useful for hand tremors than for axial tremors such as those affecting the head and neck. [7] Atenolol is useful for treating abnormally rapid heart rhythms, reducing the force of contraction of the heart muscles and lowering blood pressure, and can be taken with acetaminophen for hypertension, migraine, and myocardial infarction. [8]

One of the hypotheses to explain the antihypertensive effect of β adrenergic blocking drugs is that these drugs increase the sensitivity of the arterial baroreflex. However, this has only received consistent experimental support from animal studies, while human studies have produced diverging results. [9] While beta blockers are frequently used, they are mainly provided in conventional dosage forms. These have limitations such as hepatic first-pass metabolism, variable absorption profiles, higher frequency of administration and poor patient compliance. Transdermal delivery can be an alternative to overcome these drawbacks. [10-12]

Use of physical penetration enhancement strategies such as iontophoresis or microneedle treatment of the skin, can result in an enhanced delivery rate with a minimal lag time. Due to enhanced drug delivery, the patch size can be reduced considerably resulting in less skin reactions and increased patient compliance. In studies involving the transdermal delivery of drugs across the skin, permeation experiments are usually performed under steady-state conditions, and the results are interpreted through the use of steady-state models. However, in the clinical setting, drug delivery occurs from applications which are only 20-50 pm in thickness. Thus, even when a drug is formulated at high percentage composition, only a minute mass of drug is available for diffusion per unit area of the application. Under this condition, permeation is almost certainly a non-steady process. Two concerted, complex sets of events control the delivery of drugs across the skin in this situation. The initial kinetic factors of consequence are those that control movement of mass within the dosage form itself. Of at least equal importance are the factors that control mass transfer across the skin. A model describing drug delivery through the skin from a finite topical application should properly account for all these factors. Such a comprehensive model would aid in the design of in vitro experiments.

The primary advantages of a predictive drug model would be benefits in both time and cost. The model should provide a close estimate as to the extent of drug permeation through the skin to systemic circulation for transdermal drugs. Equations describing the diffusion of a drug through the skin from a topical formulation are based on models that assume the skin is initially devoid of any drug. This is problematic to verify as it takes time during the initial setup of the experiments to spread the formulation onto the membrane and to remove the air bubbles that form in the diffusion cell. During this time between application of the formulation and the start of the experiment, the drug has already begun to diffuse into the membrane, reducing the accuracy of the modeling. With intact skin the diffusion through the stratum corneum usually takes long enough to not be significant with respect to the experiment's setup. However, for skin treated with microperforations in preparation for permeation studies, it is more likely to affect the modeling of the initial conditions of the skin.

2.3 Materials and Methods

In vitro studies were performed with dermatomed human skin (Allosource, Centennial, OH) using vertical static Franz diffusion cells (PermeGear, Hellertown, PA, USA). [13] The frozen skin was thawed at ambient temperature for about 20 minutes. It was then mounted between donor and receptor cells with the epidermis facing the donor. The receiver chamber contained 5 ml of pH 5.2 citrate buffer and was maintained at 37^{o}C with a water circulation jacket that surrounded the lower part of the cell. The diffusion area of the skin was 0.64 cm^2. Beta blocker solution at approximately 1mg/mL was prepared with propranolol, acebutolol, atenolol, and sotalol. Standards were prepared at concentrations from 1 ug/mL to 200 ug/mL. The donor compartment was loaded with 0.5 ml of the beta-blocker solution in citrate buffer and covered with parafilm to prevent evaporation. At regular intervals, 500 µl aliquots from the receptor were collected and replenished with fresh receptor solution. The samples were stored in a refrigerator overnight until analyzed. Iontophoretic studies involved the application of direct current (Keithley Instruments, Inc, Cleveland, Ohio, USA) using Ag as an anode and Ag/AgCl as a cathode. During the iontophoresis studies, the skin was subjected to a current for 6 hours, after which the current was removed. The preliminary drug model used included several rate constants to account for multiple drug transfer pathways in the system, including passive diffusion into the skin (Kp), drug permeation due to microneedle perforation of the skin (Km), drug permeation due to the application of iontophoresis (Ki) and diffusion of the drug out of the skin into the sampling chamber (Kd). The rate constants Kp, Km, Ki, and Kd were modeled with WinNonLin software.

Analysis of the samples was performed using HPLC. The system (Waters Corp., MA, USA) equipped with an autosampler (model 717 plus), an isocratic pump (model 1525) and a PDA UV detector (model 998) was used. The system was interfaced with Empower 2 software for data collection and processing. Samples were eluted on a C8 analytical column, 5µm silica particles, 4.6 mm x 250 mm (Betasil, Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of an 80:20 mixture of 0.1 M ammonium phosphate (pH 3.1) and acetonitrile. A flow rate of 1.0 ml / min was maintained and 60 µl of the sample was spiked onto the column and detected at a wavelength of 205 nm. The cumulative amount of drug permeated through a unit area of skin was plotted as a function of time. The *in vitro* steady-state permeation flux was calculated from the slope of the linear portion of the plot. Analysis of variance was used to assess the statistical significance of the observations. For all statistical analysis, $P < 0.05$ was considered to be significant.

Data from these investigations was then modeled using WinNonLin software. A simple computational model was used to calculate the rate constants into the skin (Kp for passive diffusion and Km for microneedles) and into the collection volume (Kd), and in the case for the iontophoresis, an additional rate constant during the application of iontophoresis (Ki) studies was included.

2.4 Results and Discussion

2.4.1 Results

For the permeation of propranolol through excised human skin, the results are shown in Figure 2.1. The amount permeated through the skin by passive diffusion increased linearly, at a relatively low amount. With dermatomed skin simulating microneedle perforation of the skin, the amount permeated increased linearly at a much higher rate as compared to passive diffusion. With iontophoresis, permeation of propranolol increased rapidly, slowing after the current was turned off at 6 hours. While the current was applied, there was no noticeable difference between skin treated with iontophoresis and with both iontophoresis and microneedle perforation. After the current was shut off, permeation increased linearly similar to passive diffusion in the skin samples that received iontophoresis only, and similar to dermatomed skin in the case of being treated with a combination of both iontophoresis and microneedle perforation.

With acebutolol, similar trends were seen, as shown on Figure 2.2. Extra samples were performed with iontophoresis to confirm the results. Passive diffusion resulted in little to no drug permeation, while microneedle perforated skin showed a linear increase in the amount of acebutolol permeated through the skin. Iontophoretic treatment of the skin showed greater permeation, while skin treated with both iontophoresis and micro-perforation had the greatest amount.

Atenolol diffusion through the skin was also at a negligible level with passive diffusion. Microneedle perforation of the skin resulted in an improvement in transdermal permeation, followed by iontophoresis with an even greater amount. Skin treated with iontophoresis and microneedle perforation had the greatest amount of atenolol permeation, with differences seen even during the initial phase with the current on, as seen in Figure 2.3.

Transdermal permeation of sotalol followed similar treads for each of the permeation enhancement treatments (Figure 2.4). A few of the samples showed a much greater amount of sotalol permeating through the skin. This was suspected to be due to issues in mounting the excised section of skin in the Franz cell apparatus.

Comparisons were also made between each of the beta-blockers by the permeation enhancement treatment that was performed. Differences in passive diffusion, in Figure 2.5, should reflect the different physicochemical properties of the four beta blockers that were investigated (Table 2.1). Comparisons of microneedle treatment, iontophoresis, and iontophoresis with microneedle treatment showed less obvious differences between the beta blockers. (Figures 2.6-2.8)

The amount of beta blockers that was retained in the skin was also recorded. (Table 2.2) The retention of the drugs is related to the lag time between the application of the drug solution to the donor chamber of the Franz cell and when the drug was detected in the receptor chamber. More lipophilic drugs, like propranolol, were retained in greater amounts due to the lipid makeup of the stratum corneum.

During modeling, with few exceptions, regression of the transdermal permeability study data sets converged to provide rate constant values. A single compartment model (Figure 2.9) was used to model the data, with the appropriate rate constants used with regard to the drug permeation method being measured. Two equations were developed for the modeling, the first for the samples examining passive diffusion and microneedled skin, with Ko representing the rate constant due to passive diffusion (Kp) or microneedles (Km), depending on whether or not the skin sample was dermatomed. The second equation was used for the samples that experienced iontophoretic permeation enhancement, alone and in combination with microneedling, with Ki representing the iontophoresis rate constant, and Ko again for passive diffusion or microperforated skin:

Eqn 2.1

$$C = KoT - \frac{Ko}{K}\left(1 - e^{-KT}\right)$$

Eqn 2.2

$$C = KoT - \frac{Ko}{K}\left(1 - e^{-KT}\right) + KiTi - \frac{Ki}{K}\left(1 - e^{-KT}\right)e^{-\mathrm{K(T-Ti)}}$$

where C is the concentration, K is the rate constant, T is the time, and Ti is the time during which current was applied during the iontophoresis experiments.

Two algorithms were used while trying to determine best fit. The Gauss-Newton algorithm for non-linear least squares method was used initially, followed by the Nelder-Mead algorithm, which is a heuristic optimization approach to non-linear problems. The final rate constants determined are as described on Table 2.3. Passive diffusion modeling had correlation values of 0.998 to 0.999, with Kp values 1.08 to 18.1 ug/cm^2/hr, and Kd values ranging from 0.101 to 0.622 ug/cm^2/hr across all drugs. Transdermal enhancement with microneedles was modeled with correlation values of 0.995 to 0.999, Km from 10.9 to 33.8 ug/cm^2/hr and Kd from 0.434 to 7.76 ug/cm^2/hr. Transdermal enhancement with iontophoresis was modeled with correlation values of 0.996 to 0.999, Kp from 1.64 to 11.8 ug/cm^2/hr, Kd from 0.127 to 0.965 ug/cm^2/hr, and Ki from 148 to 200 ug/cm^2/hr. Each beta blocker showed significant increases in transdermal permeation rate constant Km with respect to Kp with the application of microneedles versus passive diffusion alone. While Kp and Kd did not significantly change with the application of iontophoresis, the greater amount of drug detected was accounted for by Ki representing the duration of iontophoresis.

2.4.2 Discussion

The model appears adequate to describe in vitro transdermal absorption for microneedle and iontophoretic treatment. The modeling techniques used in the study were shown to be a good way to examine experimental transdermal data.

Passive diffusion was greatest for propranolol, the drug with the highest partition coefficient. Also seen in Figure 2.5 is that the two beta blockers that are lipophilic, propranolol and acebutolol, pass through the skin more easily than the two hydrophilic beta blockers. The passive diffusion of the beta blockers through microporated skin were the lowest compared with the usage of permeation enhancement techniques, likely due to higher skin partitioning with the more lipophilic beta blockers. [14-17] Microneedle treatment mostly likely contributed to the skin increased the flux and decreased lag time compared to passive diffusion due to the creation of microchannels through the stratum corneum for the drug to directly permeate through. [18] Lipophilic drugs such as the beta blockers can use the microchannels created as aqueous passageways. [19] Iontophoresis enhanced overall drug passage through the skin equally for all drugs. The iontophoresis treatment increased the passive diffusion of acebutolol and decreased that of propranolol. The effect of using iontophoresis as a transdermal enhancer can therefore be seen to be dependent on the physico-chemical properties of the beta blockers. [20, 21] Microneedle treatment was less effective than iontophoresis on transdermal absorption, with a slightly greater effect for propranolol than other drugs. Diffusion out of the skin may or may not be affected by iontophoresis or microneedle treatment, depending on the drug properties. Comparisons between the drug permeation rate constants and the beta blockers' log P values were made to investigate any trends between lipophilicity and drug permeation, as shown in Figure 2.10. It can be seen that there is some relation between lipophilicity and drug permeation during passive diffusion of the drug as well as with microporated skin. However, a relationship between iontophoretic enhancement of drug permeation and the beta blockers' lipophilicity could not be determined.

To account for lag time, equations describing the diffusion of a drug through the skin from a topical formulation were based on models that assume the skin is initially devoid of any drug. As it takes time during the initial setup of the experiments to spread the formulation onto the membrane and to remove the air bubbles that form in the diffusion cell, it is more likely to affect the modeling of the initial conditions of the skin treated with microperforations in preparation for permeation studies. Preliminary drug models were developed, taking into account individual drug properties. These predictive models took into account the volume of distribution (V), clearance (Cl), and elimination rate (ke), as displayed in Figure 2.11.

Passive diffusion Eqn 2.3

$$\frac{Kp}{Cl} + \frac{Kp}{V\,(Ks - Ke)}\ e^{Ks*Tp} - Kp\frac{Ks}{Cl\,(Ks - Ke)}\ e^{Ke*Tp}$$

Microneedles Eqn 2.4

$$\frac{Km}{Cl} + \frac{Km}{V\,(Ksm - Ke)}\ e^{Ksm*T} - Ksm\frac{Km}{Cl\,(Ksm - Ke)}\ e^{Ke*T}$$

Iontophoresis Eqn 2.5

$$\frac{Ki}{Cl} + \frac{Ki}{V\,(Ks - Ke)}\ e^{Ks*Ti} - Ki\frac{Ks}{Cl\,(Ks - Ke)}\ e^{Ke*Ti}$$

Further simulations performed with the derived rate constants predicted plasma concentration values for each of the drugs during the sampling times of their original studies. The values predicted reflected the changes in concentration over time. The predicted plasma concentration values were plotted for each drug. (Figures 2.12-2.15) No model was made of the iontophoresis combined with microneedle effect due to the relative scales of the rate constants of the permeation enhancement techniques individually. In addition to the complexity of the modeling program to account for the application and cessation of the iontophoretic current, the combined equations made the program unworkable.

The model appears adequate to describe in vitro transdermal absorption for microneedle and iontophoretic treatment. The modeling techniques used in the study was shown to be a good way to examine experimental transdermal data. Passive diffusion was greatest for propranolol, the drug with the highest partition coefficient. Iontophoresis enhanced overall drug passage through the skin equally for all drugs. The iontophoresis treatment increased the passive diffusion of acebutolol and decreased that of propranolol. Microneedle treatment was less effective than iontophoresis on transdermal absorption, with a slightly greater effect for propranolol than other drugs. Diffusion out of skin may or may not be affected by iontophoresis or microneedle treatment, depending on the drug properties. Further simulations performed with the derived rate constants predicted plasma concentration values for each of the drugs during the sampling times of their original studies. The values predicted reflected the changes in concentration over time.

Figure 2.1 Permeation of propranolol through skin.

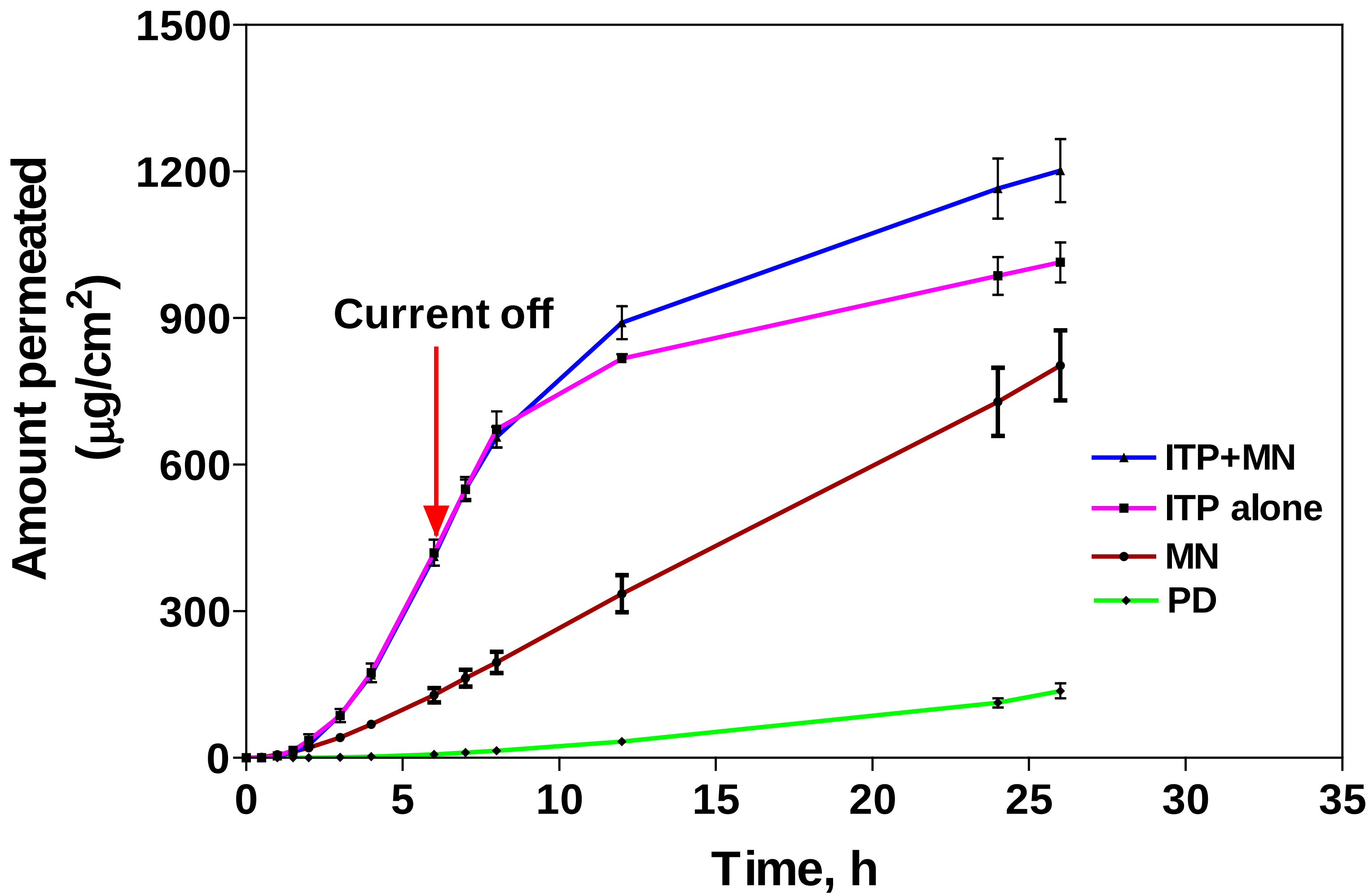


Figure 2.2 Permeation of acebutolol through skin.

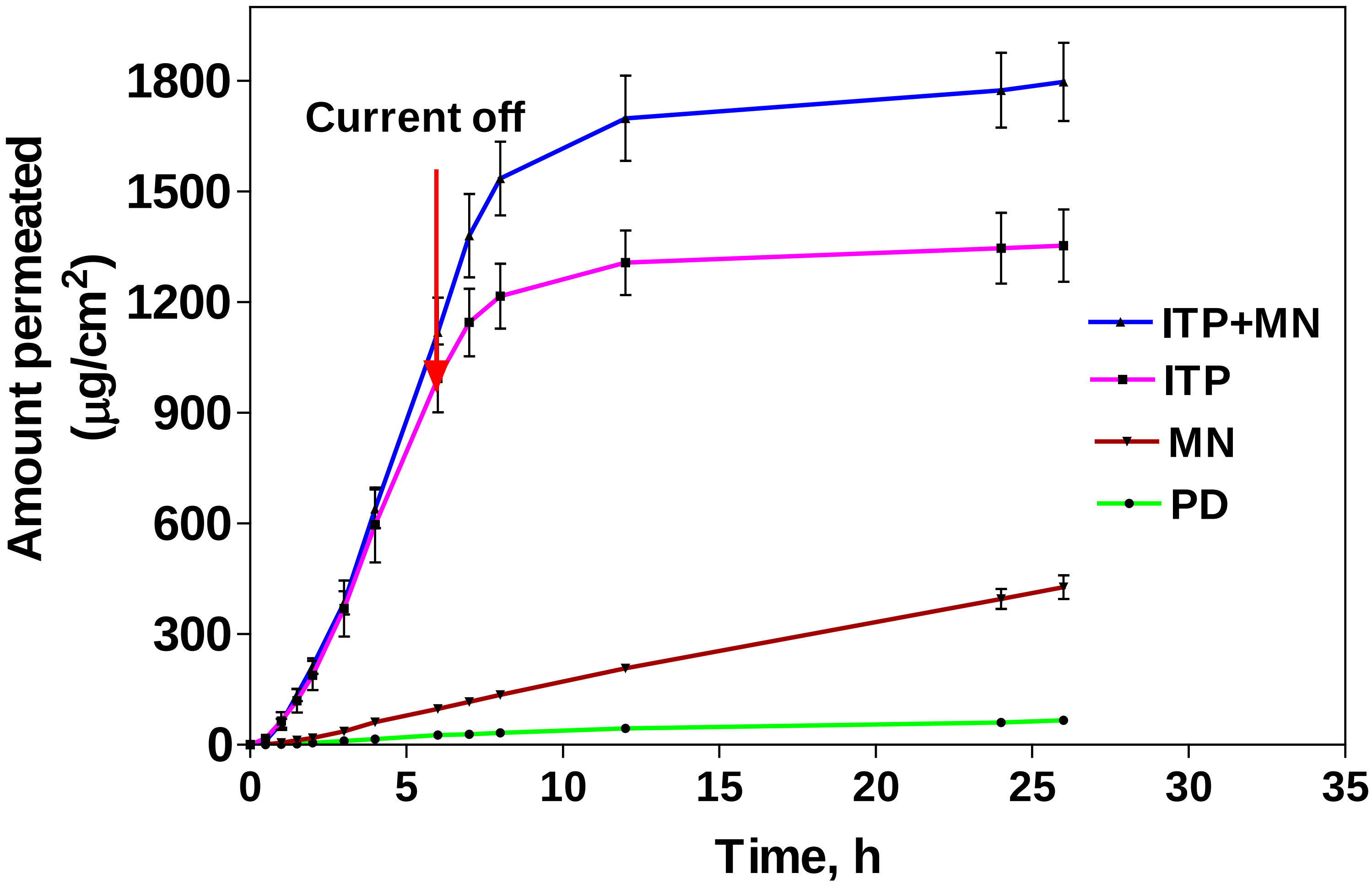


Figure 2.3 Permeation of atenolol through skin.

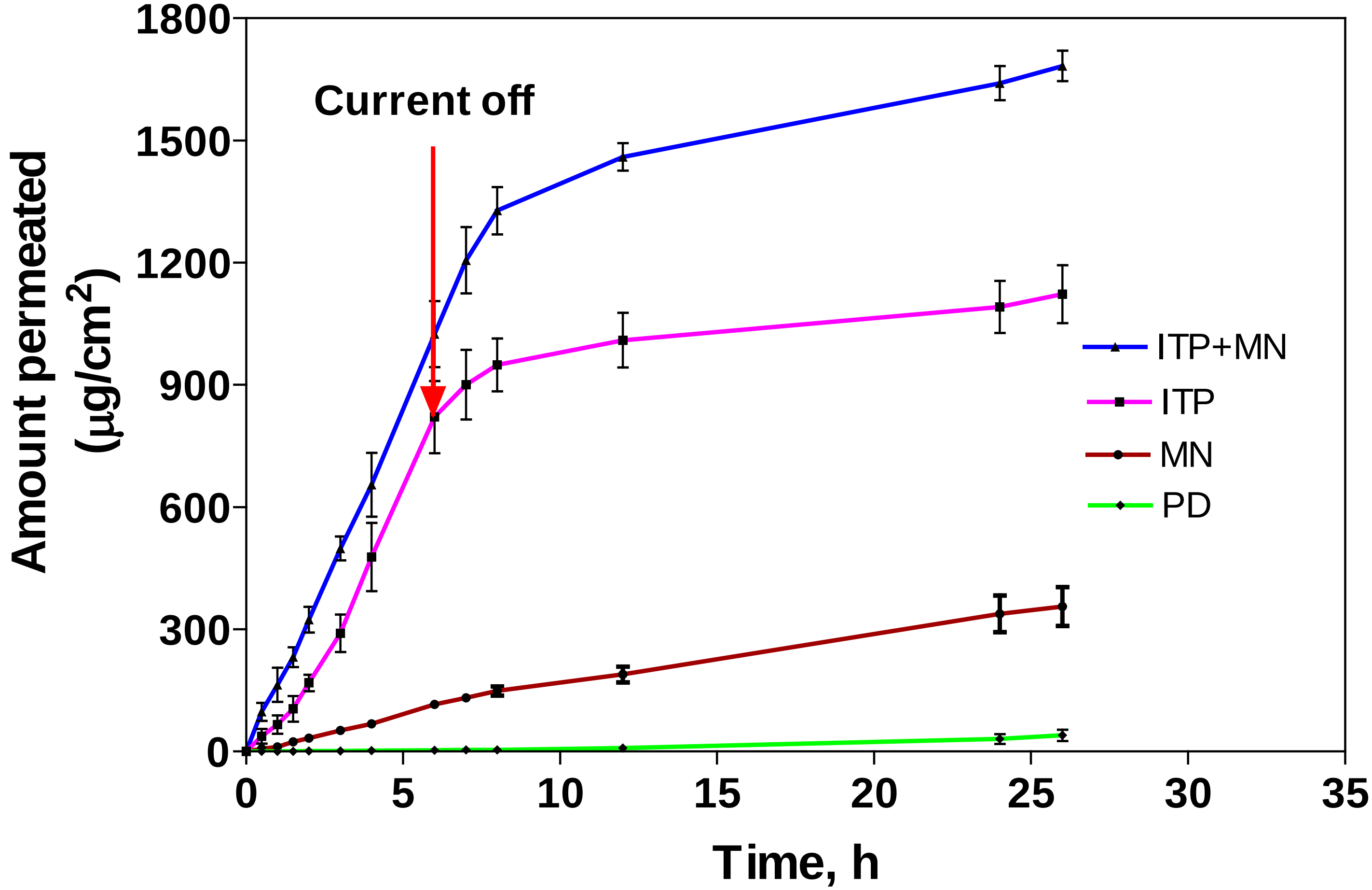


Figure 2.4 Permeation of sotalol through skin.

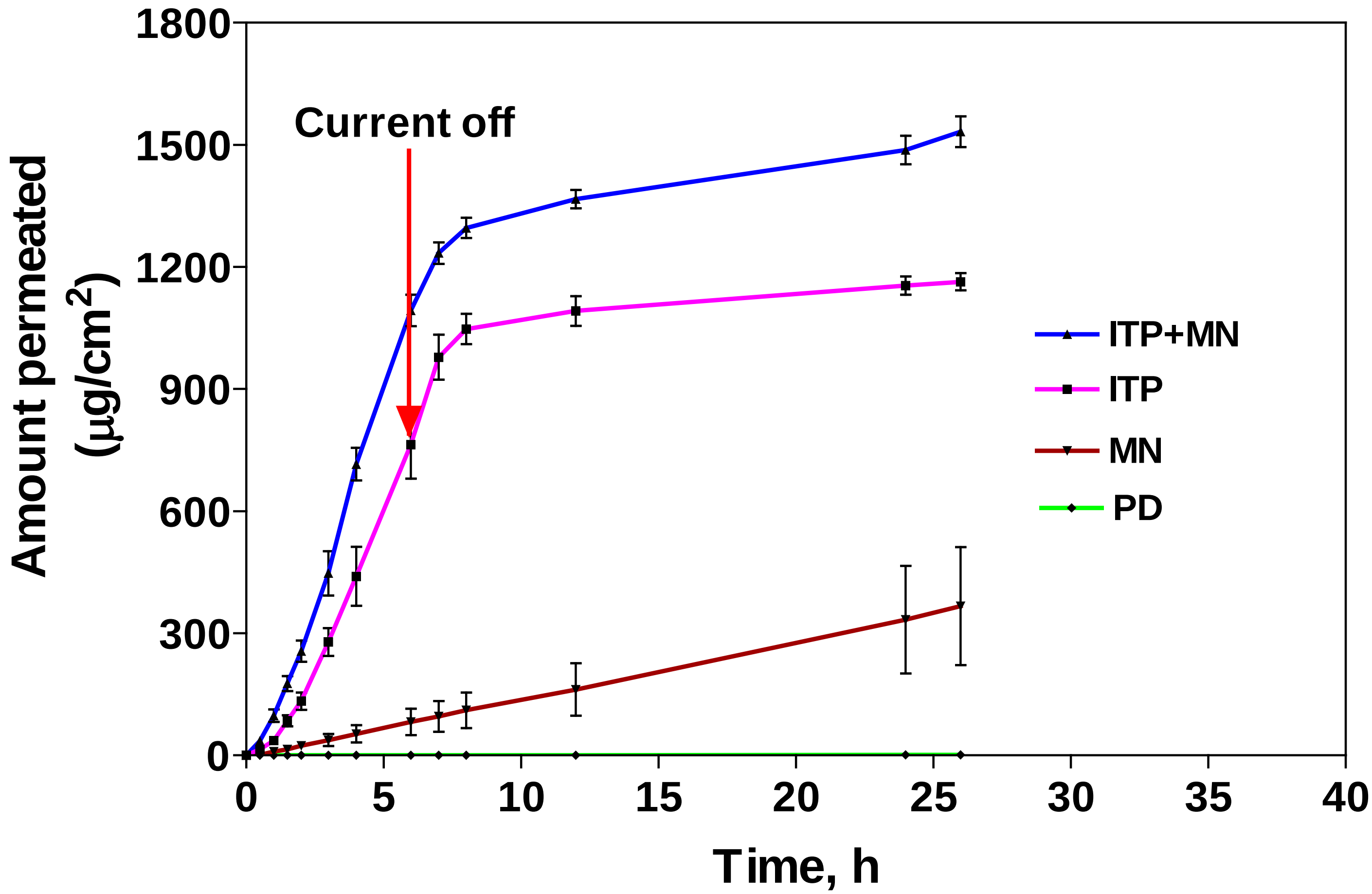


Figure 2.5. Drug permeation of beta blockers by passive diffusion

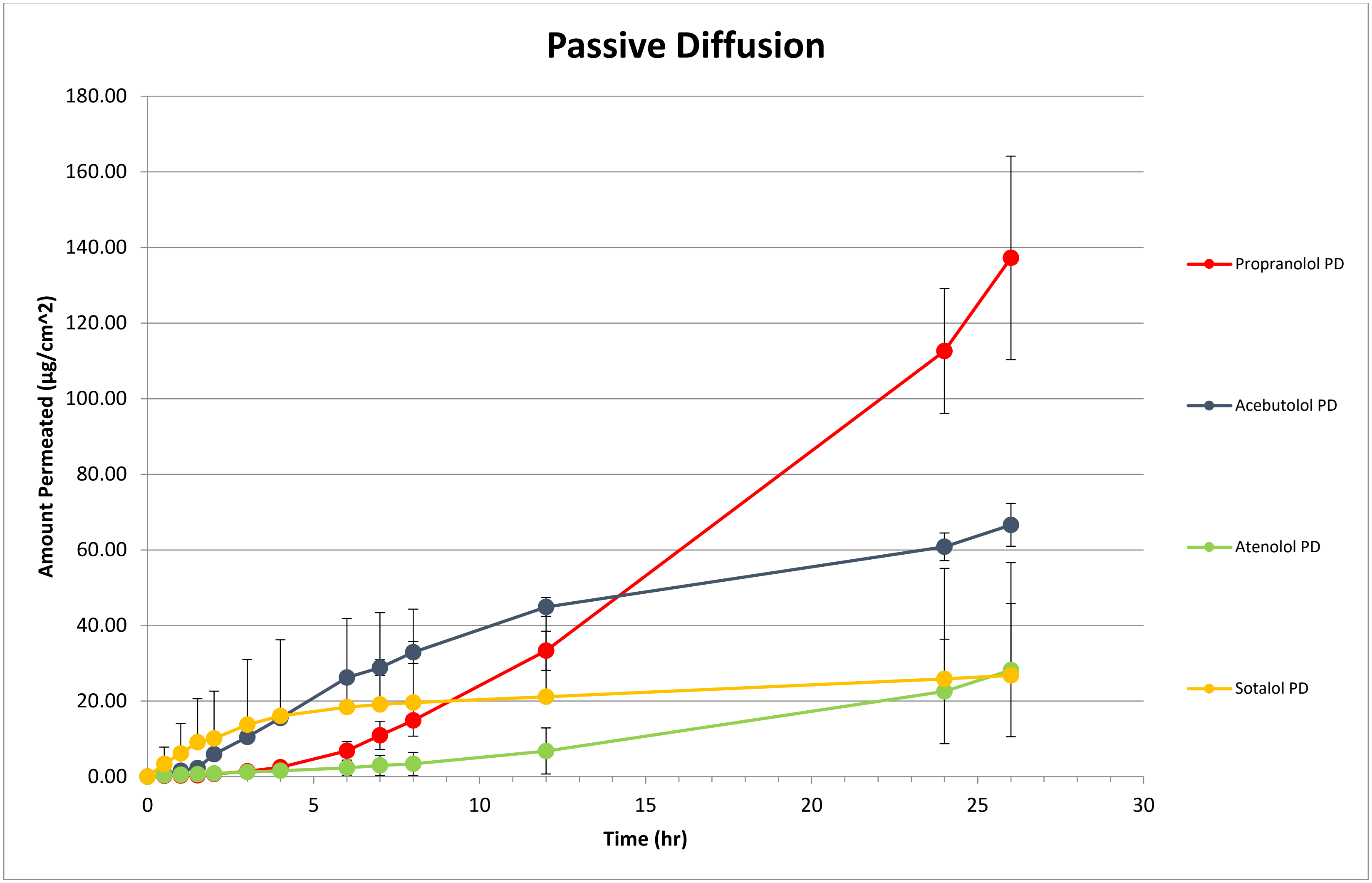


Table 2.1 Physicochemical properties of the beta blockers investigated

Physico-chemical properties and structures of beta blockers

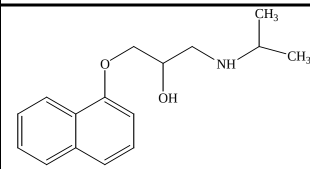
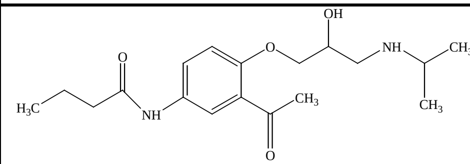
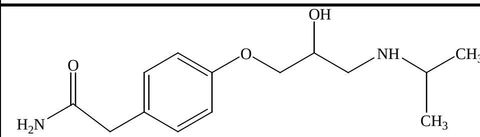
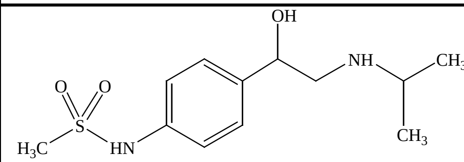
Beta blocker	**Molecular weight (free base)**	**pKa**	**Log P**	**Chemical structure**
Propranolol	259.35	9.26-9.50	2.58-3.65	
Acebutolol	336.43	9.20-9.40	1.43-1.87	
Atenolol	266	9.37-9.63	0.23-0.57	
Sotalol	272.36	9.80 (amine) 8.30 (sulfonamide)	-0.41-0.85	

Figure 2.6. Transdermal drug permeation of beta blockers with microneedle treatment

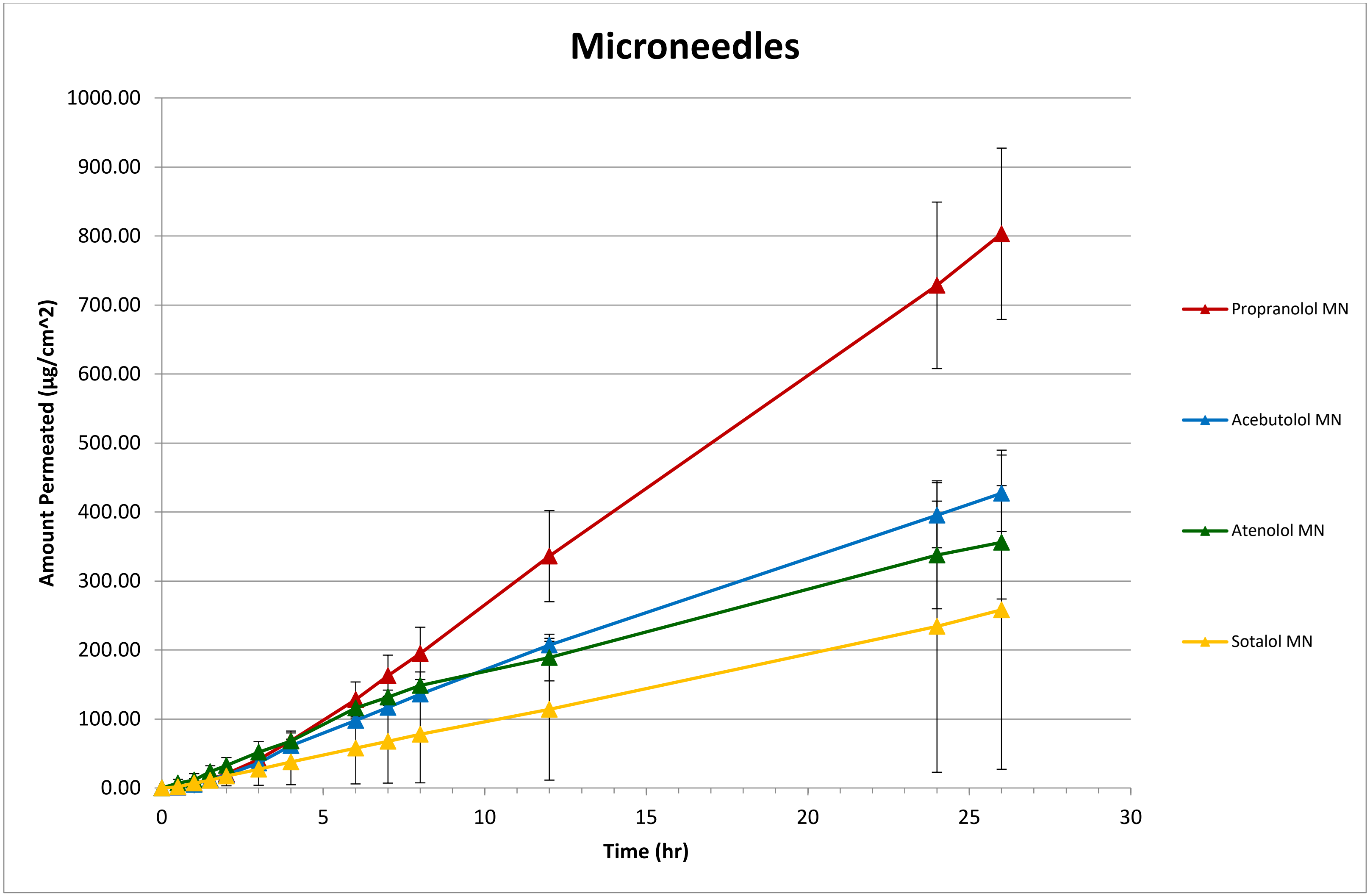


Figure 2.7. Transdermal drug permeation of beta blockers by iontophoresis

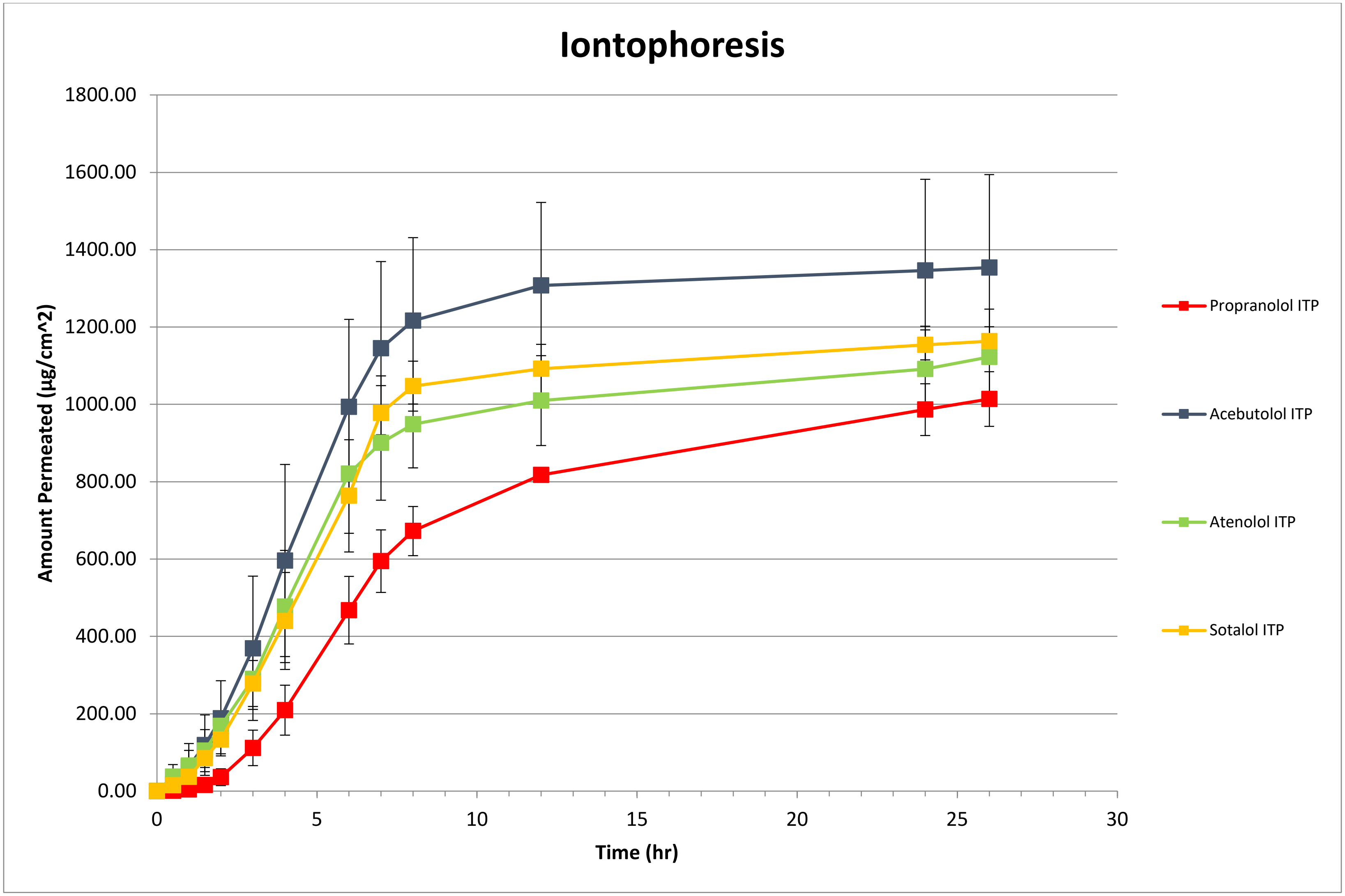


Figure 2.8. Transdermal drug permeation of beta blockers with iontophoresis and microneedle perforation

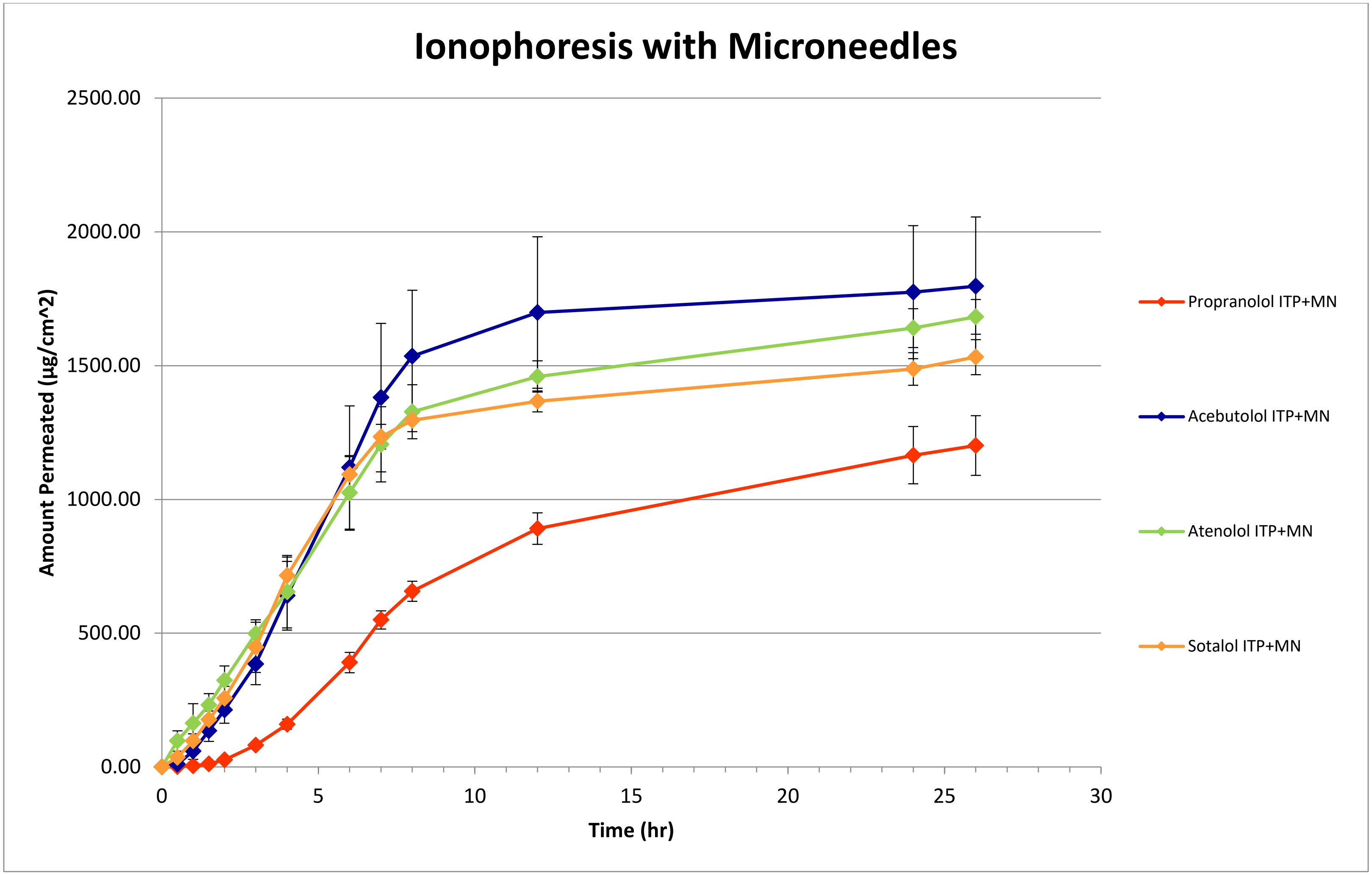


Table 2.2 Skin retention of beta blockers

Skin retention levels (µg of drug per gram of skin) of various beta blockers determined at the end of permeation study

Beta blocker	PD (µg/gm)	MN (µg/gm)	ITP (µg of drug /g of skin)		ITP+MN (µg of drug /g of skin)	
			6 h	26 h	6 h	26 h
PPL	1.67 ± 0.22	1.74 ± 0.10	20.87 ± 3.71***	4.24 ± 1.43**	28.82 ± 2.16***	5.41 ± 0.43**
ACB	0.67 ± 0.20	1.25 ± 0.30	19.79 ± 0.88***	2.18 ± 0.74**	26.77 ± 2.93***	2.00 ± 0.30**
ATL	0.26 ± 0.05	0.38 ± 0.03	17.34 ± 2.59***	2.85 ± 0.03**	20.55 ± 3.71***	3.50 ± 0.44**
SOT	0.28 ± 0.06	0.22 ± 0.07	9.99 ± 3.23***	2.42 ± 0.44**	12.60 ± 1.63***	2.55 ± 0.04**

**P<0.01; ***P<0.001 versus MN and PD of respective drugs

PPL, Propranolol; ACB, Acebutolol, ATL, Atenolol, SOT, Sotalol

Figure 2.9 Single compartment model with the rate constants used for data modeling

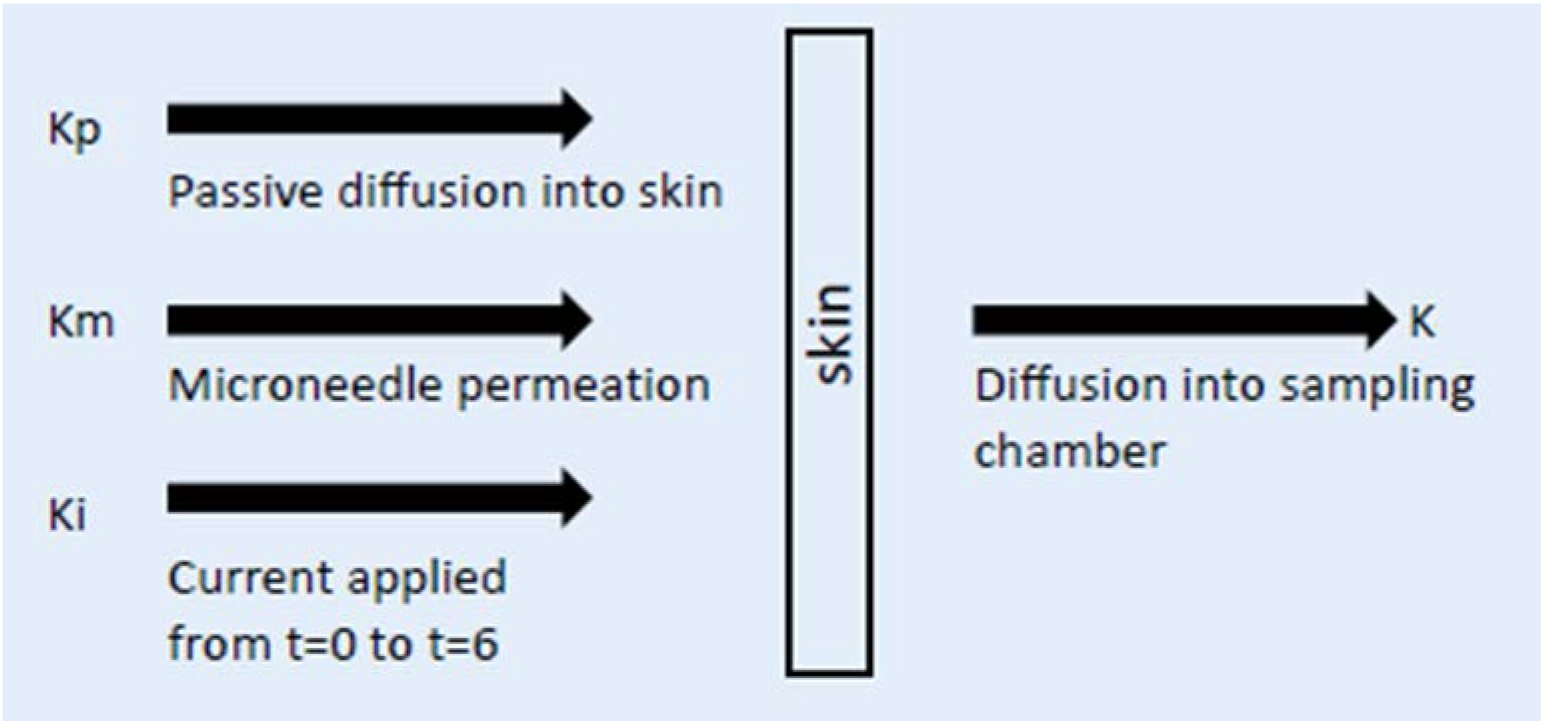


Table 2.3 Rate constants (μg/cm^2/hr) for beta blockers.

Propranolol	Log P	3.115		Vd	280				
Method	Constant	Estimate	SD	Constant	Estimate	SD	Constant	Estimate	SD
Passive Diffusion	K	0.622	0.246	KO	18.10	2.65			
Microneedles	K	0.434	0.062	Km	33.82	5.05			
Iontophoresis	K	0.308	0.109	KO	6.47	3.19	KI	144.5	6.1

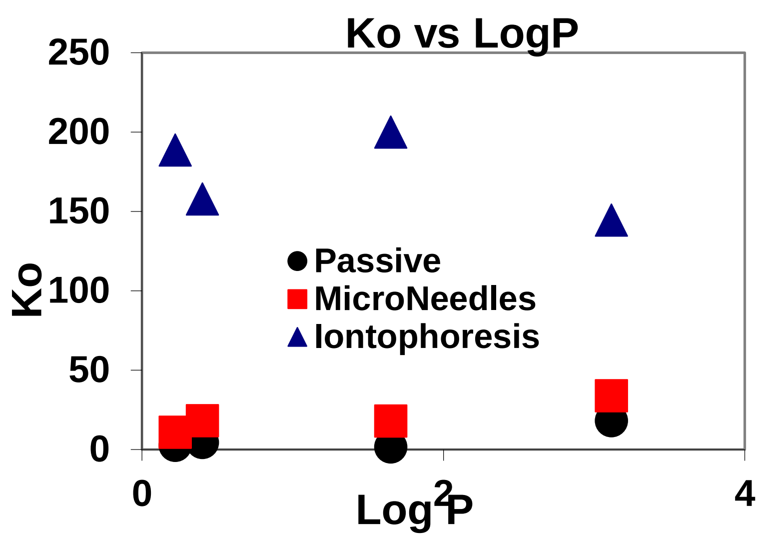
Acebutolol	Log P	1.650		Vd	81.55				
Method	Constant	Estimate	SD	Constant	Estimate	SD	Constant	Estimate	SD
Passive Diffusion	K	0.101	0.049	KO	1.08	0.79			
Microneedles	K	1.899	1.385	Km	17.91	2.39			
Iontophoresis	K	0.965	0.386	KO	11.83	8.73	KI	200.0	0.0

Atenolol	Log P	0.400		Vd	10.5				
Method	Constant	Estimate	SD	Constant	Estimate	SD	Constant	Estimate	SD
Passive Diffusion	K	0.114	0.109	KO	4.39	4.07			
Microneedles	K	7.756		Km	18.09				
Iontophoresis	K	0.922	0.395	KO	6.71	1.41	KI	157.8	15.0

Sotalol	Log P	0.220		Vd	140				
Method	Constant	Estimate	SD	Constant	Estimate	SD	Constant	Estimate	SD
Passive Diffusion	K	0.548		KO	2.62				
Microneedles	K	1.132	0.539	Km	10.87	11.57			
Iontophoresis	K	0.684	0.066	KO	1.64	0.71	KI	188.5	10.0

Figure 2.10 Drug permeation rate constants plotted against Log P values, a) comparisons between passive diffusion, microneedles, and iontophoresis treatments, b) magnified view of just the passive diffusion and microneedle treated values.

a)



b)

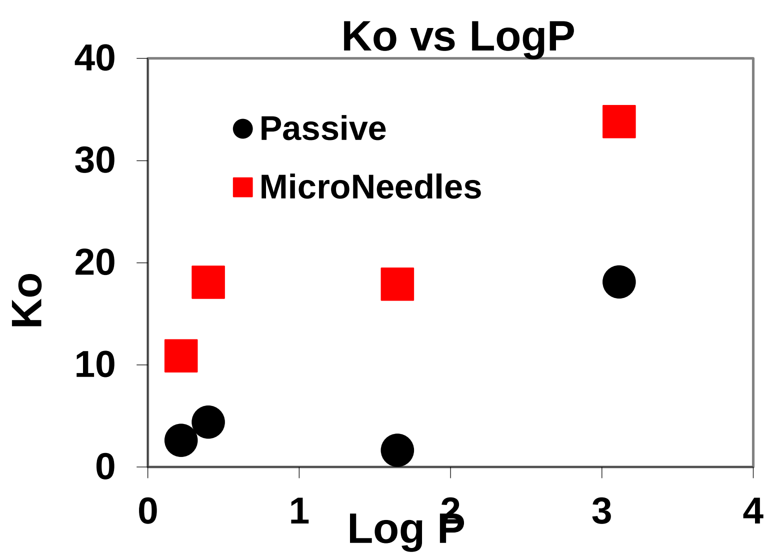


Figure 2.11 Preliminary *in vivo* drug model.

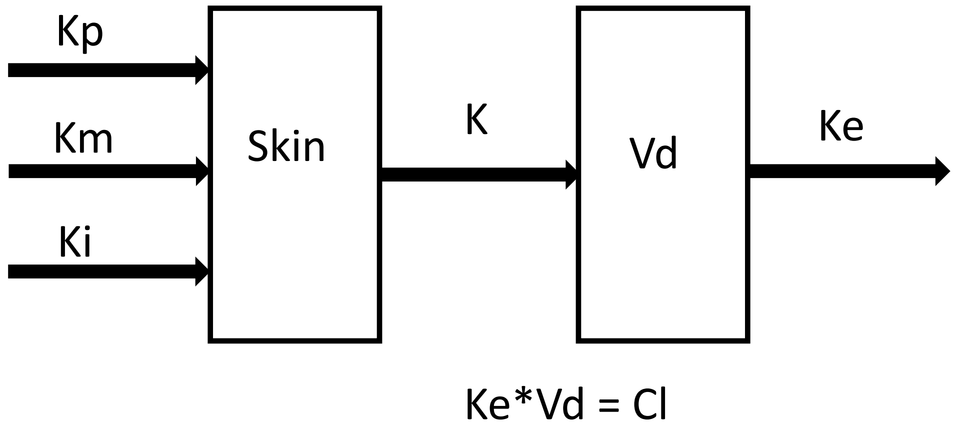


Figure 2.12 Predicted plasma concentration to achieve therapeutic goal within 24 hrs for propranolol

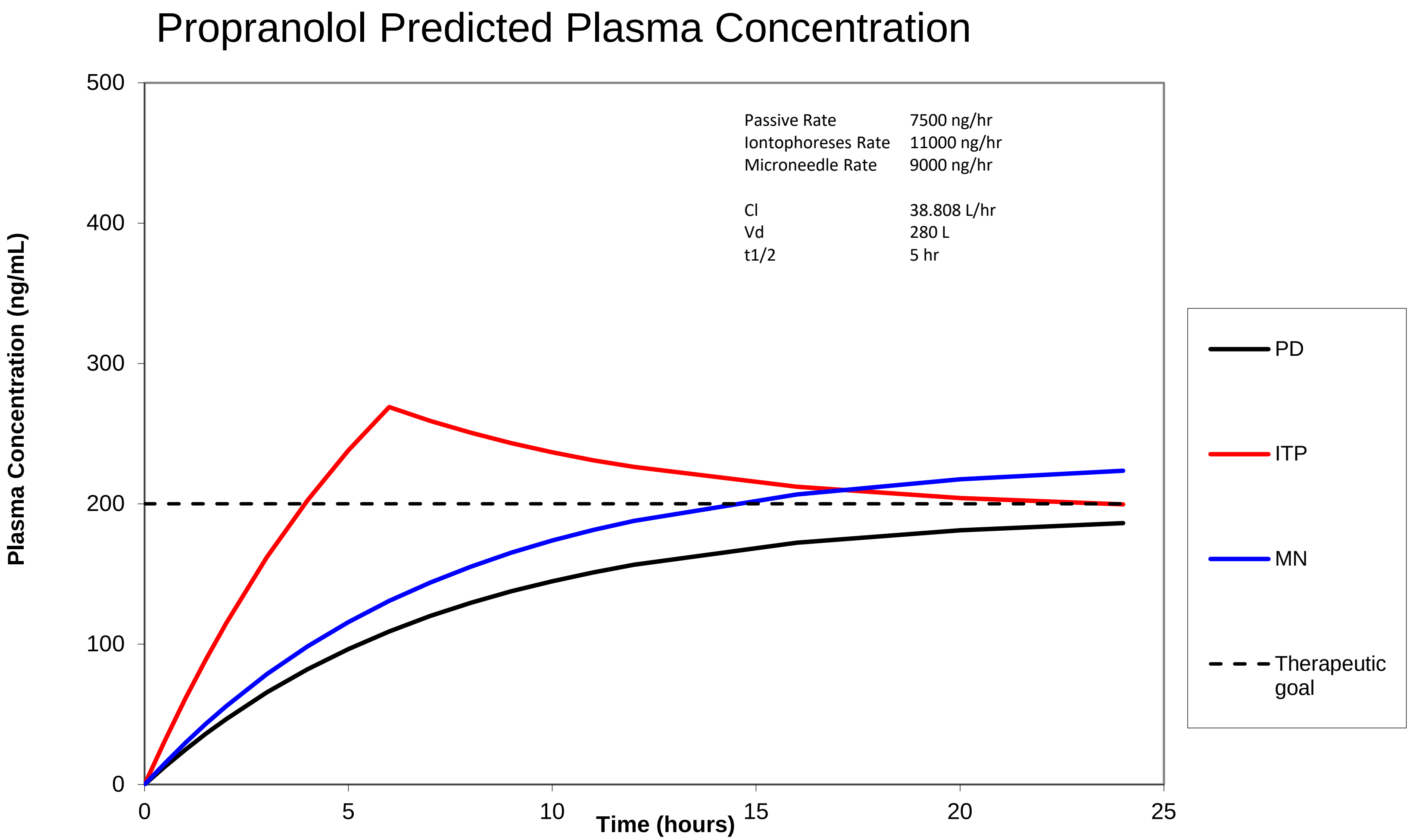


Figure 2.13 Predicted plasma concentration to achieve therapeutic goal within 24 hrs for acebutolol

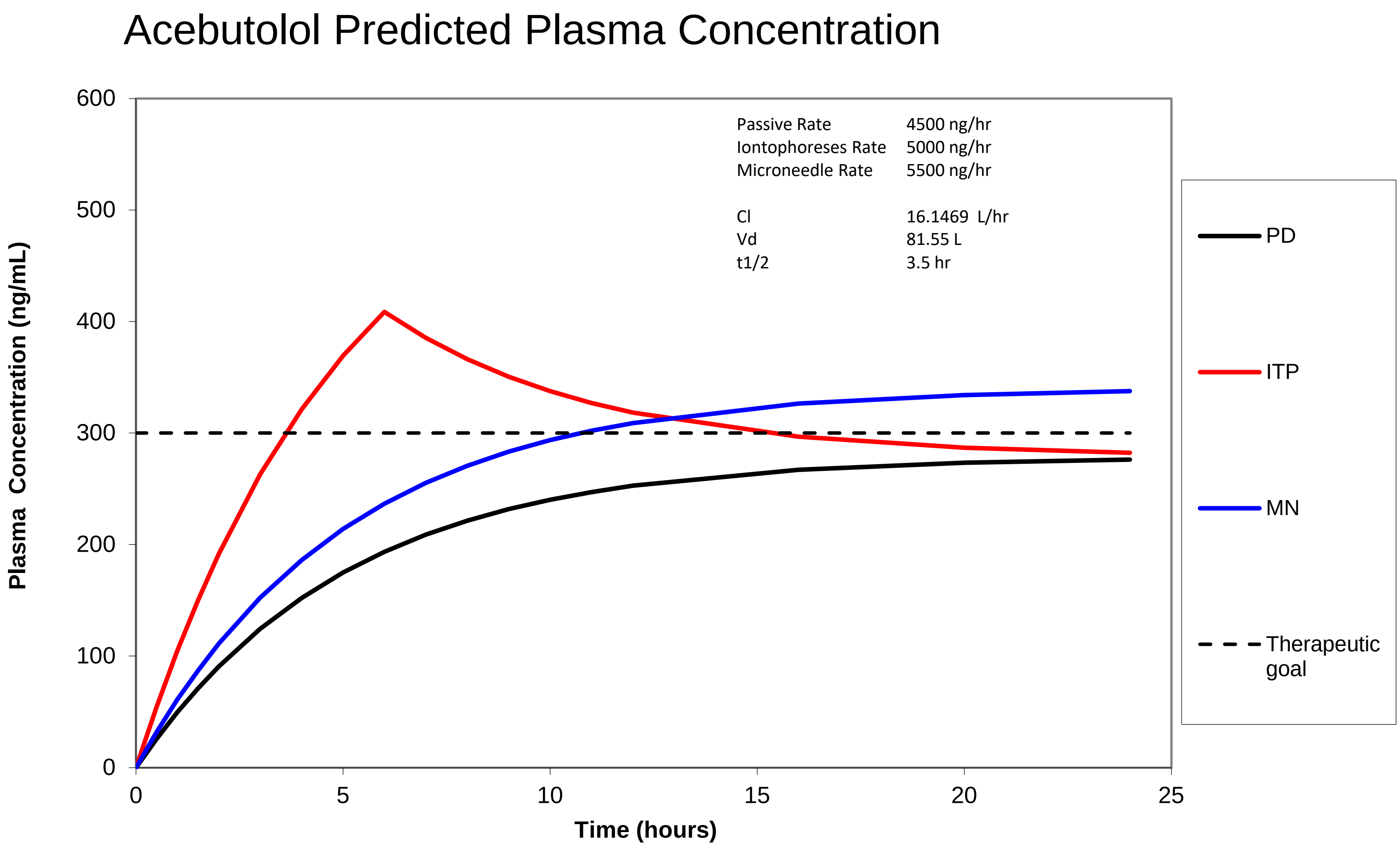


Figure 2.14 Predicted plasma concentration to achieve therapeutic goal within 24 hrs for atenolol

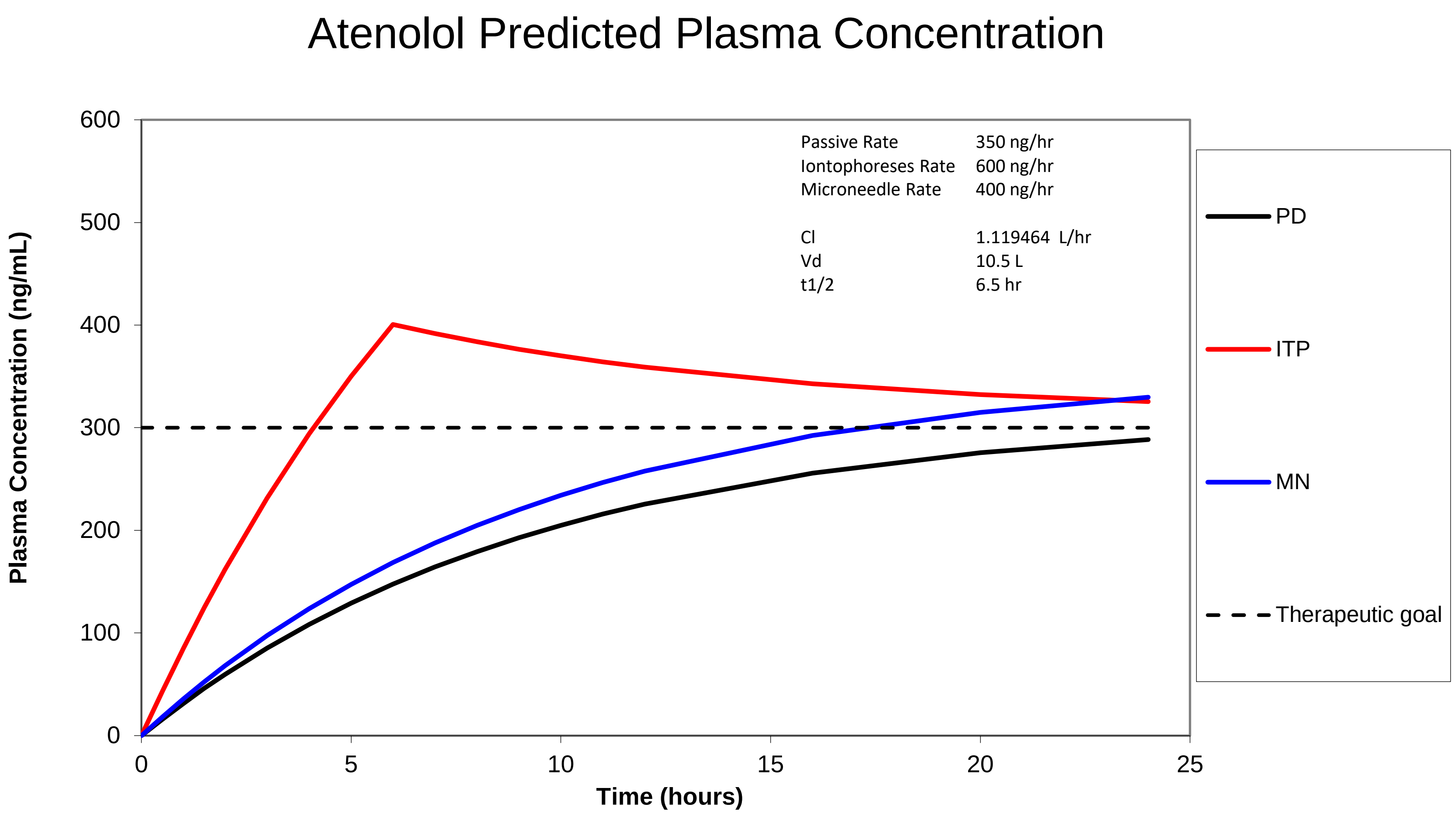
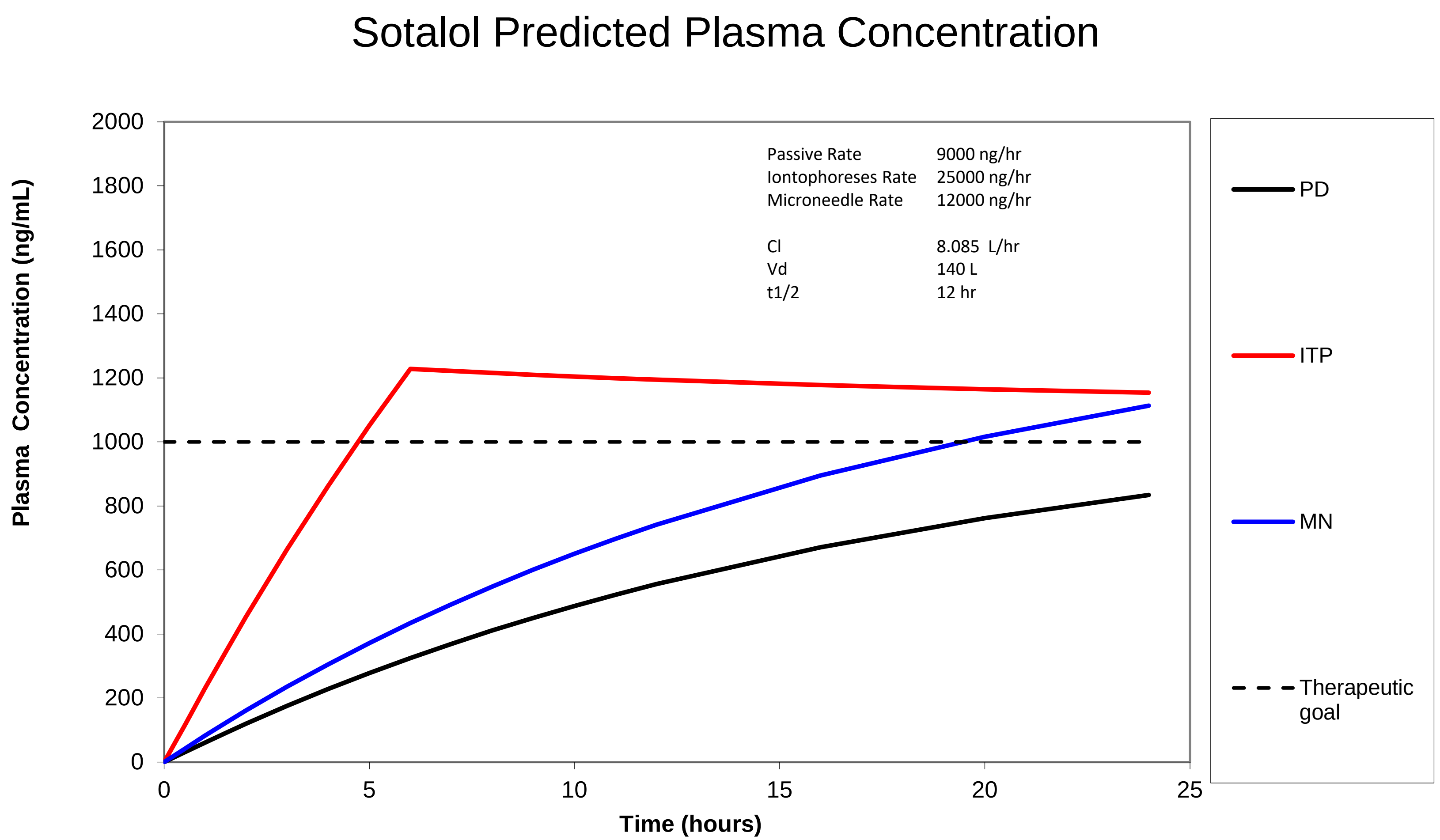


Figure 2.15 Predicted plasma concentration to achieve therapeutic goal within 24 hrs for sotalol



2.5. References

1. Bristow MR. Mechanism of action of beta-blocking agents in heart failure. Am J Cardiol. 1997 (80), 26-40.
2. Sleiman OI, Murin J, Ghanem WM. Beta-blockers in heart failure. Bratislavske lekarske listy. 2001(102), 332-337.
3. Furchgott RF. The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In Catecholamines. Springer Berlin Heidelberg. 1972, 283-335.
4. Kohsaka S, Miyata H, Motomura N, et al. Effects of preoperative β-blocker use on clinical outcomes after coronary artery bypass grafting. Anesthesiology. 2016(124), 45-55.
5. Zhang H, Yuan X, Zhang H, et al. Efficacy of long-term β-blocker therapy for secondary prevention of long-term outcomes after coronary artery bypass grafting surgery. Circulation. 2015(131), 2194-2201.
6. Contenti J, Occelli C, Corraze H, et al. Long-term β-blocker therapy decreases blood lactate concentration in severely septic patients. Crit Care Med. 2015(43), 2616-2622.
7. Justicz N, Hapner ER, Josephs JS, et al. Comparative effectiveness of propranolol and botulinum for the treatment of essential voice tremor. Laryngoscope. 2016(126), 113–117.
8. Rofaeil RR, Kamel MY, Abdelzahar WY. Different effects of selective b1-adrenoceptor antagonists, nebivolol or atenolol in acetaminophen-induced hepatotoxicity of rats. Fund Clin Pharmacol. 2017(31), 165-173.
9. Parati G, Mutti E, Frattola A, et al. β-adrenergic blocking treatment and 24-hour baroreflex sensitivity in essential hypertensive patients. Hypertension. 1994(23), 992-996.

10. Sclar DA, Skaer TL, Chin A, et al. Utility of a transdermal delivery system for antihypertensive therapy. Part 1. Am J Med. 1991(91), 50-56.

11. Sclar DA, Skaer TL, Chin A, et al. Utility of a transdermal delivery system for antihypertensive therapy. Part 2. Am J Med. 1991(91), 57-60

12. Ranade VV. Drug delivery systems. 6. Transdermal drug delivery. J Clin Pharmacol. 1991(31), 401-418.

13. Pawar KR, Smith F, Kolli CS, Babu RJ. Effect of lipophilicity on microneedle-mediated iontophoretic transdermal delivery across human skin in vitro. J Pharm Sci. 2013(102), 3784-3791

14. Abla MJ, Banga AK. Quantification of skin penetration of antioxidants of varying lipophilicity. Int J Cosmet Sci. 2013(35), 19–26.

15. Cross SE, Magnusson BM, Winckle G, Anissimov Y, Roberts MS. Determination of the effect of lipophilicity on the in vitro permeability and tissue reservoir characteristics of topically applied solutes in human skin layers. J Invest Dermatol. 2003(120), 759–764.

16. Mills PC, Magnusson BM, Cross SE. Effect of solute lipophilicity on penetration through canine skin. Aust Vet J. 2003(81), 752–755.

17. Yagi S, Nakayama K, Kurosaki Y, Higaki K, Kimura T. Factors determining drug residence in skin during transdermal absorption: Studies on beta-blocking agents. Biol Pharm Bull 1998(21), 1195–1201.

18. Banks SL, Pinninti RR, Gill HS, et al. Transdermal delivery of naltrexol and skin permeability lifetime after microneedle treatment in hairless guinea pigs. J Pharm Sci. 2010(99), 3072–3080.

19. Wagner H, Kostka KH, Lehr CM, et al. Correlation between stratum corneum/water-partition coefficient and amounts of flufenamic acid penetrated into the stratum corneum. J Pharm Sci. 2002(91), 1915–1921

20. Wang Y, Thakur R, Fan Q, et al. Transdermal iontophoresis: Combination strategies to improve transdermal iontophoretic drug delivery. Eur J Pharm Biopharm. 2005(60), 179–191.

21. Nair A, Reddy C, Jacob S. Delivery of a classical antihypertensive agent through the skin by chemical enhancers and iontophoresis. Skin Res Technol. 2009(15), 187-194.

3. Population Pharmacokinetics in Cutaneous Leishmaniasis Treatment

3.1 Abstract

A pharmacokinetic study was conducted in Peru and Panama in two phase 2 clinical trials, with two topical cream formulations, one with one aminoglycoside (PARO) and the other being the combination of two aminoglycosides (PARO/G). A basic pharmacokinetic analysis of this study was performed. [1]

The study design consisted of 26 patients at two sites who received either 15% w/w PARO or a 15%/0.5% w/w PARO/G cream for cutaneous leishmaniasis (CL). In the study, the PARO or PARO/G topical cream was applied once daily following surface cleaning of the skin lesion. The pharmacokinetics of the drugs were determined from a series of plasma concentrations drawn on day 1 and 20, shortly before application of the cream and in the minutes and hours immediately afterwards, as well as single samples periodically in between. The data set contained 573 observations, with greater than 1100 dosing events. From both of the creams, PARO pharmacokinetic results were determined from traditional noncompartment analysis. Although the pharmacokinetics and safety of both antibiotics separately were well known, it had not been determined to what extent they would be absorbed into the systemic circulation when applied to the open skin wounds of CL lesions. From the results, one aminoglycoside showed a wide range of variation, and many of the results were below the lower limit of quantification. No pharmacokinetic analysis was performed with the other as most of the plasma concentrations were below the limits of detection. An accumulation effect was another factor to consider in the study, which could result from a skin depot developing from continuous dosing, or from binding and saturation of the skin layers due to multiple doses.

The sources and correlations of variability in drug concentrations among the target patient population can be used to determine population pharmacokinetics. Certain patient features, such as body weight, excretory and metabolic functions, and the presence of other therapies, can regularly alter dose-concentration relationships. The collection of relevant pharmacokinetic information in patients who are representative of the target population can help develop a quantitative estimation of the magnitude of the unexplained variability in the patient population.

The population PK program NONMEM was used to develop a model from the study data. Data values below quantification limits were addressed, and several models were attempted. Models were selected based on lowering the objective function, standard error of the estimates, and residual plots of covariates.

A population pharmacokinetic model that describes transdermal PARO absorption and accounts for increased drug accumulation during multiple dosing was developed. The increased extent of PARO transdermal absorption could be predicted based on a tissue depot or saturable tissue binding model. Including lesion size as a covariate reduced inter- and intra-subject variability and improved the parameter estimates.

3.2 Introduction

Leishmaniasis is a parasitic disease caused by a protozoa which affects millions of people around the world in tropical and subtropical areas. [2] It can cause a wide range of effects in patients, from cutaneous lesions to severe organ damage. The incidence of CL is estimated around 0.7 to 1.2 million cases a year, with several thousand in the United States. [3] While several drugs have been identified to treat leishmaniasis, most have limitations, such as high toxicity in humans or development of resistance in the parasites. [4, 5]

Currently, there are no FDA approved drugs for the treatment of leishmaniasis in the United States. Several topical formulations have been investigated, with the primary drug being paromomycin (PARO). A new combination of two aminoglycosides, PARO and gentamicin (G), in a topical cream has been investigated for the treatment of CL, where the skin lesions resulting from the disease were approximately 2 to 5 cm in diameter.

Population pharmacokinetics is the study of the sources and correlations of variability in drug concentrations among individuals who are the target patient population receiving clinically relevant doses of a drug of interest. Certain patient features, such as body weight, excretory and metabolic functions, and the presence of other therapies, can regularly alter dose-concentration relationships. It involves the collection of relevant pharmacokinetic information in patients who are representative of the target population to be treated with the drug, the identification and measurement of variability during drug development and evaluation, the explanation of variability by identifying factors that may influence the pharmacokinetic behavior of a drug, and the quantitative estimation of the magnitude of the unexplained variability in the patient population. [6]

A search of population pharmacokinetics applied to transdermal delivery of drugs shows some fentanyl and nicotine studies, but all are single studies and in some cases modeled the pharmacological effects instead of plasma concentrations. Although a few studies mention increased systemic bioavailability from transdermal patches on multiple administration, there are no reports of determining or modeling this effect. In addition, all of the transdermal studies examine intact skin not broken or lesioned skin. [7-9]

3.3. Methods

This study included 26 patients who received either 15% paromomycin (PARO) or 15% paromomycin/0.5% gentamicin (PARO/G) cream. Patients received the cream once daily for 20 days. Infected areas were cleaned prior to each application. On days 1 and 20, blood samples were collected prior to topical cream application and at 0.5, 1, 2, 3, 4, 8, 12, and 24 h after completion of cream application.

The basic pharmacokinetic parameters were recalculated to include full datasets for each subject (Table 3.1). The dose is in grams of topical cream which correspond to the following PARO amount based on a 15% cream. The $t_{1/2}$ of PARO following IM and IV administration is 2-3 hours. The observed $t_{1/2}$ on both days for both treatments was longer, 3.4 to 7 hr. This suggest prolonged absorption where the elimination $t_{1/2}$ is shorter than the absorption $t_{1/2}$. Figures 3.1 and 3.2, respectively, show plasma concentrations for PARO and PARO/G on Day 1 and Day 20. The plasma concentrations were highly variable with no significant difference between the two creams.

Accumulation of PARO due to multiple dosing was also considered.. It was calculated as the ratio of 24 hour AUC values corrected for dose for day 20 divided by day 1. This accumulation factor could not be calculated for all subjects, since several displayed plasma concentrations below the lower limits of quantification (LLOQ). If the accumulation was based on either the elimination of 2.5 hours or observed $t_{1/2}$ of 6 hours, accumulation with once daily dosing would be minimal. Instead, it was found to be 5 to 6 fold higher (Table 3.2). This suggested that the extent of absorption is greater on Day 20 than day 1. This could then be modeled as a depot effect or a tissue binding effect.

The goal was to develop a population pharmacokinetic model to describe the transdermal absorption of PARO. The challenges included approximately 30% of the samples below the

LLOQ. In addition, the study did not include IV or IM results to determine bioavailability. Parameters from previous studies were therefore used [10],

$$Cl\ (L/hr) = 7.29 * BSA/1.73$$

$$V\ (L) = 0.375 * WT$$

with BSA as body surface area in m^2 and WT weight in kg. Furthermore, as the cutaneous lesion area was cleaned of any excess or remaining topical cream prior to the administration of the daily dose, the removal of unabsorbed drug presents a significant coding issue.

Data sets were created that contained 573 plasma sample times and 2131 events which included 1100 dosing events and 550 additional time events to monitor and predict the drug amount in the cream and skin. The population PK program NONMEM, for non-linear mixed effect modeling, has many routines for developing models and the option for differential equations was selected to allow the most flexibility in model development. Complete model analysis requires completing the covariance step in the modeling and this was included. Convergence of the model with Laplacian was recommended for complex differential equation models. First, the issues of the dose removal and the BQL values were dealt with using a simple multiple dose model. The programming language and the dataset events were modified to empty the dosing compartment 3 minutes before the next dose was administered.

To address the BQL data points, Beal's methods [11] were considered (Table 3.3). Some of these methods are intended to deal with small numbers of samples below BQL. This is particularly true for M1, M2, and M7 and were not considered since 30% of the sample times were BQL. Reviews of these methods suggest that M3 appears best and this approach was initially used. M4 is more for missed sample times. M5 and M6 are more for examining after a model is developed. By the M3 method, the BQL observations are retained but treated as censored observations with the assumption that their predicted values are normally distributed. Then the likelihood for all the values, including the likelihood of the BQL values are in fact BQL, are maximized with regard to the model parameters.

A variety of base models with modifications were proposed, developed into code, and then evaluated. Evaluation and selection of models was based on the lowering of the objective function, SE of the estimates, plots of predicted and observed concentration, and the residuals as a function of time. In the case of assessing improvement based upon the objective function, a chi-square test ($\alpha<0.01$) of the log-likelihood difference between objective functions with the degrees of freedom being equal to the difference in the number of parameters between the models was used. This corresponds to an objective function change of 3.84 for the addition of 1 parameter. Inter-individual variation was determined by an exponential error model:

$$\mathrm{IIV}\ (\%) = \sqrt{\eta j} \times 100$$

$$\mathrm{Pj} = \theta \times e^{\eta j}$$

with inter-subject variability IIV, individual subject estimate Pj, population geometric mean θ, and individual specific random error or variability (ETA) ηj. Intra-subject error was calculated as:

$$\mathrm{Cij} = \mathrm{Cmij} + \mathrm{Cmij} * \varepsilon 1\mathrm{ij} + \varepsilon 2\mathrm{ij}$$

with observed concentration in the jth subject for the ith measured observation Cij, the ith concentration predicted by the model in the jth subject Cmij, the residual error terms for the jth individual at the ith measured observation based on a constant coefficient of variation (CV) and additive (AD) error models ε1ij and ε2ij, and the intra-subject residual variability IRV being both CV and AD.

Once an appropriate model was identified, the covariance between population model parameters and demographics was examined. The continuous and categorical patient demographics included were age, WT, BSA, treatment, site, lesion size, and creatinine clearance. As an example with WT and lesion size with a scaling factor (ALLO), covariates were included in the model as modifiers to model parameters as:

$$\mathrm{Pj} = \theta \times \left(\frac{\mathrm{WTj}}{\mathrm{median\ WT}}\right)^{\mathrm{ALLO}}$$

In the equation, the terms are previously described and the covariate is included as a fraction considering the median value for that parameter.

Table 3.1. Pharmacokinetic Parameters for PARO in Cutaneous Leishmaniasis Patients

Day	Treatment	Subject	Dose (G)	CumDose (G)	Cmax (ng/mL)	Tmax (hr)	AUC24 (ng*hr/mL)	t1/2 (hr)
		N	**13**	**13**	**13**	**10**	**10**	**9**
1	**PARO**	**Mean**	**2.1**	**2.1**	**337**	**2.5**	**2318**	**3.40**
		CV%	**69.8**	**69.8**	**108.6**	**38.9**	**108.1**	**19.8**
		GeoMean	**1.7**	**1.7**	**166**	**2.3**	**1220**	**3.34**
		N	**13**	**13**	**13**	**9**	**10**	**6**
1	**PARO/G**	**Mean**	**2.6**	**2.6**	**139**	**2.9**	**1160**	**4.15**
		CV%	**54.7**	**54.7**	**74.1**	**76.3**	**79.7**	**35.2**
		GeoMean	**2.3**	**2.3**	**96**	**2.3**	**900**	**3.96**
		N	**13**	**13**	**13**	**13**	**13**	**11**
20	**PARO**	**Mean**	**3.4**	**53.3**	**1002**	**3.1**	**8575**	**5.96**
		CV%	**50.4**	**40.1**	**74.8**	**73.0**	**84.8**	**43.5**
		GeoMean	**3.0**	**48.6**	**751**	**2.5**	**6173**	**5.53**
		N	**13**	**13**	**13**	**12**	**13**	**12**
20	**PARO/G**	**Mean**	**3.4**	**57.8**	**662**	**2.5**	**6083**	**6.94**
		CV%	**55.4**	**43.7**	**73.0**	**53.1**	**63.8**	**47.6**
		GeoMean	**2.8**	**52.1**	**454**	**2.1**	**4385**	**6.21**

Figure 3.1. PARO plasma concentration on Day 1. PARO cream (○) and PARO/G cream(●).

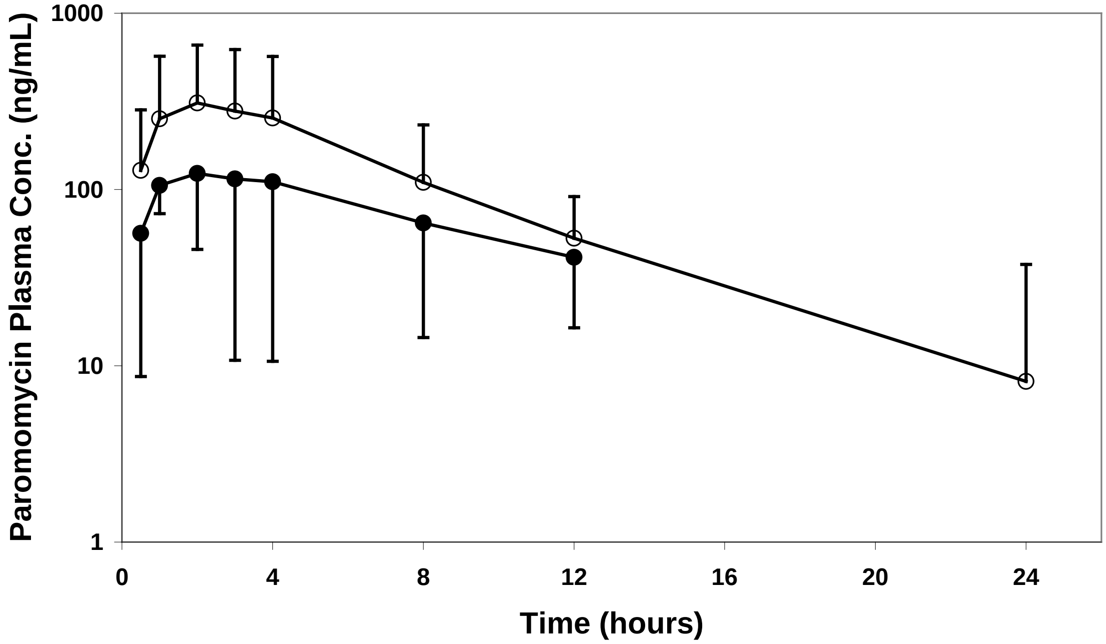


Figure 3.2. PARO plasma concentration on Day 20. PARO cream (○) and PARO/G cream(●).

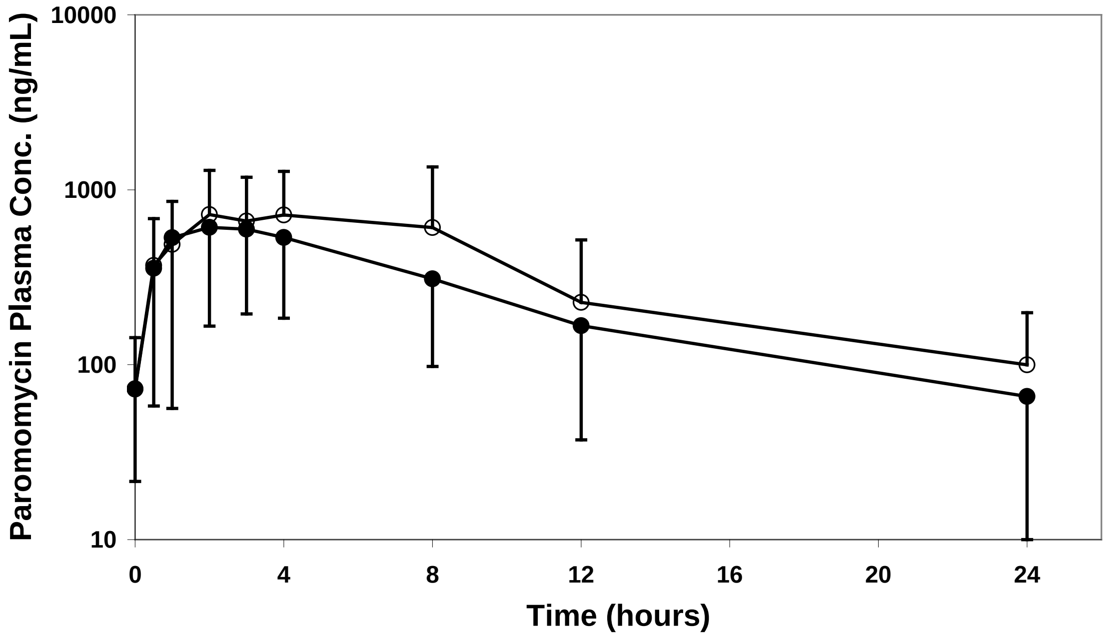


Table 3.2. PARO accumulation during multiple dosing

Day	Treatment	Subject	Dose (G)	CumDose (G)	Day 1 AUC24 (ng*hr/mL)	Day 1 AUC/D (hr/ML)	Day 20 AUC24 (ng*hr/mL)	Day 20 AUC/D (hr/ML)	Accum.
		N	13	13	13	13	13	13	10
20	PARO	Mean	3.4	53.3	2318	6.82	8575	15.6	6.26
		CV%	50.4	40.1	108.1	69.7	84.8	53.3	149.2
		GeoMean	3.0	48.6	1220	4.77	6173	13.8	3.21
		N	13	13	10	13	13	13	9
20	PARO/G	Mean	3.4	57.8	1160	2.99	6083	12.9	4.94
		CV%	55.4	43.7	79.7	47.9	63.8	74.4	89.1
		GeoMean	2.8	52.1	899.6	2.61	4385	10.3	3.82

Table 3.3. Recommended methods by Beal. [11]

M1	Ignore missing values
M2	Likelihood assumes all values are censored at LLOQ
M3	Estimate likelihood at times measurements are BLQ
M4	Like M3 but also assume measurements are ≥ 0
M5	Replace all BLQ with LLOQ/2
M6	Replace first BLQ with LLOQ/2, ignore others
M7	Replace all BLQ with zero

3.4. Results and Discussion

The first simple model developed was A, described with Figure 3.3a. This was a simple absorption model which include corrections for the removed doses and BQL values. Both the $t_{1/2abs}$ and $t_{1/2elim}$ with a geometric mean of 6.4 and 5.5 hours were longer than the reported $t_{1/2}$ of 2.6 hours. This was consistent with noncompartmental analysis. The fact that neither $t_{1/2}$'s are close to 2.6 means that it will be difficult to obtain a good estimation of ka. Also, the extent of absorption could not be estimated without using some adopted PARO PK parameters. Due to the high subject variability along with possible model misdefinition, the modeling program could not be made to fully converge (Table 3.4).

To test how well the dose correction approach and the BQL methods work with this dataset, a simple 1st order absorption multiple dose modeling was applied (Figure 3.3b). Then in order to permit the estimates of extent and rates of absorption, patient specific PK parameters for V and Cl were included in the model. In addition, day 1 and day 20 were modeled separately to evaluate the extent of absorption differences between those days. (Table 3.5) The ka rates were of the same magnitude but day 1 was a better estimate and inter-subject variability was about the same and very high. (100 and 119%). For F, as expected, the F was higher on day 20 with F = 2.5 % on Day 1 and 9.5% on Day 20. On day 1 and 20, the estimate of inter-subject variability was high and difficult to estimate. The variability is seen in the fitted plots, Figure 3.4. The predictions without observation are predicted based on likelihood approach applied by M3 for concentrations below LLOQ in patients.

The next model included a skin depot, described in Figure 3.3c. It was necessary to include a direct absorption step as well as depot absorption. This was because absorption was too rapid for purely a depot absorption since peak plasma concentrations were seen as quickly as 2-3 hr.

This may be due to the higher permeability of damaged, lesioned skin. This model had an improved objective function but IIV was still large. (Table 3.6) Figure 3.5a shows predicted concentration for each subject including IIV and IRV as IPRED and predictions based on simply using the mean absorption parameter estimate PRED. Even though data could be fitted to E2, plots of predicted versus observed in Figure 3.5b showed poor predictions at high concentration. The diagnostic measures of covariance and correlation suggested that the model was over parameterized. It became necessary to find a simpler model with less covariate parameters

Another model or approach which could explain the higher F on multiple doses would be a tissue binding model (Figure 3.3d). A tissue or skin saturation model was tried, where binding of drug to the skin would explain the lower extent of absorption on the first dose. The binding was described by Bmax, the maximum drug bound in the skin, and Kb, the amount when the sites are 50% saturated. In the model, the drug is absorbed into the skin and the passage to the body is driven by the free drug in the tissue. In order to predict free drug in the tissue, the following equation was incorporated into the model and code, with Xt = total drug and Xfree = free/unbound drug:

$$X_{free} = X_t - \left[0.5\left(\frac{1}{K_b + X_t + B_{max}}\right) + 0.5\sqrt{\frac{1}{(K_b + X_t + B_{max})^2} - 4B_{max}X_t}\right]$$

This equation was described and reported for a single binding site. [12, 13] None of these models fully converged. (Table 3.7) Estimates of the amount bound and the binding constant were quite low. Based on numerous modeling attempts it was concluded that there was not enough concentration/time information to model both Bmax and Kd.

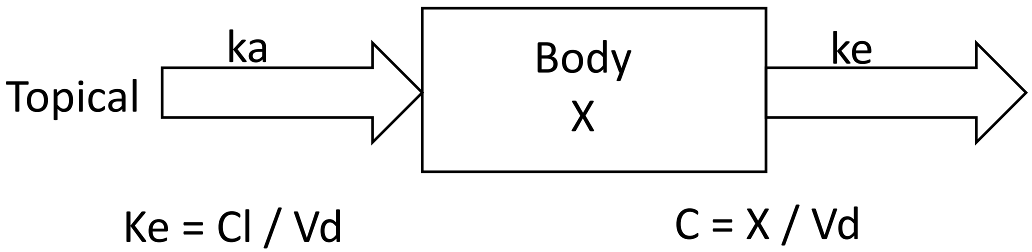
Based on the experiences with these models, it appeared that the F was lower on the first dose than on the following doses, probably due to tissue binding and saturation. Other studies have shown that drug bound in the tissue can have a significant effect on the drug model developed. [14, 15] The next set of models (Figure 3.3e) included a saturated tissue step by which a proportion of the dose was lost to binding on the first dose and the bioavailability improves after this first dose. This was consistent with in vitro Franz cell studies. For these models, the F was modeled for the first dose (FS) and a separate F for remaining doses (FF). The Bmax was estimated from the difference. Of all the models attempted, this approach gave the most consistent and complete modeling of the results. (Table 3.8) This model gave the lowest objective function value of all tested models with a small SE. Covariance between absorption parameters was evaluated by plots of ETA values for parameters. There was no apparent covariance of FS and FF with Ka. (Figure 3.6) However, based on ETA plots and statistical output, there appears a significant covariance between FS and FF, which might be expected. This was added to the model (F1) and significantly lowered the objective function value. Including the other covariances (ka FS, Ka FF, and all included) did not improve the objective function significantly. The PRED and IPRED values versus observed concentration was plotted from the best of the models in Figure 3.7. Based on the error models used, it was decided to also examine the LOG-LOG plot for predicted and observed concentration (Figure 3.8). Considering the variability of the data, this showed reasonably good individual prediction (IPRED). In addition, residuals as IPRED minus observed were examined. (Figure 3.9) Although there is large error, this error is distributed relatively evenly across time. Based on all the modeling, it was felt that model F1 was best for future demographic and parameter covariance evaluations.

Using model F1, preliminary covariate analysis was performed for the demographic characteristic of age, weight, body surface area, treatment, site, lesion size, and creatinine clearance. Covariate effects on inter-subject variation (ETA) for FS, FF, and Ka of Model F1 were examined. Treatment of PARO or PARO/G appeared to have no effect on the PARO transdermal absorption. (Figure 3.10) The only covariate influence was noted with the lesion size affecting the value of the FF, first dose extent of absorption, with no effect on Ka or FS. This relationship is shown in Figure 3.11. Lesion size with a scaling factor, ALLO, was added to the model F1 and this produced a significant decrease in the objective function (3962). As with other population pharmacokinetic studies, the demographic based covariates did not show significant influence over the system, while disease specific covariates in afflicted patients, in this case, lesion size, did have a notable influence on the developed model. [16-18]

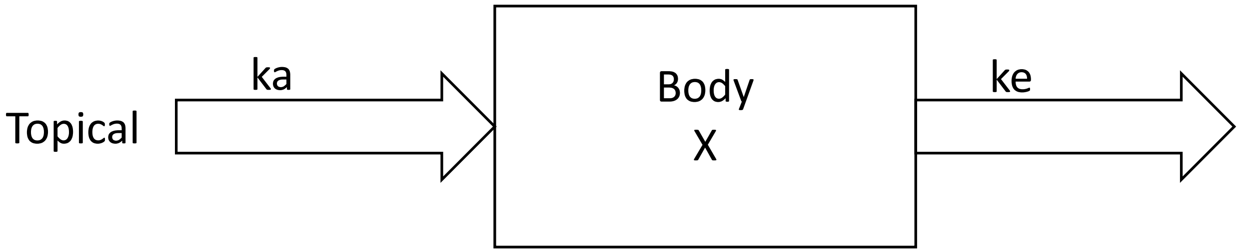
In conclusion, a population pharmacokinetic model was developed that describes transdermal PARO absorption and accounts for increased drug accumulation during multiple dosing. The increased extent of PARO transdermal absorption could be predicted based on a tissue depot or saturated tissue binding. Future in vitro studies with intact and microneedle treated skin will compare PARO absorption from the 2 topical cream formulations. Skin treated with different extents of microporation would be used to simulate varying severity of stratum corneum damage caused by skin lesions.

Figure 3.3. Compartment model flow diagrams. a) First, simple model series A, b) first order absorption model series B, c) depot compartment model series E, d) saturable tissue binding model C, e) saturated tissue binding model series F.

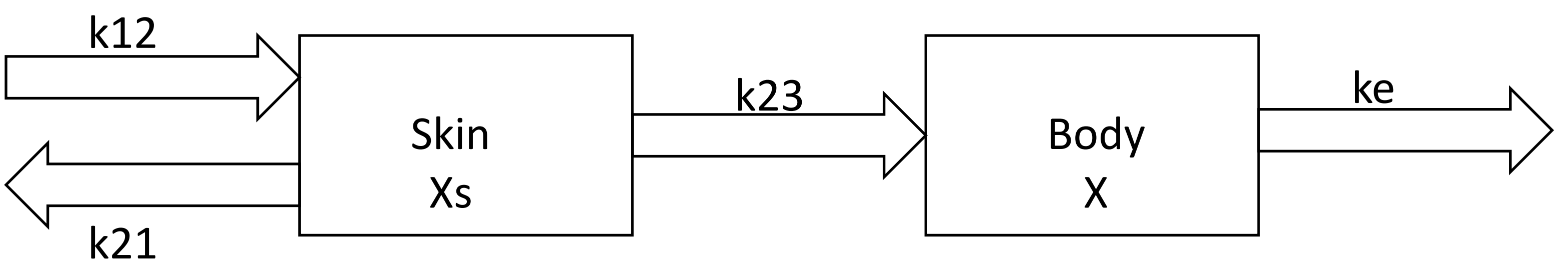
a)



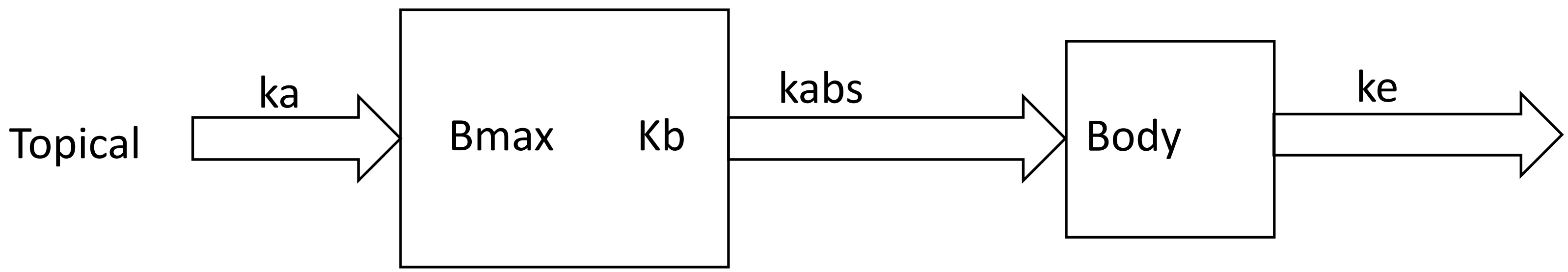
b)



c)



d)



e)

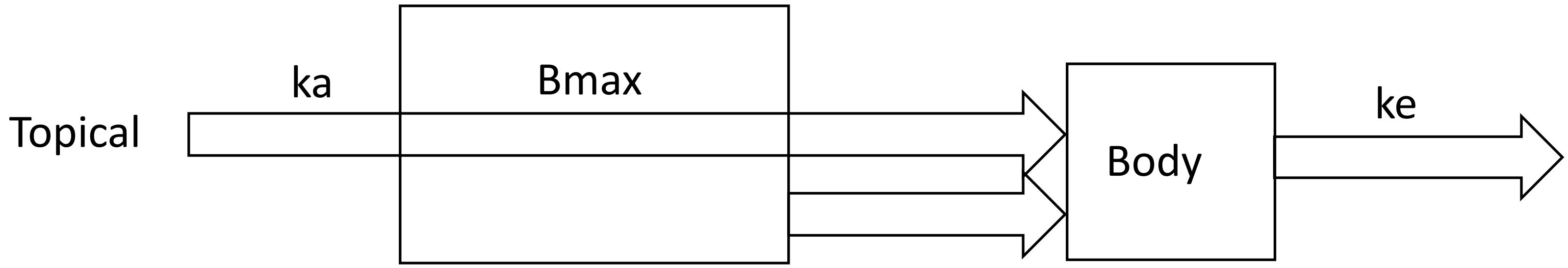


Table 3.4 First model series A

Model		Ka	CL	V	IRV CV%	IRV add	Objective Function
A0	Estimate	0.0821	94.3	69.4	83.9	0.047	4195.641
	SE%						
	IIV	53.9	51.3	41.7			

Table 3.5. First-order absorption model series B with individual PK parameters

Model		Ka (1/hr)	F	IRV CV%	IRV ADD	Objective Function
	Estimate	0.193	0.0816	80.1	36.4	
B0	SE%	27.2	8.0	13.7	31.0	4245
	IIV	130.0	53.6			
	SE%	51.3	39.4			
	Estimate	0.428	0.0251	26.0	17.6	
BD1	SE%	18.4	14.7	22.3	3.20	1086
	IIV	100.0	90.0			
	SE%	27.5	22.5			
	Estimate	0.351	0.0954	29.4	65.3	
BD21	SE%	237.9	72.9	223.1	57.9	2330
	IIV	118.7	57.4			
	SE%	232.6	569.7			

Figure 3.4. B series models of observed and predicted concentration a) Day 1, b) Day 20

a)

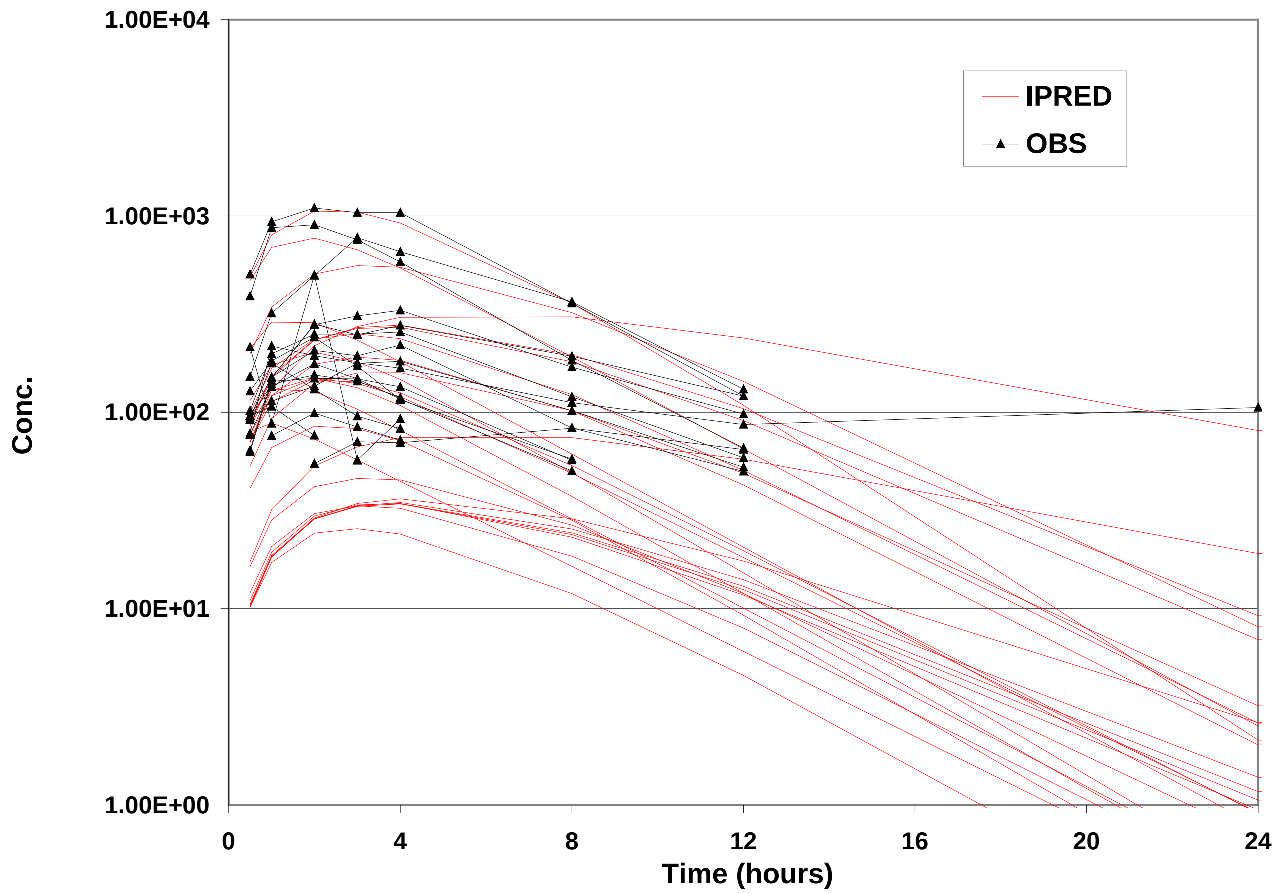


Figure 3.4 cont.

b)

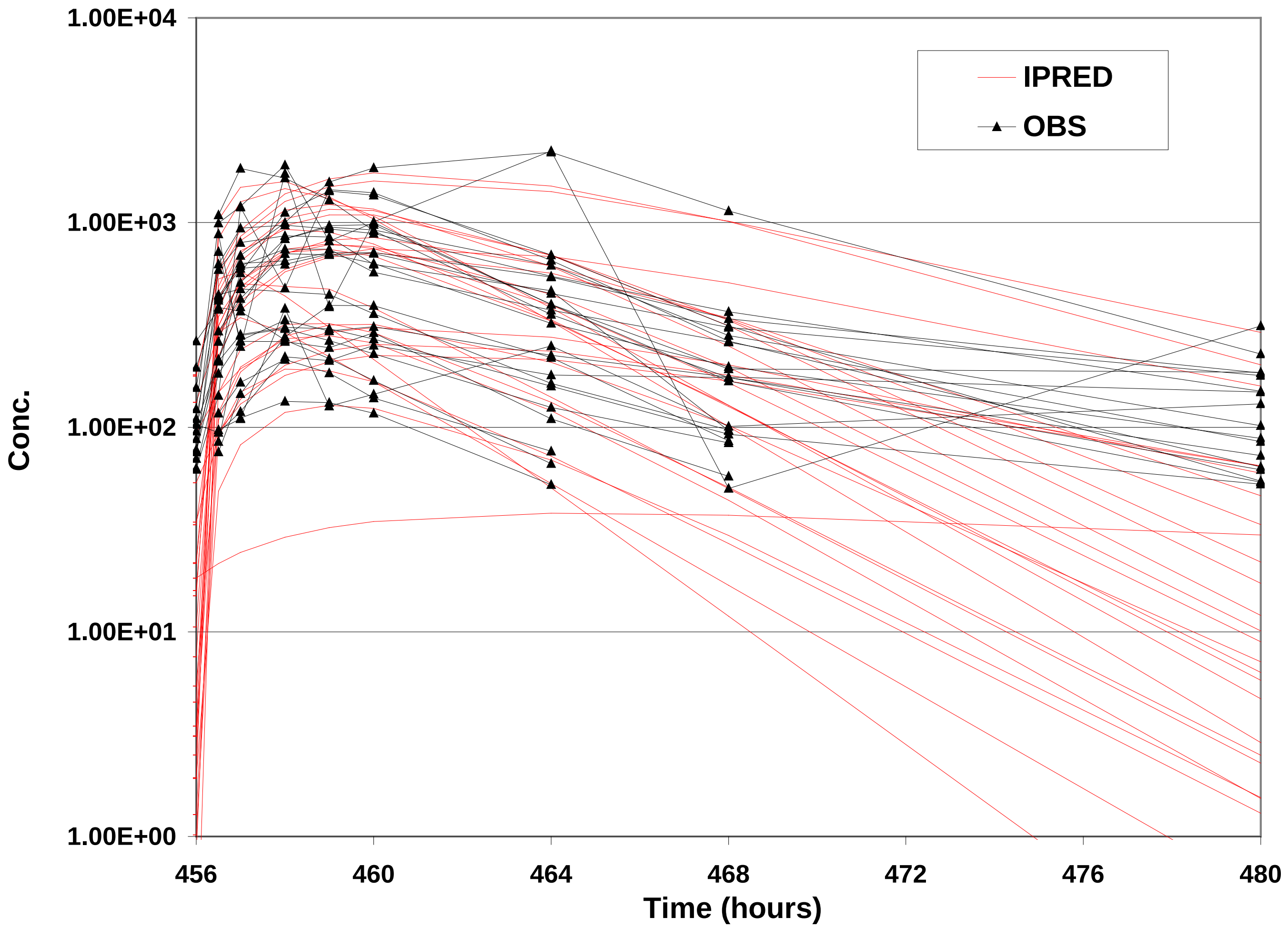


Table 3.6. E series model

Model		Ka (1/hr)	F	K12 (1/hr)	K23 (1/hr)	K21 (1/hr)	IRV CV%	IRV add	Objective Function
	Estimate	0.129	0.152	0.478	0.00116		62.3	0.0500	
E2	SE%								4,018
	IIV	105.8	47.5	73.2	58.8				
	SE%								
	Estimate	0.134	0.150	0.485	0.0012	0.000022	62.3	0.2250	
E1	SE%	4.7	9.1	22.7	25.7	136.4	8.4	44.9	4,018
	IIV	102.5	45.6	75.7	63.8				
	SE%	47.8	65.4	42.2	33.9				
	Estimate		0.114	181	0.0400	0.0014	85.2	0.0506	
E0a	SE%								4,252
	IIV		57.8	55.1	240.8	<u>56.1</u>			
	SE%								

Figure 3.5. E1 model, a) IPRED vs observed values, b) observed and predicted concentration for model E1

a)

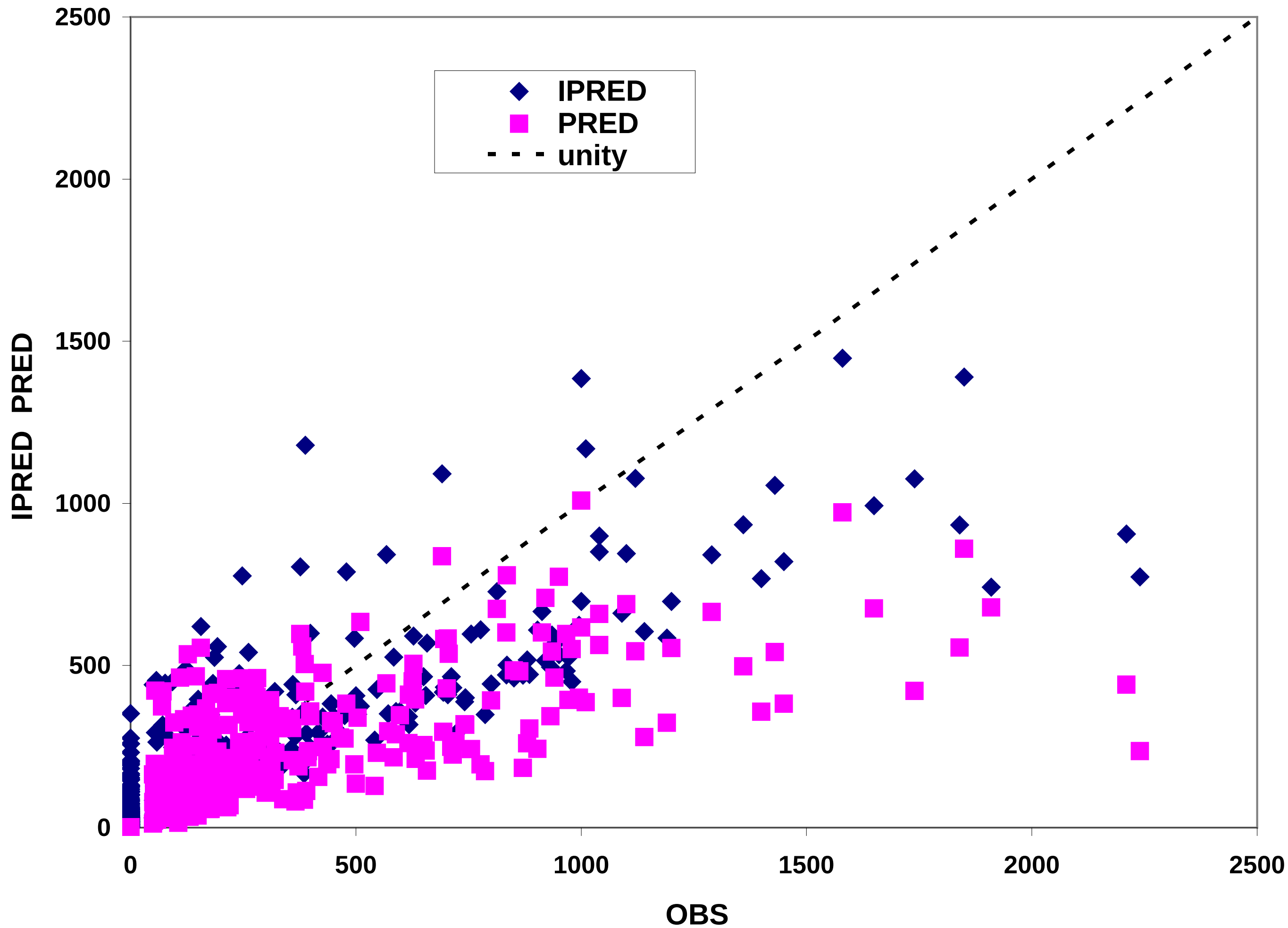


Figure 3.5 cont.

b)

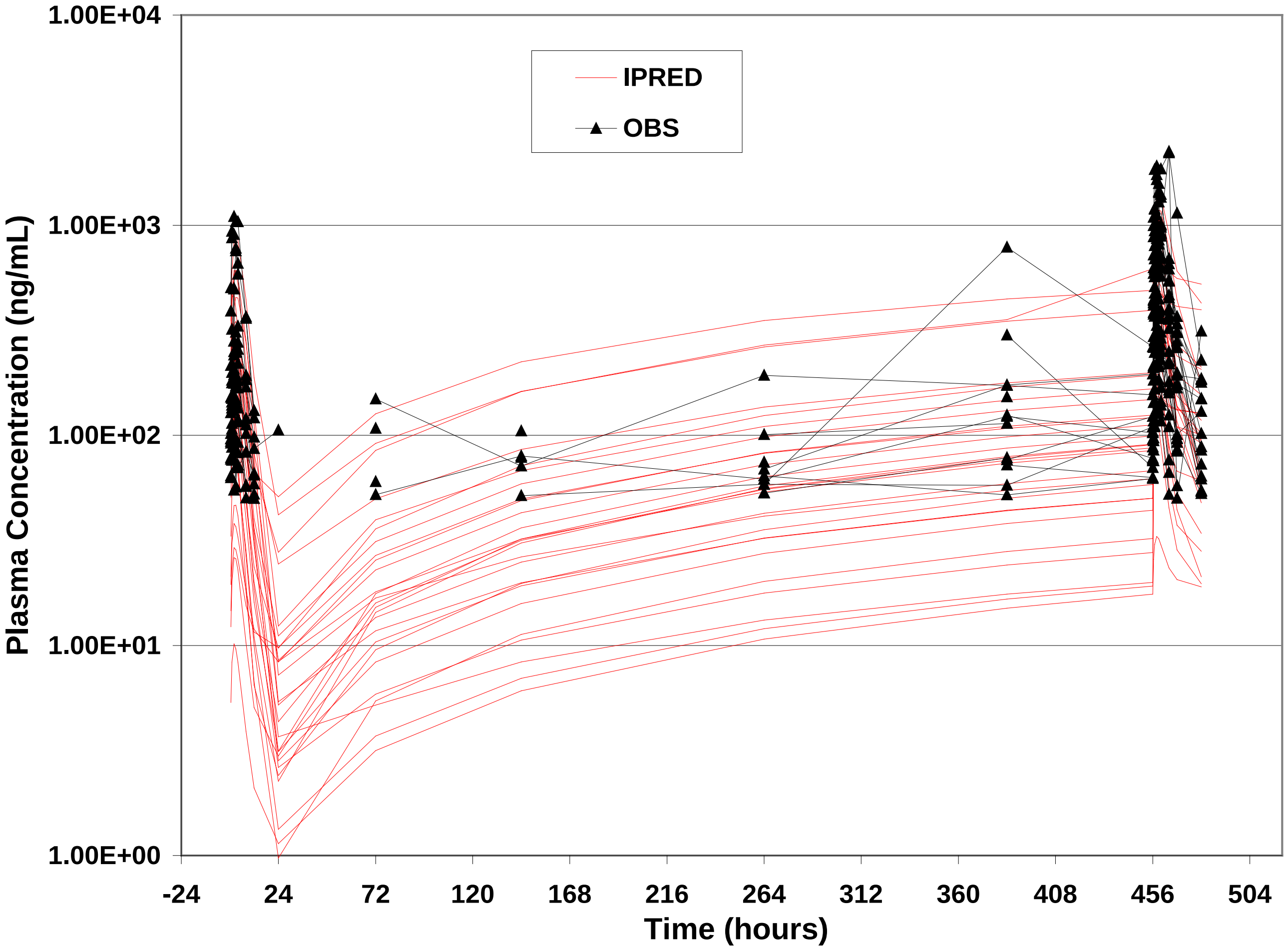


Table 3.7. Saturable tissue model series C

Model		Ka (1/hr)	F	Kabs (1/hr)	Bmax (mg)	KB (mg)	IRV CV%	IRV add	Objective Function
	Estimate	0.318	0.0635	99.00	1.740	0.0042	72.8	72.3	
C3	SE%								4300
	IIV	107.7	65.4		41.6				
	SE%								
	Estimate	0.0825	0.109		5.120	0.288	68.6	31.9	
C2a	SE%								4393
	IIV	116.6	60.0						
	SE%								
	Estimate	0.0836	0.0946		6.240	2.600	89.6	31.4	
C3a	SE%								4301
	IIV	171.2	65.7		558.6				
	SE%								

Table 3.8. Saturated tissue binding model series F

Model		Ka (1/hr)	FS	FF	Bmax (mg)	IRV CV%	IRV add	Model Covariate	Corr. Covariate	Objective Function
	Estimate	0.231	0.104	0.0266	97.8	43.9	37.4			
F0	SE%	1.0	0.4	0.1	7.0	4.4	8.8			3986
	IIV	133.8	56.5	87.2						
	SE%	2.4	0.3	6.3						
	Estimate	0.250	0.107	0.0257	115.5	43.3	38.0	FF FS	0.416	
F1	SE%	8.4	12.0	34.0	4.7	20.5	13.3			3981
	IIV	113.6	56.5	120.4						
	SE%	156.6	7.0	61.5						
	Estimate	0.258	0.107	0.0247	102.5	42.2	40.6	KaFS	-0.411	
F5	SE%	79.5	16.6	34.0	6.5	32.0	29.3			3979
	IIV	129.6	54.9	56.5				FSFF	0.425	
	SE%	76.8	-34.6	186.2						
								KaFF	0.0484	
	Estimate	0.242	0.109	0.0257	97.0	42.3	40.1	KaFS	-0.382	
F2	SE%	38.8	12.5	34.9	7.2	23.1	32.4			3983
	IIV	125.3	54.4	116.2						
	SE%	62.4	24.2	38.6						
	Estimate	0.246	0.108	0.0240	97.3	42.2	41.3	KaFF	0.0424	
F3	SE%	35.3	12.9	35.0	7.1	22.2	30.0			3986
	IIV	116.6	55.0	122.1						
	SE%	54.6	26.8	38.7						
	Estimate	0.268	0.108	0.0242	102.6	41.6	41.2	KaFS	-0.445	
F4	SE%	7.9	24.2	36.2	6.5	30.0	27.4			3980
	IIV	126.1	56.3	120.4				FSFF	0.406	
	SE%	83.6	35.6	38.8						
	Estimate	0.251	0.105	0.0244	112.1	43.6	40.6	KaFF	0.197	
F6	SE%	29.5	19.5	31.6	5.0	24.5	27.6			3981
	IIV	114.0	55.1	120.8				FFFS	0.436	
	SE%	56.5	34.9	37.1						
	Estimate	0.247	0.106	0.0216	220	43.1	38.2	FF FS	0.708	
F1LESN	SE%	7.6	1.3	5.4	17.0	4.13	1.61			3962
	IIV	115.0	56.8	76.9						
	SE%	57.8	23.7	17.0						
	ALLO	1.800								

Figure 3.6. ETA plots for model F1. a) IPRED vs observed values, b) ETA of FS and FF versus Ka, c) FF versus FS

a)

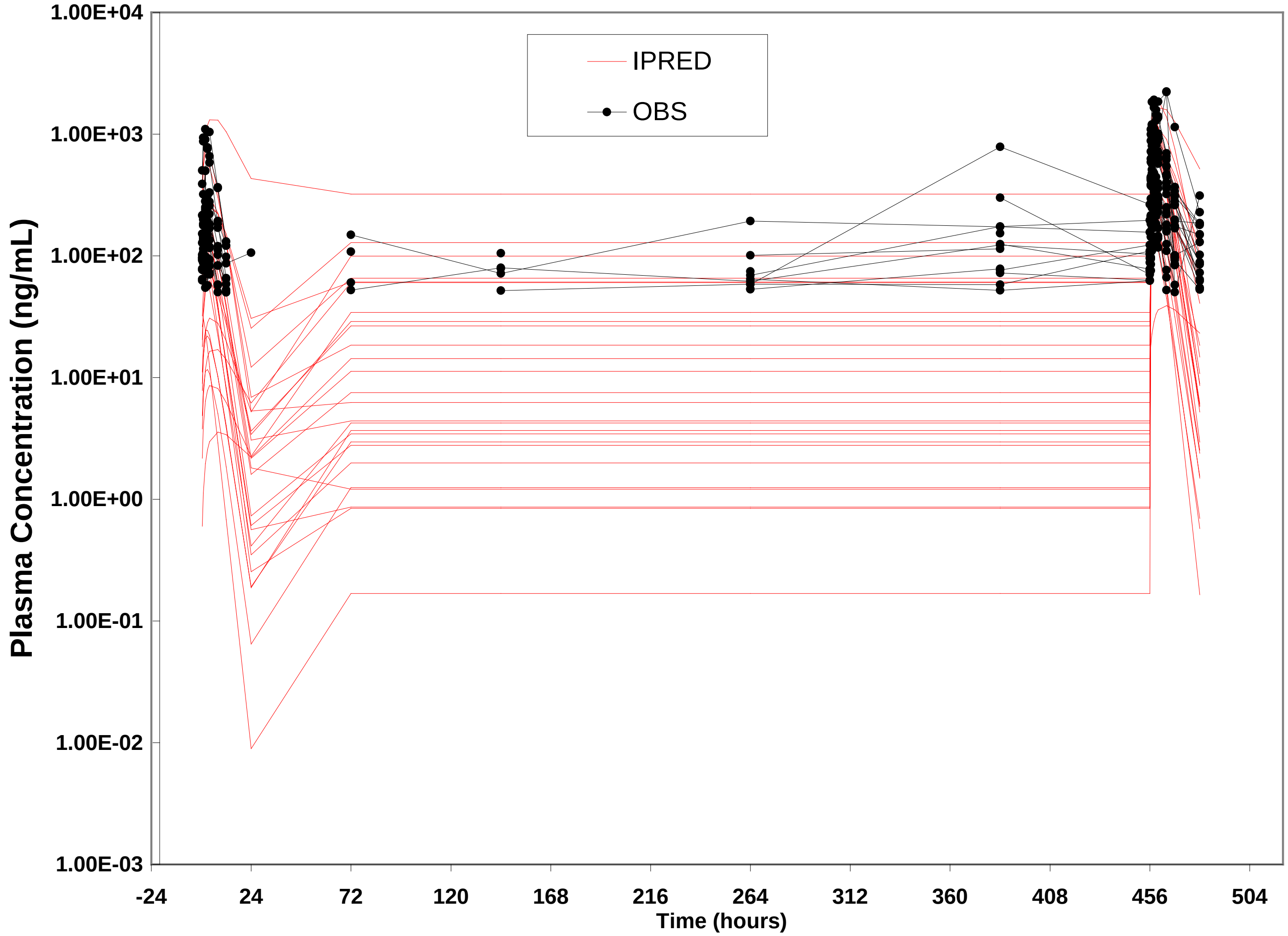


Figure 3.6 cont.

b)

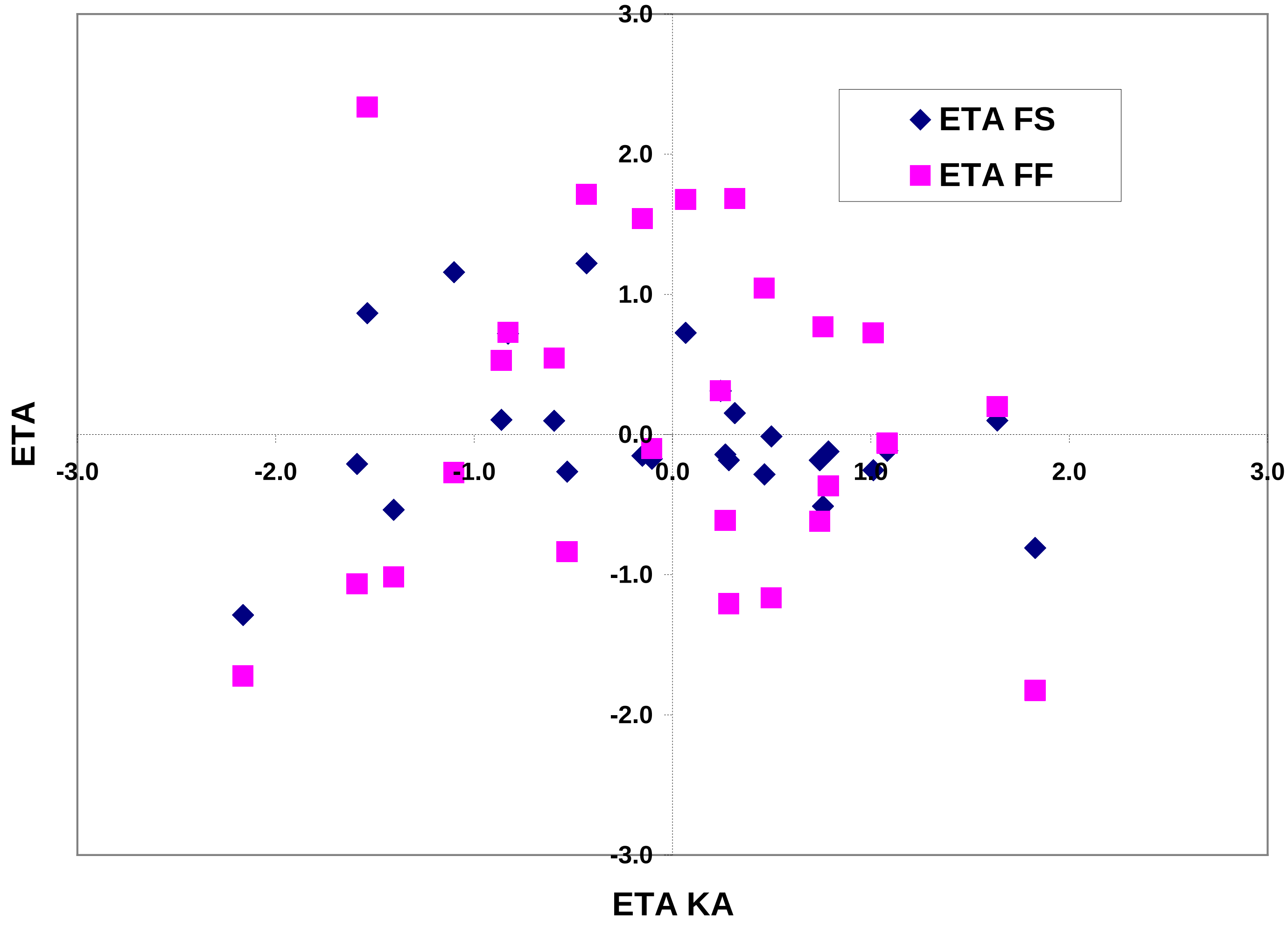


Figure 3.6 cont.

c)

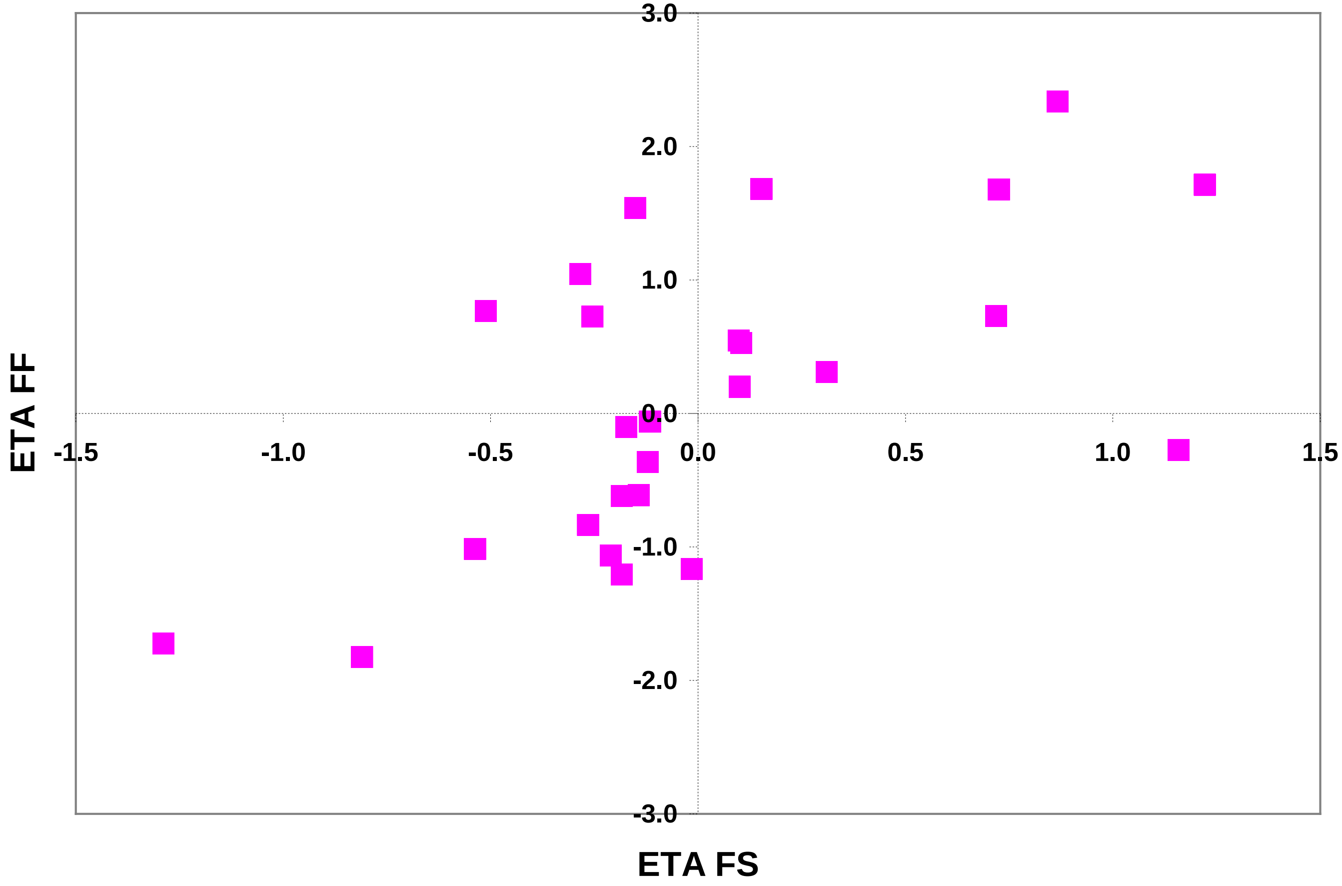


Figure 3.7. PRED and IPRED values versus observed concentration from the best of the F1model

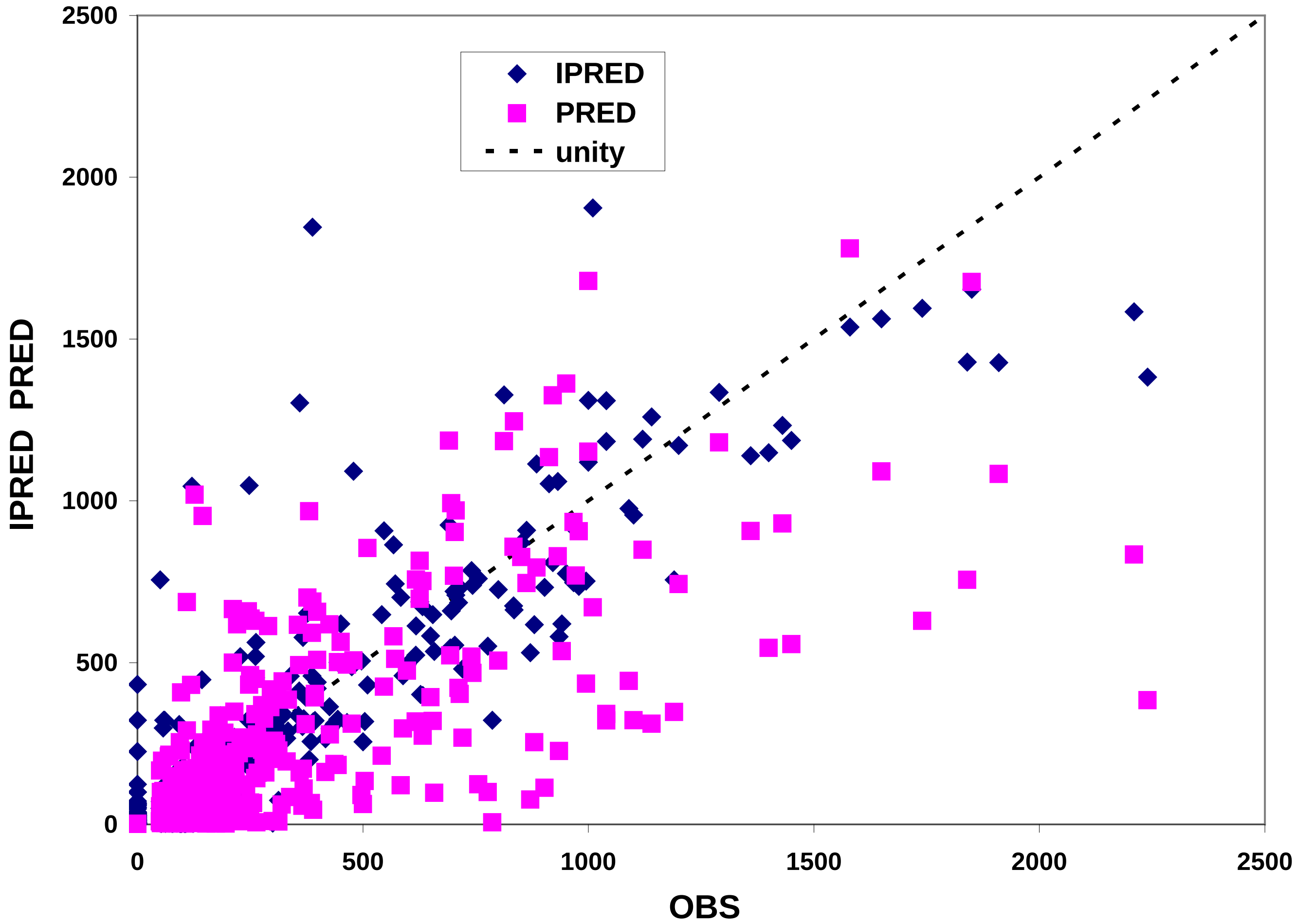


Figure 3.8. Log-log plot for predicted and observed concentration from best of F1 model

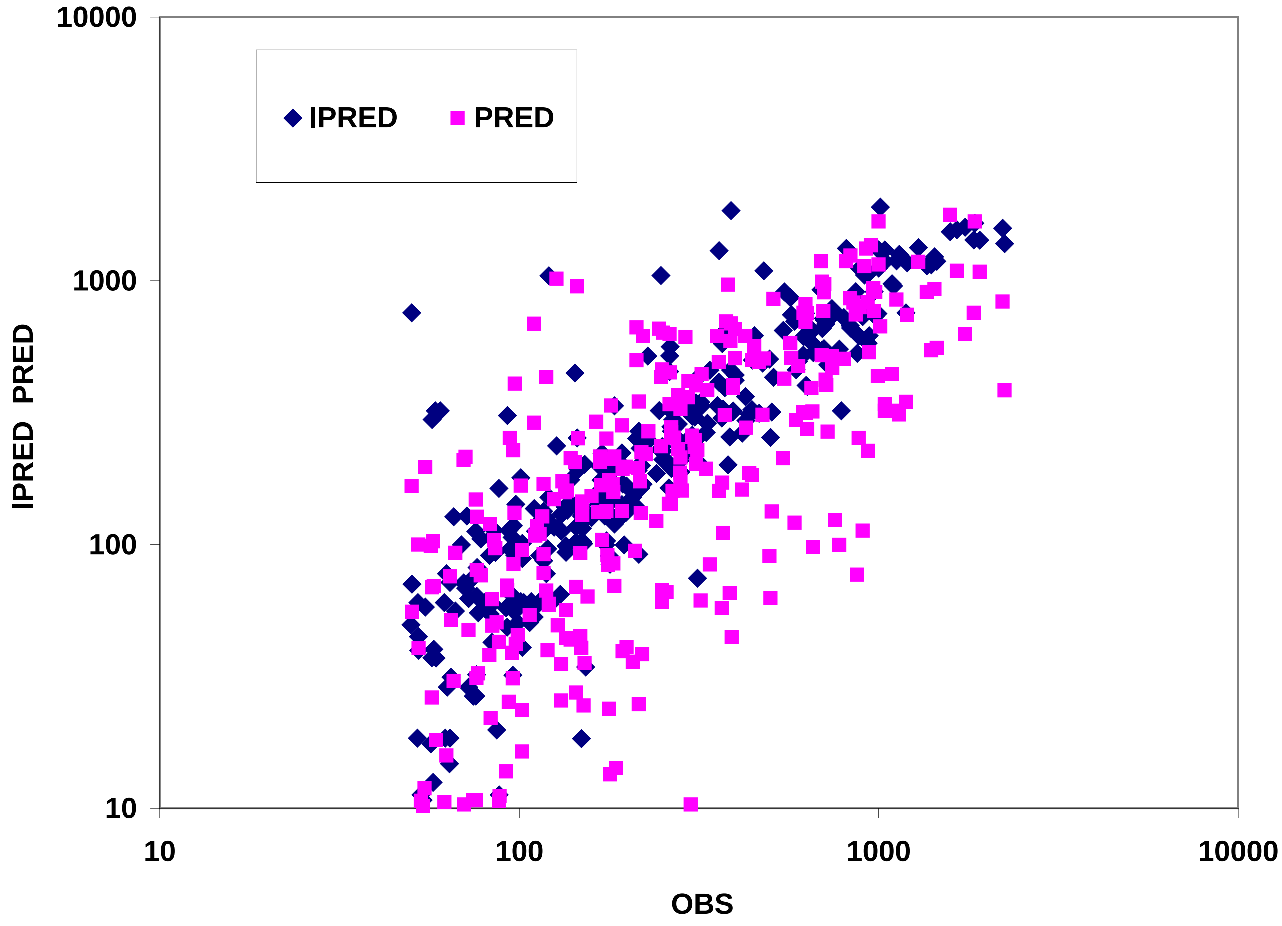


Figure 3.9. Residuals as IPRED minus observed values for the F1 model.

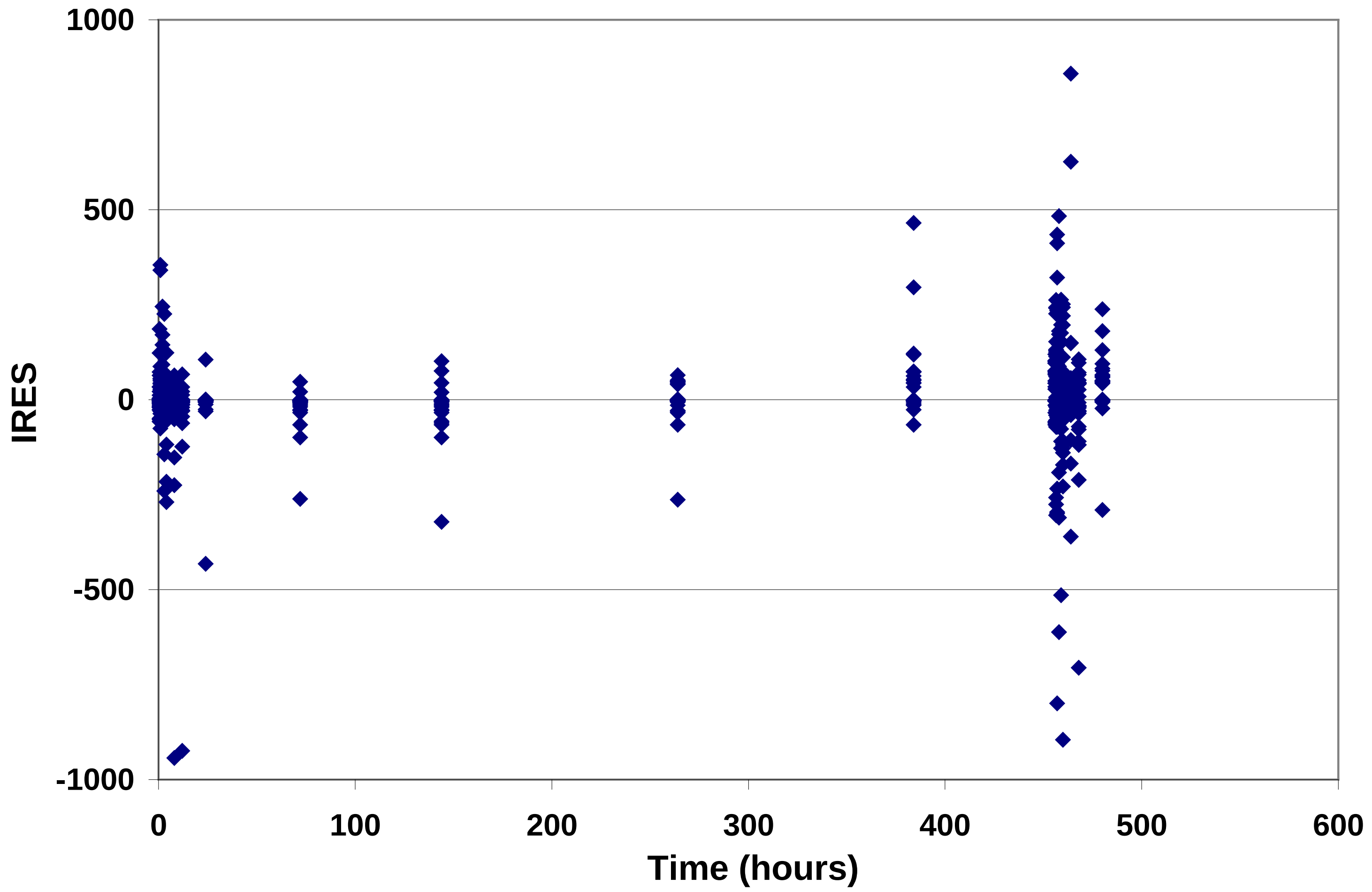


Figure 3.10. Influence of treatment, PARO (1) and PARO/G (2), on the extent of absorption for the First Dose (FF).

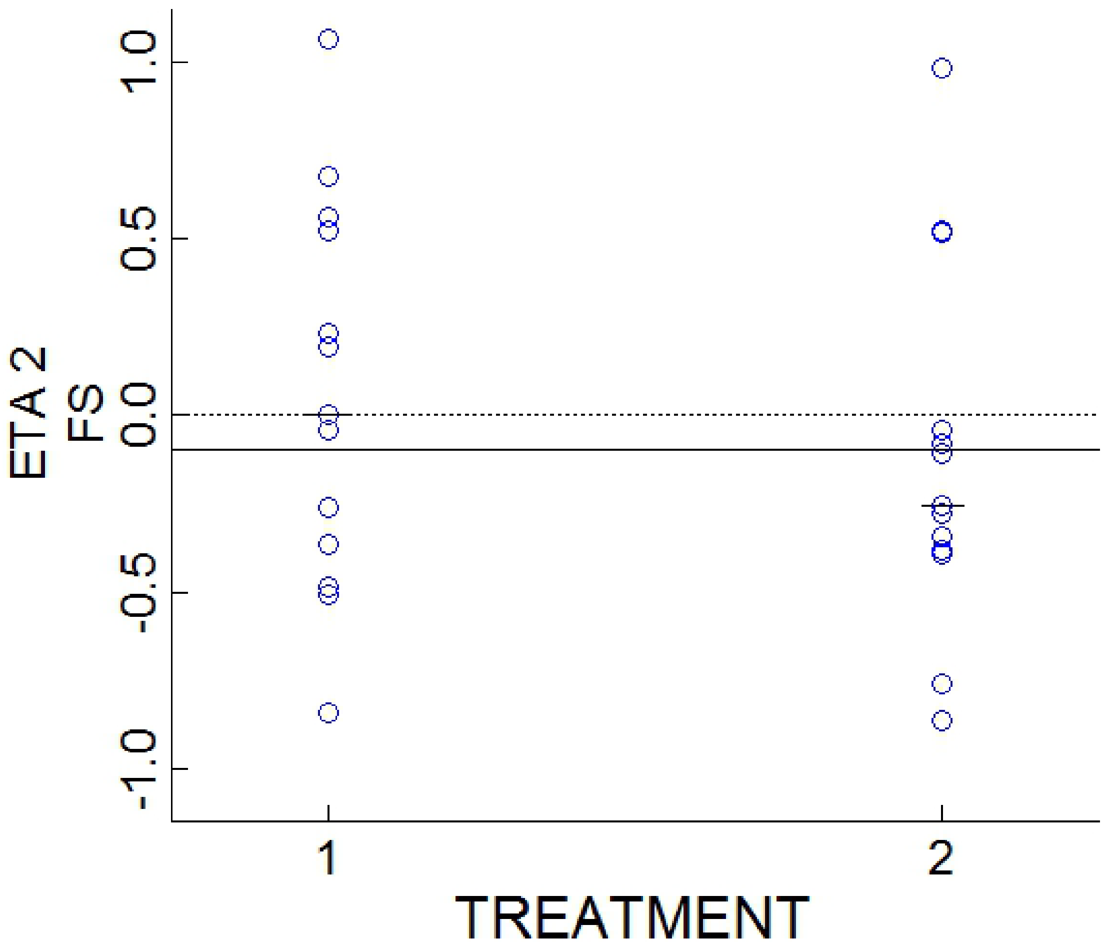
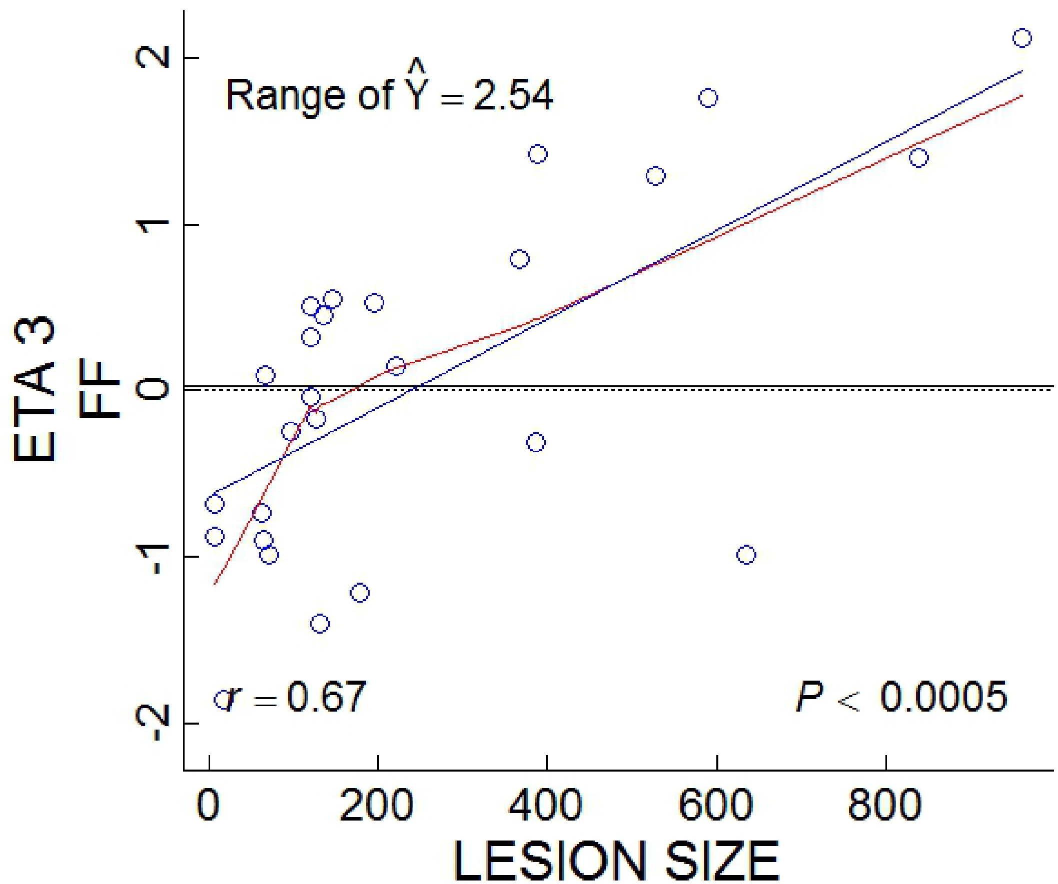


Figure 3.11. Influence of Lesion Size on the Extent of Absorption of the First Dose (FF). Lesion size in mm^2. Blue line is simple linear regression. Red line is smoothing curve function applied by XPOSE.



3.5 References

1. Ravis WR, Llanos-Cuentas A, Sosa N, et al. Pharmacokinetics and absorption of paromomycin and gentamicin from topical creams used to treat cutaneous leishmaniasis. Antimicrob Agents Ch. 2013(57), 4809-4815.
2. Croft SL, Coombs GH. Leishmaniasis–current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol. 2003(19), 502-508.
3. Alvar J, Vélez ID, Bern C, et al. Leishmaniasis worldwide and global estimates of its incidence. Plos One. 2012(7), e35671.
4. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clinical microbiology reviews. 2006(19), 111-126.
5. Polonio T, Efferth T. Leishmaniasis: drug resistance and natural products. Int J Mol Med. 2008(22), 277-286.
6. Sheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. J Pharmacokinet Biop. 1977(5), 445-79.
7. Bista SR, Haywood A, Hardy J, et al. Exposure to fentanyl after transdermal patch administration for cancer pain management. J Clin Pharmacol. 2016(56), 705-713.
8. Kokubun H, Ebinuma K, Matoba M, et al. Population pharmacokinetics of transdermal fentanyl in patients with cancer-related pain. J Pain Pall Care Pharmacother. 2012(26), 98-104.
9. Freise KJ, Linton DD, Newbound GC, et al. Population pharmacokinetics of transdermal fentanyl solution following a single dose administered prior to soft tissue and orthopedic surgery in dogs. J Vet Pharmacol Ther. 2012(35), 65-72.

10. Kanyok TP, Killian AD, Rodvold KA, et al. Pharmacokinetics of intramuscularly administered aminosidine in healthy subjects. Antimicrob Agents Ch. 1997(41)982–986.

11. Beal S. Ways to Fit a PK Model with Some Data Below the Quantification Limit. J Pharmacokinet Phar. 2001(28), 481-504.

12. Behm HL and Wagner JG. Parabolic equation relating free and total drug concentrations in cases of nonlinear plasma protein binding. J Pharm Sci. 1981(70), 802–804.

13. Parsons DL and Ravis WR. Direct calculation of bound drug from total drug based on the langmuir-scatchard model with two classes of sites. Academy of Pharmaceutical Sciences, Annual Meeting, Miami Beach, FL, November 1983.

14. Hope WW, Goodwin J, Felton TW, et al. Population pharmacokinetics of conventional and intermittent dosing of liposomal amphotericin b in adults: a first critical step for rational design of innovative regimens. Antimicrob Agents Ch. 2012(56), 5303-5308.

15. Vogelsinger H, Weiler S, Djanani A, et al. Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion. J. Antimicrob. Chemother. 2006(57), 1153–1160.

16. Rubino CM, Bhavnani SM, Moeck G, et al. Population pharmacokinetic analysis for a single 1,200-milligram dose of oritavancin using data from two pivotal phase 3 clinical trials. Antimicrob Agents Ch. 2015(59), 3365-3372.

17. Samara E, Shaw JP, Barriere SL, et al. Population pharmacokinetics of telavancin in healthy subjects and patients with infections. Antimicrob Agents Ch. 2012(56), 2067-2073.

18. Van Wart SA, Owen JS, Ludwig EA, et al. Population pharmacokinetics of tigecycline in patients with complicated intra-abdominal or skin and skin structure infections. Antimicrob Agents Ch. 2006(50), 3701-3707.

4. In Vitro Absorption of Vitamin B12 Derivatives through Human Skin

4.1 Abstract

The objective of this research was to study the skin permeation of various cobalamins across intact and microporated dermatomed human cadaver skin. Preliminary data for linear pharmacokinetic modeling was gathered to describe the in vitro absorption of four cobalamin derivatives: cyanocobalamin, hydroxocobalamin, methylcobalamin, and adenosylcobalamin. Such a model would enable formulators to predict the effects of rate of drug delivery on drug pharmacokinetics and assist with dosage form design.

A Franz diffusion cell apparatus was used to investigate the in vitro transdermal permeability of the four cobalamins. Passive diffusion through the skin and the effect of permeation enhancement by perforating the skin with microneedles were compared. Drug retention in the skin was also measured as another factor to consider for potential modeling.

The results showed that the four cobalamins did not readily diffuse through the skin, though microperforation of the skin allowed for transdermal permeation. Skin retention results showed that cyanocobalamin and hydroxocobalamin were relatively impermeable while methylcobalamin and adenosylcobalamin were absorbed into the skin. Further modeling was not attempted as no measurable cobalamin levels were detected by passive diffusion to use as a control. However, varying the extent of microporation was postulated as a potential basis for future experiments and modeling.

4.2 Introduction

Vitamin B12 has a key role in the normal functioning of the brain and nervous system via the synthesis of myelin. B12 is involved in the metabolism of every cell of the human body, affecting DNA synthesis, and fatty acid and amino acid metabolism. As medication, it is used to treat B12 deficiency and cyanide poisoning. Deficiency of this vitamin can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than normal, a range of symptoms such as fatigue, lethargy, depression, poor memory, breathlessness, headaches, and pale skin, among others, may be experienced, especially in elderly people. While vitamin B12 deficiency is most commonly caused by low intakes, it can also result from malabsorption, certain intestinal disorders, low presence of binding proteins, and use of certain medications. A diagnosis of vitamin B-12 deficiency is currently defined as a concentration < 148 pmol/L (200 pg/mL) and marginal status defined as a concentration of 148–221 pmol/L. [1] It is mainly prevalent in the older generation with a incidence of around 40%, due to changes in their digestive system; and in strict vegans, as it is available only from natural animal products such as eggs, fish and meat. Patients with pernicious anemia and other intestinal disorders also commonly develop vitamin B12 deficiency because of their inability to absorb small amounts of the vitamin. When left untreated, this may lead to anemia and irreversible nerve damage. [2] In these cases, where the oral delivery of cobalamin may not be feasible, as discussed below, transdermal absorption of B12 can be used as an alternative to treating the vitamin deficiency.

The synthetic form of the vitamin B12 molecule, cobalamin, is cyanocobalamin. Cyanocobalamin is usually given after surgery to remove part or all of the stomach or intestine, to ensure adequate levels of vitamin B12 in the body. Cyanocobalamin is one of the most widely manufactured vitamers of B12, due to it not being susceptible to oxidation in its crystalline form.

Its stability leads it to be used in many food additives and multivitamins. Hydroxocobalamin is also a commonly used form of B12. Adenosylcobalamin and methylcobalamin are the two active forms of B12 that occur naturally within the body. [2]

While cyanocobalamin is the main mode of treatment for vitamin B12 deficiency, the oral dosage form has been associated with concerns related to irregular and unpredictable absorption in patients with pernicious anemia. [2] Most people with vitamin B12 deficiencies are treated with intramuscular or deep subcutaneous injections, which requires assistance from healthcare professionals. Hence, the treatment is expensive and makes it less patient-compliant. Transdermal drug delivery is very convenient and patient compliant for many drug molecules. [3] Various physical and chemical enhancement techniques were attempted to successfully deliver B12 across hairless rat skin. Enhancers such as ethanol (50%), oleic acid (10%) and propylene glycol (40%) showed significant improvement in the permeation of B12. [4] In addition, iontophoresis and microneedle treatments resulted in enhanced permeation levels compared to passive controls. Similarly, the permeation across rat skin was significantly higher from Capryol, labrafil, oleic acid and propylene glycol containing 0.07% vitamin B12 [4]. The permeation of liposomal gel adenosylcobalamin in the treatment of atopic dermatitis in mice was very effective suggesting significant permeation across mice skin [5]. Many formulation approaches such as microemulsions [6], polymer nanofibers [7], and solid lipid nanoparticles [8] were attempted for transdermal delivery of vitamin B12.

Most of the published literature on transdermal vitamin B12 delivery used animal skin in the permeation experiments. Also, there is no report that compares the skin permeation of various cobalamins (cyanocobalamin, methylcobalamin, hydroxocobalamin, adenosylcobalamin).

4.3. Materials and Methods

4.3.1. Materials

All chemicals were of analytical grade and purchased from VWR International (Suwannee, GA, USA): cyanocobalamin (B12), hydroxocobalamin (B12a), methylcobalamin (MeB12), adenosylcobalamin (AdB12), acetonitrile (HPLC grade), phosphate buffered saline (PBS), Whatman® nylon syringe filters (0.2 µm), and octanol. Water was obtained from a Millipore water purification unit.

4.3.2. Preparation of B12 formulations

Cyanocobalamin, hydroxocobalamin, methylcobalamin or adenosylcobalamin in a 10 mg quantity was dissolved in 10 mL of PBS pH 7.4 by vortex mixing followed by immersion in a sonicator bath.

4.3.3. Log P determination

Log P was determined with the shake flask method. [9] Known amount of each cobalamin was dissolved in 10 ml each of water and octanol. The flask was inverted 50 times, and the liquids were allowed to separate overnight. The concentration of each vitamin B12 in both water and octanol was measured by HPLC.

4.3.4. Skin permeation and retention study

Human cadaver skin was harvested from the thighs and backs of the donors within 8 hours of death by an electro-dermatome set at 0.5 mm thickness. The skin was preserved in a cryopreservation medium (50% glycerol in normal saline) and immediately stored at -80°C

(Allosource, Cincinnati, OH, USA). Prior to experiments, the skin was thawed at room temperature for 30 min and transferred to pH 7.4 PBS and equilibrated for 30 min, then thoroughly rinsed with PBS to remove any traces of glycerol. The skin surface was examined for any bruises or cuts under a 10x magnifying lens. In vitro skin permeation studies were performed using vertical static Franz diffusion cells (PermeGear Inc, Bethlehem, PA, USA). The skin was cut, rinsed with PBS and sandwiched between donor and receptor cells with the epidermis facing the donor cell. The receptor cell contained PBS (5 ml, pH 7.4), maintained at 37°C with a water circulation jacket that surrounded the cell. The available diffusion area of the skin was 0.64 cm^2. The donor cell contained the B12 solution, approximately 1.0 mg/ml, in PBS buffer. The cell was covered with Parafilm® to prevent solvent evaporation. The receptor cells were stirred with magnetic bars at 300 rpm. At regular intervals, 500 µl aliquots from the receptor were collected and replaced with fresh PBS pre-equilibrated at 37°C. The collected samples were stored in a refrigerator and analyzed within 24 h. All experiments were performed with at least 3 replicates.

Residual B-12 in the skin was determined at the end of the permeation study. The B12 solution remaining on the surface of the skin was removed using cotton swabs. The surface was washed with 10% ethanol and gently wiped with a cotton swab. Afterwards, 200 µL of PBS was added to the surface and dabbed with a fresh cotton swab. This rinsing and dabbing was repeated five times. Following the washing procedure, the active diffusion area was excised. The collected skin was weighed, minced using scissors and transferred into a borosilicate glass vial. To each vial, 1 mL of PBS was added, sonicated for 15 min and allowed to stand overnight. They were re-sonicated for 30 min, filtered through a 0.45 µm membrane filter, and the filtrate was analyzed by HPLC.

4.3.5. Microporated skin permeation and retention

Stainless steel microneedles (250 µm long, 100 µm width at the base, 192 needles assembled in 8 rows) were used for pre-treating the skin (Dermaroller™ Deutschland s.a.r.l., Germany). The Dermaroller™ was passed over the epidermal surface of the skin 20 times in multiple directions and the treated skin was cut to size and mounted on the Franz diffusion cells. The in vitro permeation and skin extraction studies were conducted as described above.

4.3.6. HPLC analysis

The HPLC system (Waters Corp., MA, USA) was equipped with an autosampler (model 717 plus), an isocratic pump (model 1525) and a PDA UV detector (model 998). The system was interfaced with Empower 2 software for data collection and processing. Samples were eluted on a C8 analytical column, 5µm silica particles, 4.6 mm x 250 mm (Betasil, Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of an 86:14 ratio of pH 5.2 phosphate buffer with acetonitrile at a 1.0 ml/min flow rate. The detection wavelength was 360nm and the injection volume was 5µL.

4.3.7. Statistical analysis

The results are presented as mean ± standard error of mean (SEM). The cumulative amount of B12 permeated ($\mu g/cm^2$) was plotted as a function of time (t). The permeation rate ($\mu g/cm^2/h$) was calculated from the slope of the linear portion of the plot. The amount of B12 (mg) retained in the skin was normalized to 1 gram of skin. Analysis of variance was performed to determine the level of significance between the means. Mean differences with $P < 0.05$ were considered to be significant.

4.4. Results and Discussion

In unmodified skin, all four of the cobalamin molecules showed negligible passive diffusion below the HPLC detectable limits. This was supported visually as each donor solution possessed a clear, red coloration, while the receptor cell remained clear and colorless. These results are in contrast to the published results on cyanocobalamin dissolved in PBS in an identical quantity (1 mg/ml). The hairless rat skin permeation of 1 mg/ml cyanocobalamin in PBS was 10 $\mu g/cm^2$ in 24 hours. [2] Similarly, pig ear skin permeation of 1 mg/ml cyanocobalamin in PBS was around 4 $\mu g/cm^2$ in 6 hours. [10] The permeation across hairless mouse abdominal skin for vitamin B12 was 22 $\mu g/cm^2$ in 24 hours. [11] The studies were conducted under identical conditions as the published reports, but used human skin and the permeation was negligible. The skin retention levels of cobalamins in the unmodified skin was: methylcobalamin > adenosylcobalamin > cyanocobalamin > hydroxocobalamin. The skin concentrations of adenosylcobalamin and methylcobalamin were about 15 and 25 fold higher than cyanocobalamin, respectively. This suggests that these compounds form a depot in the skin layers. Based on the log P data (Table 4.1), methyl and adenosyl compounds are slightly more lipophilic than cyanocobalamin and hydroxocobalamin.

With microporated skin, the more hydrophilic compound cyanocobalamin showed the greatest diffusion, followed by hydroxocobalamin, adenosylcobalamin, and, finally, methylcobalamin. (Figure 4.1). There is a clear correlation between log P and skin permeation. The area of the skin exposed to the formulation during diffusion showed a small amount of cyanocobalamin and hydroxocobalamin retained in untreated skin, with visibly larger amounts of methylcobalamin and adenosylcobalamin being retained (Figure 4.2, Table 4.2). In microporated skin, the skin retention levels of cobalamins was: adenosylcobalamin > methylcobalamin >

cyanocobalamin > hydroxocobalamin. The skin concentrations of methylcobalamin and adenosylcobalamin were about 7 and 9 fold higher, respectively, than cyanocobalamin. Other data [2, 10, 11] for cyanocobalamin passive diffusion were also in amounts well below therapeutic levels, and only through the application of permeation enhancement methods were therapeutic levels achieved. In addition, this study has also shown that other chemical forms of B12, such as hydroxocobalamin, methylcobalamin, and adenosylcobalamin, do not readily diffuse through human skin.

The use of microneedles to create a physical channel through the stratum corneum was effective in improving transdermal delivery of all types of B12 vitamins. There was an inverse relationship between the amount diffused and log P of each of the B12 vitamins. Retention in untreated skin shows that cyanocobalamin and hydroxocobalamin are relatively impermeable, while methylcobalamin and adenosylcobalamin are absorbed. The saturation point of methylcobalamin and adenosylcobalamin in unmodified skin was not explored, so it is unknown whether passive diffusion into the receptor cell would occur once the exposed skin area was saturated. Variations in the extent of microporation may provide usable data for pharmacokinetic modeling. However, diffusion of B12 through the skin without permeation enhancement would be difficult to model as no measureable levels of any of the B12 vitamins were detected.

Figure 4.1. Permeation of vitamin B12 compounds across microporated human skin

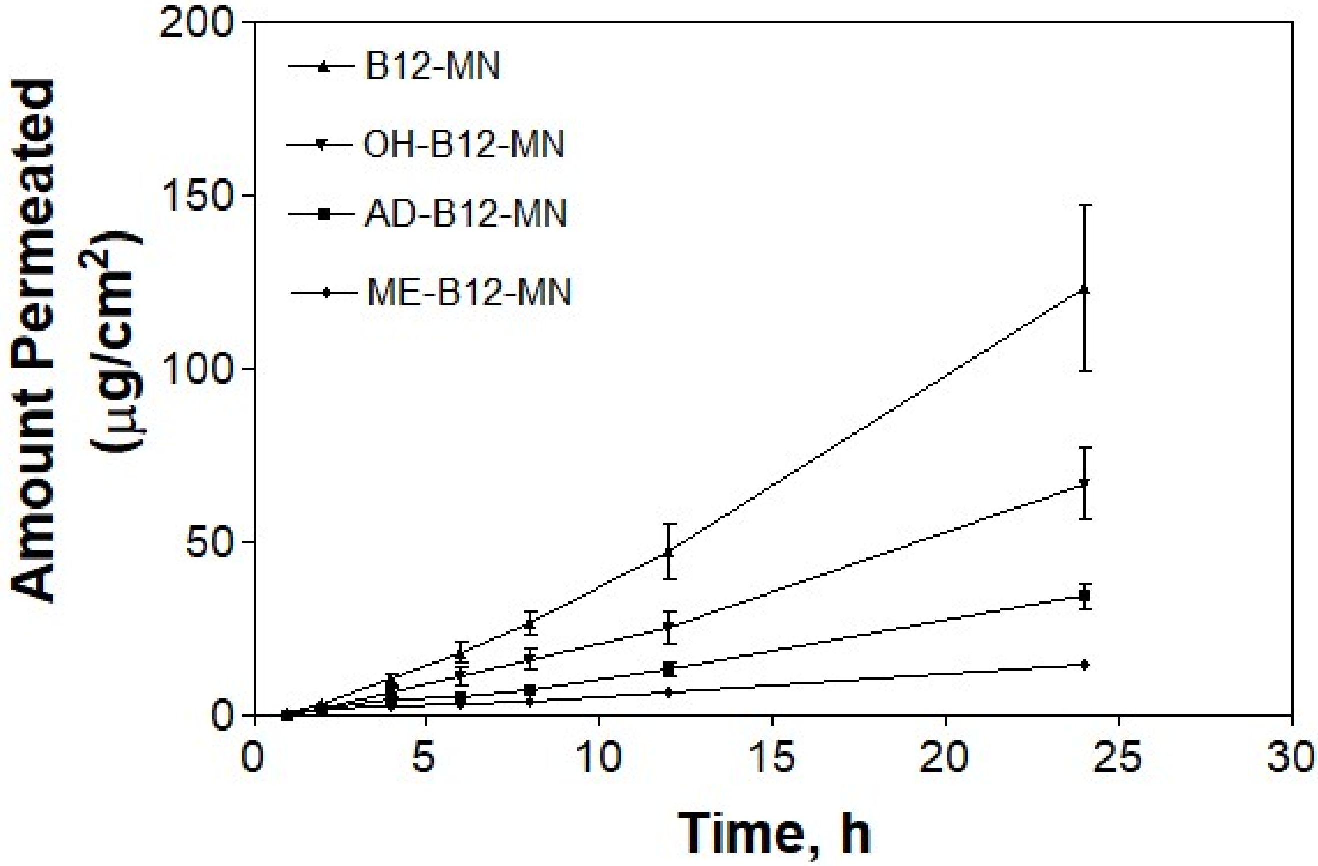


Figure 4.2. Skin concentration of vitamin B12 compounds after 24 h. Across untreated (PD) and microporated (MN) skin

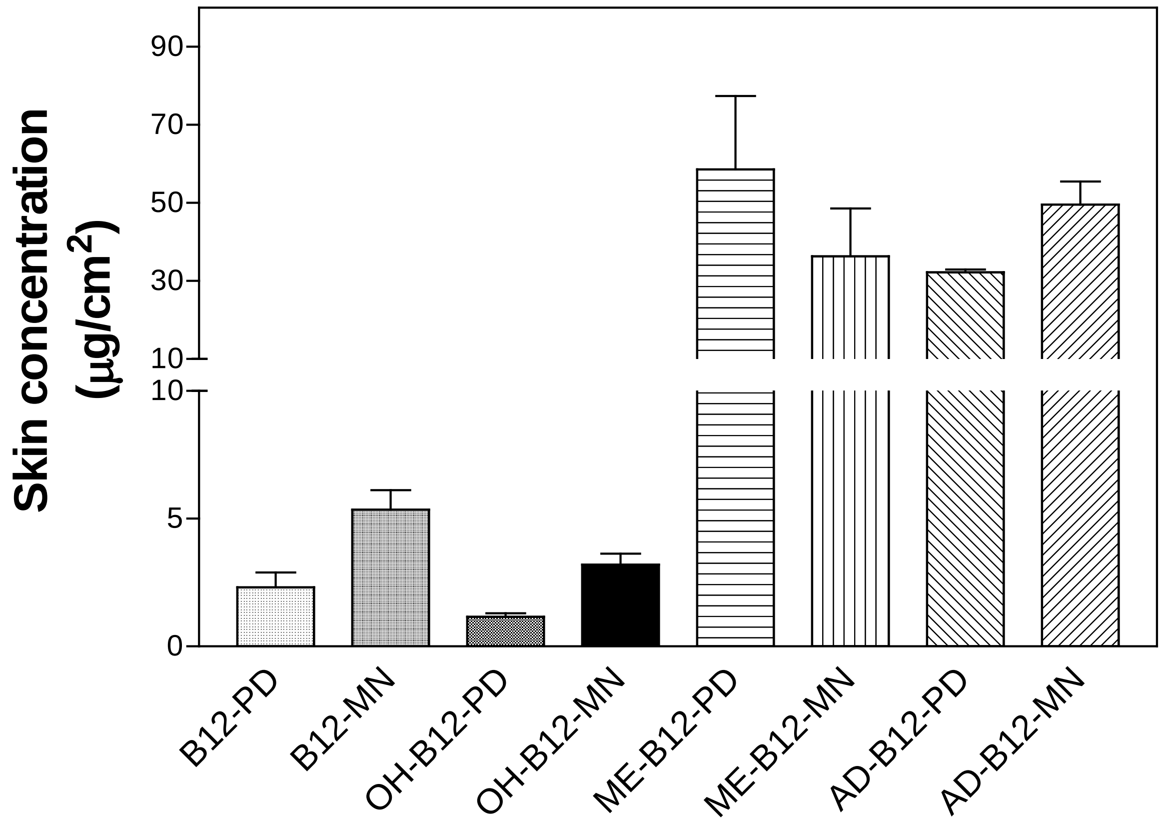


Table 4.1 Log P of vitamin B12 compounds

B12 vitamin	Molar Mass (g/mol)	log P
Cyanocobalamin	1355.38	-0.504
Hydroxocobalamin	1346.37	-1.108
Methylcobalamin	1344.40	-0.293
Adenosylcobalamin	1579.58	-0.277

Table 4.2: Skin concentration of vitamin B12 compounds after 24 h permeation study

B12 Vitamin	Avg Concentration (µg/cm^2)	
	Untreated Skin	Microporated Skin
Cyanocobalamin	2.31±0.591	5.36±0.759
Hydroxocobalamin	1.16±0.142	3.2±0.421
Methylcobalamin	58.63±18.769	36.36±12.262
Adenosylcobalamin	32.24±0.737	49.59±5.884

4.5 References

1. Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr. 2009(89), 693-696.
2. Yang Y, Kalluri H, Banga AK. Effects of chemical and physical enhancement techniques on transdermal delivery of cyanocobalamin (vitamin b12) in vitro. Pharmaceutics 2011(3), 474-484.
3. Wiedersberg S, Guy RH. Transdermal drug delivery: 30+ years of war and still fighting! J Control Release. 2014(190), 150-156.
4. Moghimipour E, Salimi A, Zadeh BS. Effect of the various solvents on the in vitro permeability of vitamin b 12 through excised rat skin. Trop J Pharm Res. 2013(12), 671-677.
5. Jung SH, Cho YS, Jun SS, et al. Topical application of liposomal cobalamin hydrogel for atopic dermatitis therapy. Pharmazie. 2011 (66), 430-435.
6. Salimi A, Zadeh BS, Moghimipour E. Preparation and characterization of cyanocobalamin (Vit B12) microemulsion properties and structure for topical and transdermal application. Iran J Basic Med Sci. 2013 (16), 865.
7. Madhaiyan K, Sridhar R, Sundarrajan S, et al. Vitamin B 12 loaded polycaprolactone nanofibers: a novel transdermal route for the water soluble energy supplement delivery. Int J Pharm. 2013(444), 70-76.
8. Genç L, Kutlu HM, Güney G. Vitamin B12-loaded solid lipid nanoparticles as a drug carrier in cancer therapy. Pharm Dev Technol. 2015(20), 337-344.
9. EPA Product Properties Test Guidelines OPPTS 830.7550 Partition Coefficient (n-Octanol/Water), Shake Flask Method. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J6ZW.PDF?Dockey=P100J6ZW.PDF. 1996.

10. Mohamied A. Effect of iontophoresis, microporation and combined iontophoresis/microporation on the transdermal delivery of cyanocobalamin (Doctoral dissertation, Long Island University, The Brooklyn Center). 2012.
11. Miyagi T, Hikima T, Tojo K. Effect of molecular weight of penetrants on iontophoretic transdermal delivery in vitro. J Chem Eng Jpn. 2006(39), 360-365.

5. Summary

Diffusion and compartmental models have been used to describe the pharmacokinetics of transdermal drug delivery. Limited information has been reported on pharmacokinetic modeling of skin permeation from microneedle or iontophoresis based skin delivery, nor has there been many studies regarding damaged skin, all of which can affect the diffusion constant of Fick's law. Population pharmacokinetics can also be used as a modeling tool to address differences in cutaneous physiology among the patient population. By addressing these issues, more accurate models for transdermal drug delivery can be developed to improve dosage form design.

The modeling techniques used in the beta blocker study were shown to be a good method for examining transdermal data. Microneedles and iontophoresis both improved the transdermal permeability of the beta blockers studied, with iontophoresis showing a greater effect. The correlation between drug properties such as lipophilicity, as Log P values, and diffusion through the skin can be seen, and may affect parameters such as skin retention of the drug and the lag time between application of the drug formulation and diffusion into the donor chamber. It remains to be seen whether alterations in the extent of microporation or the strength of the applied current with iontophoresis will have a significant effect on the amount of drug permeated.

With the use of population pharmacokinetics, transdermal absorption of an aminoglycoside antibiotic was described, even with limited data due to sampling limitations in the clinical study, as well as low to undetectable concentrations of the drug in the plasma. Transdermal absorption of the aminoglycoside can lead to systemic drug exposure when the skin is damaged as in the case of lesions. Different compartmental models were developed and evaluated to improve the accuracy of the transdermal model to account for drug accumulation during multiple dosing. On adding covariates to the model, such as the influence of age, sex, weight, treatment, and lesion size, the addition of lesion size to the model improved its ability to predict plasma concentrations. Once a transdermal model can be defined, population pharmacokinetic modeling can improve dosage regimen design by considering factors such as the lesion size and damage, as well as the varying demographics in patient populations.

The B12 derivatives in the study showed improvement in transdermal permeability with the use of microneedle treated skin. In addition, the lipophilicity of the B12 derivatives is related to the retention of the drug in the skin. Modeling the transdermal permeability might be a useful way to predict plasma concentrations to treat B12 deficiency. However, while studies have shown cobalamins to passively diffuse through various types of animal skin (rat, mice and porcine), passive diffusion through human cadaver skin was negligible. Without passive diffusion data, transdermal modeling of B12 derivatives was not pursued.

As the ability to gather data improves with technology, more information about both patient and drug concentration in plasma can be acquired. Drugs with limited oral administration feasibility due to first pass metabolism, short half-lives, or gastrointestinal adverse effects, can be made available without inconvenient methods with poor compliance such as intravenous dosing. Use of modeling techniques, applied with skin penetration enhancers, will allow future developments and a more customized dosage form design for the patient.