TOPICAL DELIVERY OF A MODEL PHENOLIC COMPOUND: ALKYLOXYCARBONYL PRODRUGS OF ACETAMINOPHEN

By

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This document is dedicated to my parents Charles and Barbara, my brother Shaun and my sister Christine.
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Looking back, it is clear that my success has depended upon the kindness and support of more friends and coworkers then I could possibly name here. Even so, a few individuals were so profoundly important that they deserve a special mention. First, I would like to thank my parents, Charles and Barbara for fostering my love of science and for providing me with a lifetime of unwavering support. In addition, I would like to thank Charles Schmidt and Ian Tebbett, for their advise and the early opportunities they provided, Nancy Szabo for years of encouragement and many campaigns on my behalf, Carolynn Diaz, for always carrying more than her share of the burden, my committee members Margaret O. James and Stephen M. Roberts, who have been helpful and accommodating of my many idiosyncrasies and John Perrin, who selflessly agreed to accept the responsibility of chairing my committee with the fore knowledge that my work would do little to advance his own research.

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Chair: John Perrin
Major Department: Medicinal Chemistry

Topical delivery is an attractive route of administration for a number of therapeutic agents. However, many drugs possess physical and chemical properties that limit their ability to permeate the skin. By masking select functional groups on a drug with proper moieties, it is possible to create prodrugs with physical and chemical properties that greatly improve topical delivery. Efficient development of these prodrugs requires knowledge of how the physical and chemical characteristics of a drug influence dermal absorption. In response to this need, solubility and diffusion experiments were performed on prodrugs of 5-flurouracil, 6-mercaptopurine and theophylline to develop a model that predicts maximum flux of these compounds through hairless mouse skin when delivered from isopropyl myristate (IPM). The purpose of this work was to expand this database to include phenol containing compounds and refine this model by synthesizing and characterizing a series of alkyloxy carbonyl derivatives and a set of methoxyalkyloxy carbonyl derivatives of a model phenolic compound, acetaminophen.
In addition, two new predictive flux models, a solubility based model to predict flux from water and a new model based upon solubility and flux through polydimethylsiloxane (PDMS) membrane as an additional parameter, were developed. As with the other prodrug series studied, the acetaminophen prodrugs with the best combination of lipid (S_{IPM}) and water (S_{AQ}) solubility, the methyloxycarbonyl and the methoxyethylxoy carbonyl derivatives, had the highest flux through mouse skin from both IPM (J_{MAQ}) and water (J_{MIPM}). The addition of APAP and its prodrugs to the IPM and aqueous databases produced Roberts-Sloan equations of \( \log J_{MIPM} = -0.501 + 0.517 \log S_{IPM} + (1 - 0.517) \log S_{AQ} -0.00266 \text{MW} \) and \( \log J_{MAQ} = -1.665 + 0.657 \log S_{IPM} + (1 - 0.657) \log S_{AQ} -0.00409 \text{MW} \) for flux through hairless mouse skin from IPM and water, respectively. These models can predict flux of a drug through hairless mouse skin from IPM with an average error of prediction of 0.16 log units and from water with an average error of 0.17 log units. A simple model was found to relate flux through PDMS membrane (J_{PAQ}) and flux through hairless mouse skin from water. Using the equation, \( \log J_{MAQ} = -1.156 + 0.245 \log S_{AQ} + 0.409 \log J_{PAQ} \), flux from water through hairless mouse skin was predicted with an average absolute error of 0.13 log units.
CHAPTER 1
BACKGROUND AND SPECIFIC AIMS

The Case for Topical Delivery

The route of administration can have a profound effect upon the utility of a drug. For a given drug, the preferred method of administration will be determined by that method’s influence on a number of factors including bioavailability, rate of drug delivery, ability to reach the physiological target and patient compliance. Historically, oral delivery has been the most common means of drug administration and it continues to be the preferred target route when developing new pharmaceutical formulations. However, despite this prevalence, there are numerous situations where an alternative route, namely topical delivery, has advantages.

Topical delivery is an obvious consideration when formulating therapies to treat skin diseases or disorders. The accessibility of the skin makes site-specific delivery relatively easy compared to internal organ systems. If a drug has sufficient ability to permeate into the skin, it is possible to achieve a high local drug concentration in target areas with a minimum amount of drug. In addition to this greater efficiency, limiting exposure to the surrounding and distant tissues in this way reduces systemic toxic effects. In some cases, even agents that cause significant complications when given orally or by injection can potentially be used topically without causing significant adverse effects. For example, the topical application of 5-FU is effective in treating actinic keratosis (Jorizzo et al, 2004) and psoriasis (Ljundggren and Moller, 1972) in amounts that spare the body from most systemic effects.
Orally delivered drugs encounter numerous physiological processes that can potentially limit their bioavailability. The acidity of the stomach and the enzymes of the intestinal lumen can degrade a drug before it has an opportunity to reach the intestinal epithelium. The intestinal epithelium can prevent absorbed drugs from reaching the intestinal vasculature by returning them into the gut immediately following absorption through p-glycoprotein efflux transporters (Kunta and Sinko, 2004). As a final hurdle, drugs entering the intestinal vasculature from the epithelium are passed via the portal hepatic vein to the liver before reaching systemic circulation. While passing through the liver, they are subjected to the body’s highest concentration of biotransforming enzymes. In particular, the lack of substrate specificity in the hepatic CYP enzymes makes numerous drugs potential targets for premature metabolism and is responsible for many, if not most, instances of low oral bioavailability (Wrighton and Stevens, 1992).

For some drugs, solving the problem of low bioavailability is no more difficult than merely increasing the dose accordingly. However, for other drugs, especially compounds that require complex synthesis or purification, this is simply not an option. A great deal of effort and ingenuity has been invested to develop delivery systems to circumvent the limitations inherent to oral delivery. However, despite this effort there are many examples of therapies that require such extensive or expensive formulation before they are amenable to oral dosing that oral delivery is not cost effective. Peptide based drugs (e.g. insulin) and some steroid based drugs (e.g., estradiol) are such instances. In these cases, delivery by an alternative route becomes attractive.

Topical delivery is one alternative that potentially can circumvent these difficulties. Topically absorbed compounds enter systemic circulation without undergoing an initial
metabolism by the liver. There is enzymatic activity in the skin, but with regard to the transformation of foreign compounds, it is primarily limited to nonspecific esterase activity. In addition, while some epidermal cells do express p-glycoprotein transporters (Laupeze et al, 2001), there is no systematic arrangement of these cells or the transporters at the skin surface, which would allow them to actively clear drugs in an efficient manner. Finally, the reported pH of the skin surface does vary widely (pH 3 to 6, Hemmingway and Molokhia, 1987), but in general it is close to 4.5, far less acidic than the interior of the stomach. Given this relatively gentle environment, dermally absorbed drugs are more likely to reach systemic circulation intact than are intestinally absorbed drugs.

Instead of high enzymatic activity and active clearance, the skin possesses a specialized barrier to prevent the permeation of foreign materials. While the skin’s permeation barrier does limit the rate with which drugs enter the skin, this same barrier can be used in a beneficial way. Since movement through the skin is slower than absorption through the epithelial cells of the intestine, dermally delivered drugs often show a more sustained and consistent serum level than orally delivered drugs. This is an obvious advantage in a number of conditions (arthritis, hypertension, chronic pain, etc.) where maintaining a constant or near constant serum drug level for an extended time is the most effective dosage regimen.

**Approaches to IncreasingTopical Delivery**

Formulation, or manipulation of the delivery vehicle, is the most popular method for increasing topical delivery. These approaches can be further divided into two categories: formulations that interact with or alter the skin and those that do not. With non-interacting formulations, the basic principle is to adjust the polarity of the vehicle by
altering its composition until optimum partitioning of the drug into the skin is achieved. However, there is an inherent limitation associated with a partitioning driven system. According to calculations based in regular solution theory, a 10-fold increase in flux is the maximum improvement accessible by altering vehicle polarity before damage to the skin occurs (Sloan, 1992). With interactive formulations, or penetration enhancers, the vehicle components are intended to reduce the skin barrier. The disruption of the barrier can be caused by the movement of solvent into the skin and/or by the leaching of components from the skin that are essential for maintaining the barrier. This is a common effect and it is well established that prolonged contact with many different solvents, both polar and non-polar, disrupts the skin and increases the flux of drugs in a reproducible manner. For example, the flux of theophylline through hairless mouse skin that has been in contact with isopropyl myristate (IPM) is approximately 50 times higher than through mouse skin that has been exposed to water (Sloan et al, 2003).

More recently, several physical methods of circumventing the stratum corneum have been designed as an alternative to interactive chemical modification. Electrical potential (Riviere and Heit, 1997) has been applied across the skin to provide an electromotive force capable of driving charged molecules though the stratum corneum. Both ultrasonic (Mitragotri and Kost, 2004) and laser (Doukas and Kollias, 2004) energy can reportedly induce temporary defects in the lipid barrier large enough to allow the transdermal delivery of macromolecules, including therapeutically significant amounts of insulin. Using techniques developed for the fabrication of computer chips, micron-sized needles have been coupled to standard transdermal patches (Prausnitz, 2004). When these patches are affixed to the skin surface, the needles pierce the skin to a depth just
beneath the stratum corneum and provide a conduit from the drug reservoir directly to the viable epidermis. The drawback of increasing flux by any disruptive method is that higher skin flux is achieved at the cost of greater damage or perturbation to the skin’s permeation barrier.

The preceding methods attempt to overcome the poor physical properties of a drug by changing the environment from which it is delivered or by changing the nature of the skin. While these methods do increase delivery under the right circumstances and can be used to improve stability, no external modification can adequately overcome limited intrinsic water or lipid solubility of the drug which limits its solubility in the skin. The most efficient method to increase solubility is to chemically modify the drug. Unfortunately, such modification often reduces or eliminates the drug’s beneficial activity. In addition, even if the modified drug remained active, it is a new entity and its pharmacokinetics and toxicity may be quite different from those of the original drug. A modified drug that persists during delivery into the body, but reverts to the original drug after absorption, would reduce the potential for such complications. Such an entity is referred to as a prodrug after the term used by Alfred nearly 50 years ago to describe a pharmacologically inactive molecule that becomes active following some biological transformation. For the purpose of drug delivery, the ideal prodrug has favorable physiochemical properties, is at least 1000 times less potent than the parent drug, is stable enough to resist premature conversion, has no more toxicity than that attributed to the parent drug and will completely revert to the parent in vivo.
Basic Theoretical Considerations of Prodrug Design for Topical Delivery and Previously Synthesized Prodrugs

There are many strategies to prodrug design, but the most direct involves conjugation of the parent drug to a moiety using a linkage that is susceptible to chemical or enzymatic hydrolysis. In such cases, the linkage and its associated side chain are collectively referred to as the promoiety. Hydroxyl, amine, amide, carboxylate and cabonyl groups are the usual functional group targets for conjugation though any sufficiently nucleophilic or electrophilic site can be used. A great number of potential promoieties have been investigated ranging from relatively simple ester type derivatives to multi-functional groups requiring a number of sequential activation and hydrolysis steps before regenerating the parent drug. As one may expect, much of the fundamental research regarding topical delivery has been accomplished using less complex promoieties. When evaluating the utility of a promoiety, it is a common practice to study a promoiety by preparing a series of produgs that share the same parent drug, attachment point to the drug and promoiety linkage, but differ by sequential addition of methylene units to the promoiety’s alkyl side chains. These homologous series of compounds have been useful for they exhibit systematic changes in some key physical properties (i.e., partition coefficient and polarity) and therefore make correlations between these properties and therapeutic behavior easier to identify.

Before an effective prodrug can be synthesized, one must first have an understanding of why the parent drug behaves poorly. Initial chemical analysis of skin components had determined that the dermal barrier was composed primarily of lipophilic compounds (Downing, 1992). This agreed with empirical observations that highly hydrophilic drugs with low lipid solubility exhibited poor topical delivery.
Consequently, attempts to improve topical delivery have centered upon preparing prodrugs that show greater lipid solubility than the parent molecules.

The ability of a drug to form strong intermolecular bonds with itself is the major obstacle to solubility in non-polar media. The strength of these intermolecular forces is reflected in the compound’s heat of fusion ($\Delta H_f$). Assuming that $\Delta H_f$ remains constant from the temperature of the solution ($T$) to the compounds melting point ($T_M$), $\Delta H_f$ can be correlated to the mole fraction solubility ($X$) of non-electrolytes through the relationship:

$$\ln X = -\frac{\Delta H_f}{RT_M} (T_M - T) - \ln \gamma$$  \hspace{1cm} (1-1)

where $\gamma$ is the activity coefficient. From equation 1, it is clear that increases in heat of fusion, melting point and activity coefficient will reduce solubility. Heat of fusion is not a routinely measured property during characterization of a new compound, but it can be eliminated from (1) by using Gibb’s relationship, $\Delta H_f = \Delta S_f T_M$

$$\ln X = -\frac{\Delta S_f}{RT} (T_M - T) - \ln \gamma$$  \hspace{1cm} (1-2)

Therefore, melting point is a conveniently determined measure of the heat of fusion and, to a first approximation, any change to the molecule that decreases melting point without disproportionately increasing the activity coefficient will improve solubility.

Hydrogen-bonding functional groups are the most common structural features that contribute to high lattice energy and the resultant low lipid solubility of non-ionic drugs. If the masking of such a group were capable of disrupting its ability to hydrogen bond, then it would be a reasonable first choice for modification. This choice is reinforced by two fortunate attributes of hydrogen bonding functional groups. First, as they are often reasonably nucleophilic and, given the number of commercially available electrophilic
reagents, they lend themselves to simple synthetic schemes that can be used to produce a variety of promieties. Second, masking even a single hydrogen-bonding site can have a profound effect on solubility. A good example of this is the difference in isopropyl myristate (IPM) solubility of 6-mercaptopurine (6-MP) and its 6-S-methyl carbonyloxyethyl derivative, where masking the SH group alone reduces the melting point from 320ºC to 124ºC and increases IPM solubility from 0.022 to 1.05 mM (Waranis and Sloan, 1988).

Until recently, work in this lab has focused upon improving the topical delivery of the heterocyclic drugs 5-FU, 6-MP and theophylline by masking amide, imide and thioamide groups on the respective parent molecules.

![Figure 1-1. Structures of the database parent molecules and the pKa values for potential promoiety attachment sites.](image)

The hydrogen-bonding moieties on these three compounds are bonded directly or through resonance to multiple electron withdrawing groups, which significantly lowers their pKa values. Being approximately as acidic as a phenol, these moieties are readily converted to their anionic form. This allows them to function readily as nucleophiles and makes their derivatization relatively simple. In addition, the stability of these anions
makes them good leaving groups, which aids the subsequent regeneration of the parent molecule from the prodrug.

By 1999, seven homologous 5-FU, 6-MP and theophylline prodrug series had been prepared and characterized. The promoieties, which were used to prepare these series, could be classified as one of two general types. In the first type, the nucleophilic site on the parent molecule was bonded directly to a carbonyl group resulting in an acyl type promoiety. In the second group, the nucleophilic site on the parent molecule was separated from the carbonyl by a methyloxy spacer to form a soft alkyl promoiety. As separate entities, these promoieties are fairly simple chemical systems. However, when they are coupled to the parent molecule, the resulting prodrugs displayed a wide range in their chemical behavior and, subsequently have different stabilities to chemical and enzymatic hydrolysis.

Figure 1-2. Probable hydrolysis mechanisms of the 1-N-acyl 5-FU prodrugs at pH 7.4.
Figure 1-3. Probable hydrolysis mechanisms of the soft alkyl promoieties.

Figure 1-2 illustrates likely hydrolysis mechanisms of the N-acyl prodrugs of 5-FU at physiological pH and demonstrates how functional groups on the parent drug can interact with the promoiety to result in unexpected behavior in the prodrug. If each 5-FU prodrug hydrolyzed via a typical addition elimination mechanism, then the expected order of stability for the N-acyl promoieties would be AAC > AOC > AC. Empirically, however, the AAC 5-FU prodrugs exhibit lower chemical stability than the AOC type prodrugs and possess half-lives of only 8 to 11 minutes, which is inconsistent with such a mechanism (Sloan et al, 1993). As an alternative, it has been suggested that once the acidic N³ H of the substituted 5-FU (pKa ≈ 6.6) (Burr and Buungard, 1985) becomes ionized, it is capable of acting through resonance with the C² oxygen as a general base in an intra-molecular E1cb type hydrolysis mechanism (Sloan et al, 1993). This is significant since it is only after the addition of the promoiety to the N¹ that the N³ becomes acidic enough to be predominantly ionized at physiological pH.
The AOC 5-FU compounds are believed to hydrolyze through an addition elimination mechanism with water acting as the nucleophile as shown in Figure 1-2 (Buur and Bundgaard, 1986). Since the AC 5-FU prodrugs have a more electrophilic carbonyl than the AOC prodrugs and are therefore better targets for nucleophilic attack, a similar mechanism would be expected for the hydrolysis of the AC promoiety. However, increasing steric bulk in the alkyl portion of the AC promoiety increases the rate of their hydrolysis which is more consistent with an $S_N1$ mechanism (Buur and Bundgaard, 1984).

The soft N and S alkyl prodrugs of theophylline and 6-MP are believed to regenerate their respective parent drugs by the initial hydrolysis of the ester portion of the promoiety followed by the decomposition of the resulting hydroxymethyl compound shown as mechanism 1 in Figure 1-3 (Sloan and Wasdo, 2003). This mechanism tends to be found in those ACOM derivatives where the masked functional group on the parent molecule is reasonably acidic (Bundgaard et al, 1985). It is of interest to note that for amide prodrugs wherein the masked amide functionality has a pKa of approximately 15, the ACOM promoiety hydrolysis mechanism changes to the $S_N1$ process shown in mechanism 2 of Figure 1-3 (Bundgaard et al, 1991). Since both of these mechanisms occur relatively slowly at neutral pH, the ACOM prodrugs are stable for several hours in aqueous solution.

In addition to the wide range of chemical properties, the heterocycle prodrugs demonstrated a wide range of physical properties. Melting points for these compounds range from 57.5-212 °C, IPM solubility ranged from 0.3-174 mM and aqueous solubilities ranged from 0.001-182 mM (Roberts and Sloan, 1999).
Along with their physical properties, the abilities of these prodrugs to permeate hairless mouse skin from saturated solutions of prodrug in IPM were also measured. The seven homologous series contained a total of 39 prodrugs. To these compounds, solubility and IPM flux data for 5-FU, 6-MP, theophylline and the pivaloyl ACOM 5-FU were added to produce the largest set of compounds for which physical properties and *in vitro* flux data had been measured under the same experimental conditions by the same laboratory.

The purpose of compiling this database was to provide empirical data for new predictive flux models. Flux and permeation models for topical delivery had been developed by a number of researchers and their development will be discussed in detail in chapter 3. For now, it is sufficient to note that each model was based upon lipid solubility being the principle predictor of absorption into the skin. Whether lipid solubility was determined from solubility in a model lipophilic solvent or from a partition coefficient, these models shared a common deficiency in predicting the behavior of homologous series. For each series in the 43 compound database, the more water-soluble member of the series had the highest flux through mouse skin (Roberts and Sloan, 1999). The previously published flux models failed to predict this qualitative observation. Using the permeability model of Potts and Guy as a foundation, Roberts and Sloan were able to produce a flux model that overcame this deficiency and quantified the positive influence of aqueous solubility in determining flux (Roberts and Sloan, 1999). In subsequent work, this same model was applied to data obtained from drugs delivered from mineral oil through human skin *in vivo* (Roberts and Sloan, 2001). As with the *in vitro* mouse skin data, aqueous solubility was shown to exert a positive influence.
Specific Objectives and Preliminary Work

First Objective

The first objective of this work is to expand the 43 compound database with an additional series of prodrugs based upon a new type of parent molecule. Given the benefits and limitations inherent to topical delivery, it seemed reasonable to examine situations for which sustained long-term delivery is the preferred regimen. Of course, the treatment of disease is not the only instance were it is desirable to administer compounds in this way. It is well established that the two leading causes of death in the United States in 2001 according to the CDC’s National Center for Health Statistics, cancer and heart disease, are conditions that develop over many years. Once either of these diseases reach an advanced state, they are often impossible to treat. Given the difficulty of treatment for advanced conditions, a prophylactic approach to prevent or slow their onset is desirable. In the last decade, there has been an increased interest in the use of natural products to provide such prevention and to increase general health. While the beneficial attributes of many natural preparations are undoubtedly over-stated, the benefits of some naturally occurring compounds merit further research.

A number of epidemiological studies report a lower cancer rate among individuals whose diets are rich in certain fruits and vegetables (Lui, 2004). While non-dietary influences in these studies are hard to control and they often suffer from poor data collection (Michels, 2005), several compounds are suspected of being responsible for these benefits. Specifically, the polyphenolic flavanoids, or more commonly polyphenols, are thought to play an important role in chemoprevention (Yang et al, 2001). Polyphenols are a large class of plant-derived compounds that contain either a flavone or flavanone structure and multiple hydroxyl groups. The phenolic groups are
often glycosylated or alkylated by the plant (presumably to protect them from oxidation) and it is these forms that they are principally found in the diet. During digestion, the hydroxyl groups are uncovered. This is an important process, as many of the beneficial effects attributed to polyphenols are likely to require the presence of at least one free phenolic hydroxyl group.

Polyphenols demonstrate an influence on numerous physiological processes \textit{in vitro} and \textit{in vivo}. They can function as antioxidants and they have been shown to protect against oxidative stress \textit{in vitro} and \textit{in vivo} (Vinson, 1998), although the clinical significance of this has not been determined. Polyphenols reportedly have the potential to modulate radical mediated signaling pathways. Of these, the ability to inhibit nitric oxide synthase may be significant due to its participation in inflammatory processes (Wu and Meninger, 2002). Specific polyphenols have been reported to modulate enzymes involved in carcinogenesis, namely tyrosine kinase (Lin, 2004) and NF kappa B (Kundu and Surh, 2004). Perhaps more importantly, polyphenols inhibit CYP-450 enzymes and may reduce the bioactivation of procarcinogens. This inhibition is presumably the mechanism by which green tea phenols prevent methylcholanthrene and phorbol acetate induced tumor genesis in mouse skin (Wang et al, 1989). While it is still unclear which effect or combination of effects is responsible for the perceived benefit of polyphenols, if they are efficacious, then having a chronic supplementation based upon this class of compounds and a method to dermally deliver them would be useful.

The triphenolic flavanone naringenin (4’, 5, 7-trihydroxyflavanone), a well-studied inhibitor of hepatic CYP-450 3A4 (Geunerich and Kim, 1990), was investigated for our initial experiments.
The melting point of naringenin is 247-250°C. When it is reacted with excess acetyl chloride, the resulting triacetal product has a melting point of 72-85°C (unpublished results), indicating a substantial drop in lattice energy. However, even with the substantial drop in melting point, the aqueous and IPM solubility of the triacetal as well as its flux through mouse skin remained very low (unpublished results). When attempts to prepare and purify diesterified derivatives of naringenin and other flavanoids proved especially difficult, it was decided to seek a simpler phenol containing model compound. Hydroquinone was considered as a model compound, but it was discarded after the mono-acetate was found to disproportionate into the diacetate and hydroquinone during purification. Acetaminophen (APAP) was eventually chosen as a replacement. Though APAP itself is not polyphenolic, it was considered relevant because it possesses structural features similar to polyphenols. In particular, APAP contains one phenol, has a predominantly planar shape, aromatic character and an amide group, which, along with the phenol, allows it to form multiple hydrogen bonds. More importantly, it was anticipated that the difference in pKa values between the phenol and amide groups (approximately 10 versus 13 respectively) would allow selective ionization and
consequently derivatization of the phenolic oxygen without a significant amount of competing derivatization at the amide nitrogen.

**Second Objective**

The choice of promoiety was guided by the second objective of this work. Although the Roberts-Sloan equation was developed specifically for delivery from IPM and was initially applied to a relatively specialized group of compounds delivered from a single vehicle, its theoretical foundation suggested that it should have wider applicability. If an aqueous vehicle is assumed in the derivation of the Roberts-Sloan model, the final form of the equation remains unchanged. As a result, the model could be used to predict flux from IPM or water depending upon which values are used for the x, y and z coefficients. To estimate the coefficients for the aqueous model, Sloan et al (2003) collected the eighteen most chemically stable ACOM prodrugs present in the 43 compound database and measured their flux through hairless mouse skin from unbuffered water. To make the coefficients of the model more significant, more compounds would be needed in the dataset. In order to add the APAP prodrugs to this group, they would need to contain promoieties that were resistant to hydrolysis in aqueous solution.

Despite their prevalence among commonly prescribed drugs, very few phenolic compounds have been converted to prodrugs in an effort to improve their topical delivery. Phenol masking prodrugs of only four related compounds, morphine (Drustrup et al, 1991), bupenorphine (Stinchcomb et al, 1996), nalbuphine (Sung et al, 1998) and naltrexone (Stinchcomb et al, 2002) have been prepared and studied in flux experiments. The promoieties for most of these studies have been limited to simple alkyl esters. Although they improved topical delivery of these narcotic analgetics, simple alkyl esters, especially esters of phenols, are too unstable for prolonged exposure to aqueous solution.
Swintowski (in Dittert et al 1963) synthesized some simple alkyl esters of acetaminophen along with the corresponding alkyl carbonate esters and studied their stability to chemical and enzymatic hydrolysis. As expected, he found that the less electrophilic carbonate esters hydrolyzed times more slowly in pH 7 aqueous buffer than the more electrophilic alkyl esters and both groups were susceptible to enzymatic hydrolysis. Even though Swintowski did not synthesize all of the alkyl homologs, he did synthesize the methyl carbonate, which is expected to undergo chemical hydrolysis more rapidly than any of the longer chain carbonates. Given the long half-life of the methyl carbonate, we concluded that the carbonate functional group had adequate stability to serve as a promoiety for the APAP prodrugs.

Since the importance of increasing biphasic solubility when optimizing topical delivery is now well established, it may seem counterproductive to only study promoieties that contain alkyl side chains of ever increasing length. While these promoieties can produce compounds with reduced crystal lattice energy, their other properties are counter productive to good water solubility. When a compound dissolves in water, each solute molecule unavoidably disrupts a number of water-water hydrogen bonds by displacing water molecules from the volume it must occupy. Thermodynamically, this process has a substantial negative enthalpy. Once in solution, any functional groups on the solute molecule that are capable of forming new interactions with surrounding water molecules can reduce this enthalpy cost. Alkyl groups are only capable of interacting through relatively weak Van der Waals forces and, therefore, do little to recover the enthalpy that was lost during dissolution. It can be conjectured that
replacing the alkyl side chains with entities that are capable of forming beneficial interactions would increase aqueous solubility.

The challenge behind this approach is to find a functional group that has a large enough dipole moment to interact favorably with surrounding water molecules without unduly increasing crystal lattice energy. Polyether functional groups have these properties. Straight chain alcohols larger than propanol are immiscible with water. By contrast, polyethyleneglycols (PEGs) continue to be soluble in water even when their molecular weight grows over several thousand amu. Greenwald has used conjugation to large PEG molecules to improve the water solubility of a number of poorly soluble amine, imide and hydroxyl containing compounds (Greenwald et al, 2000). However, Greenwald favors the use of high molecular weight PEG conjugates (>20,000 amu), with the promoiety comprising the overwhelming majority of the prodrug’s total mass. These large PEG prodrugs remain water-soluble primarily because their characteristics remain close to those of the unconjugated PEGs. For promoieties containing only a few or even one ether promoiety, the question becomes to what extent will the replacement of a methylene group by an oxygen improve water solubility? To address this question, two additional promoieties will be synthesized that have such a modification; a methoxyethoxycarbonyl APAP and a methoxyisopropoxy carbonyl APAP. It is hypothesized that for both types of prodrugs the best performing compounds will be those that possess the best combination of lipid and water solubility.

**Third Objective**

Mammalian skin continues to be the membrane of choice for assessing drug permability *in vitro*. Although there are substantial differences in the thickness of the various skin layers between species, the general histology, chemical composition and
physiochemical properties of the skin barrier are reasonably similar for all mammals. Therefore, while absolute permeability varies with skin thickness, the relative flux of compounds through mammalian skin remains consistent. In other words, those compounds that diffuse most readily though human or pig skin are likely be the same compounds which diffuse most readily though mouse or rat skin. In particular, if the compounds of a given homologous series are ranked according to their flux through hairless mouse skin, it has been well established that this order will match the rank order of their flux through human skin (Scheuplein and Blank, 1973, and Sloan et al, 1997).

This ability of hairless mouse skin to predict the best performing members of a homologous series is one of the attributes that has made it a popular choice for flux experiments. In contrast to human skin, mouse skin has much less variability and data collected from mouse skin experiments can be used without normalization. This obviates the multiple additional control diffusion cells, which must be run with in vitro human skin experiments. This consistency also allows even small differences in fluxes to be discerned. Unlike human skin, which is usually dermatomed or exposed to heat or enzymatic digestion to isolate the outermost skin layers prior to use, hairless mouse skin can be used full thickness and requires little more preparation than removal from the mouse.

Despite these advantages, the use of mouse skin, or any mammalian skin, does have drawbacks. The care of the animal prior to use is expensive and special care must be taken to maintain the integrity of the skin for the duration of the experiment. Increasing regulatory requirements and ethical concerns about the use of animals in research makes finding an artificial membrane that can be used in the place of animal
tissue desirable. Most attempts to predict skin permeation using an artificial membrane have focused upon finding a surrogate material whose properties are sufficiently similar to human skin to allow a direct comparison. Untreated lipophilic polymers such as polydimethyl siloxane (PDMS) (Geinoz et al, 2002, and Cronin et al, 1998) and solvent modified lipophilic polymers (Twist and Zatz, 1990, Maitani, 1996, and DuPlessis, 2001) have been popular systems for this purpose. However, none of these systems have proven particularly useful at predicting flux through skin.

The complexity of the skin makes it unlikely that simply knowing the flux of a drug through a simple polymer, modified or not, will be sufficient to predict topical delivery. Despite this limitation, there are aspects of any compound’s diffusion behavior which hold true regardless of the nature of the membrane. For example, the kinetics of a compound’s dissolution, as well as its propensity to cluster or stack in solution, can affect its flux in a manner that is difficult to determine from its solubility alone. Therefore, despite its inability to predict flux through skin directly, a compound’s flux through a polymer membrane may still contain information that is useful for predicting its flux through skin. However, extracting and utilizing this information will likely require a more sophisticated treatment then has yet been reported.

Thus, it is the final objective of this research to produce a predictive model for flux through hairless mouse skin that is based upon its flux through PDMS membrane in addition to its solubility properties. PDMS membrane is a rubbery polymer composed of cross-linked chains of repeating Me₂SiO units. As a rubbery polymer, the chains that comprise the membrane are flexible and return quickly to an equilibrium position after being disturbed. Like diffusion through the skin, diffusion in such a material is believed
to be Fickian in nature (Crank, 1975). It is expected that the additional information provided by PDMS flux data will improve the predictability of the solubility-based models and this will serve as first step in creating an artificial membrane system that will be useful in screening for topical delivery.
CHAPTER 2
BASIC ANATOMY OF THE SKIN

Taken in its entirety, the skin is the largest organ in the human body. On average, it accounts for approximately 10% of the total adult body weight and covers a total area of 1.5 to 2 m² (Schaefer and Redelmeier, 1996). As the principle interface with our environment, the skin must be capable of simultaneously providing sensory input and functioning as a barrier to the transfer of materials into and out of the body. Specifically, the skin allows the body to effectively control fluid loss, regulate body temperature, protect against physical trauma, defend against microbial infection and provide tactile sensation. To perform these functions, the skin contains numerous specialized cells and tissues arranged with a specific architecture. While modern imaging techniques have revealed that the skin is composed of a complex microanatomy, to the naked eye, the skin appears as three superimposed layers. From deepest to most shallow, these layers are known as the hypodermis, the dermis and the epidermis.

While the skin contains a wide variety of cells and microstructures, many of them do not significantly affect the bioavailability of topically applied substances. In the following discussion, greater attention will be paid to what defines the environment of the skin and to those elements that are most likely to impact topical absorption

Hypodermis

The hypodermis, or subcutaneous tissue, contains loose connective fibers and associated adipose tissue. This connective tissue serves to anchor the overlying layers of the skin to the body and the adipose tissue functions as a carbohydrate source and thermal
insulator. It varies greatly in thickness throughout the body and between individuals. It is usually thickest around the trunk of the body and thinnest on the backs of the hands and feet. The hypodermis has a less extensive vasculature than the outer layers of the skin but it does house the larger blood vessels that ultimately feed the rest of the integument. Since the hypodermis does not contain the same specialized tissues as the other skin layers and since it does not actively participate in the regulatory functions of the skin, it is usually no longer classified as properly belonging to the skin.

**Dermis**

The dermis is responsible for the skin’s structural integrity and, with the exception of controlling water loss, performs most of its critical functions.

The tensile strength and flexibility of the dermis are the result of a tightly knit mesh of collagen and elastin fibers (Ushiki, 2002). This collagen lattice defines the dermis and provides a relatively fixed scaffolding onto which the other structures of the dermis are anchored. Both collagen and elastin are synthesized from water-soluble precursors that are secreted into the intracellular space by the most common cellular component of the dermis, the fibroblast. After leaving the fibroblast, these precursors undergo modification and are assembled into thin fibrils. In the deeper region of the dermis (the reticular dermis), primary collagen fibrils are further assembled into thick, closely grouped, and extensively cross-linked strands that run roughly parallel to the skin surface. Nearer to the epidermis (the papillary dermis), the strands become thinner and less heavily cross-linked to allow space for the proliferation of capillaries and nerve endings.

Surrounding the collagen lattice and filling most of the dermal intracellular space are extremely hydrophilic macromolecules known as proteoglycans. Proteoglycans are a group of fibroblast derived compounds that contain numerous unbranched polysaccharide
chains covalently bound to a common polypeptide backbone (Iozzo, 1998). The polysaccharide side chains are composed of several hundred repeating glycosaminoglycan disaccharides that have been sulfated to a high degree. The large number of sulfate and carboxylate groups gives a strong anionic charge to the polysaccharide side chains and causes them to repel one another. In response to this repulsion, the side chains are forced to nearly full extension from the peptide backbone and the molecule, in turn, resists compression. In addition, a large amount of water remains associated around the sulfate and carboxylate groups in hydration spheres. The ordering of water around the ionized proteoglycans gives the environment of the dermis properties similar to those of a hydrophilic gel. Small water-soluble molecules move with relative ease but highly lipophilic or high molecular weight molecules are impeded.

A multitude of anatomical structures is found within the dermal matrix (Thibodeau and Patton, 2003). Corpuscular nerve endings that are sensitive to vibration and pressure (Meissner and Pacinian corpuscles) are present as well as free nerve endings that are sensitive to pain and temperature. Smooth muscles cells control the dilation of blood vessels and follicular arrector pili cause the familiar appearance of goose bumps. Resident immunologically active Mast cells and phagocytic macrophages provide a second line of defense against foreign microorganisms as do dermal dendrocytes and circulating t-lymphocytes. However, of the many structures of the dermis, the three that have the highest potential impact on topical delivery are the hair follicles, the sweat glands and the capillary vasculature.

Hair follicles and exocrine sweat glands are rooted in the reticular dermis, but they both have pores that extend to the skin surface. These pores provide openings in and
through the epidermis and its permeation barrier. Bypassing this barrier allows free
diffusion down the sweat duct or hair follicle and allows direct access to the dermis.
However, this access is balanced by the physical dimensions of the pores. While there
are several million follicles and exocrine glands distributed over the skin surface, the
individual pore size is so small that the total cross sectional area of all pores is less then
0.1% of skin surface area (Schaefer and Redelmeier, 1996). Therefore, the pore-
mediated pathway should only be important for compounds whose ability to penetrate the
epidermal barrier is very low.

The epidermis contains no vasculature of its own and must depend upon dermal
blood vessels to supply essential compounds and remove waste material (Thibodeau and
Patton, 2003). To facilitate this process, the border between the dermis and the epidermis
is uneven with papillae from each layer interlocking with one another. Inside the dermal
papillae are capillary plexuses from which nutrients and oxygen move by diffusion. The
papillary structure of the border assures that epidermal cells surround each plexus. This
arrangement increases the likelihood of dermal nutrients reaching epidermal cells and
increases the efficiency with which epidermal waste products reach the dermal
capillaries. Similarly, this design increases access to the capillaries for any exogenous
compound that reaches the lowest level of the epidermis. With several thousand dermal
plexuses per each square centimeter of skin, this route is the principle entry into systemic
circulation for topical agents.

In addition to being the most readily available path to systemic circulation, the
capillary plexus is also an effective sink. In a static system, the volume of blood
contained in the vessels of an individual plexus is small and only a nominal mass of
absorbed material is necessary to produce a high local concentration. However, in the living system, the blood flow through the plexus prevents stagnant accumulation and dilutes absorbed compounds into systemic circulation. For poorly water-soluble molecules, serum proteins such as albumin should facilitate this process in a manner similar to what is observed with in vitro experiments (Cross et al, 2003). Many soluble proteins have nonpolar pockets into which lipophilic compounds can partition. Therefore, soluble proteins represent an additional phase that reduces the thermodynamic activity exhibited by bloodborne non-polar molecules and, concurrently, increases the blood’s capacity to carry them.

**Epidermis**

Unlike the multi-functional dermis, the epidermis is primarily designed to perform one function; provide a barrier that prevents the loss of water and essentially seals the skin against the entrance of foreign materials. The epidermal barrier covers over 99% of the body surface and it is this barrier that must be circumvented to effectively deliver drugs topically. Ironically, most of the body the permeation barrier is contained in only the outermost 10%-20% of the epidermis (Elias and Friend, 1975). The formation of these few essential µm of integument represents the final stage in a well-orchestrated transformation of viable epidermal cells into an inert and physiologically unique matrix.

Keratin-producing epithelial cells, keratinocytes, account for over 95% of the epidermal cell volume and are ultimately responsible for the formation of the barrier (Steinert et al, 1991). They are formed from resident stem cells attached to the basement membrane, which is a specialized collagen structure that defines the lower limit of the epidermis and connects it to the dermis. When a keratinocyte stem cell divides, it produces a daughter stem cell, which remains attached to the basement membrane, and a
transit-amplifying cell that begins to differentiate once it detaches from the membrane (Watt, 1989). As the daughter stem cells repeat this process, the formation and migration of new transit-amplifying cells forces older cells closer to the skin surface. Since the rates with which both stem cells divide and transit cells mature are coordinated, keratinocytes at similar stages of development are found at roughly equivalent depths of the epidermis. The difference in morphology between the various stages of keratinocyte development has resulted in the epidermis being divided into the five visually distinct regions

The first region, the stratum basale or stratum germinativum, is comprised of the epithelial stem cells and recently formed transit-amplifying cells. These cells appear columnar and have large nucleii. In the stratum spinosum (the next 2 to 7 cell layers), the keratinocytes have lost the columnar organization of the basal cells and have begun to elongate. The name of this layer refers to the many surface protein complexes (desmosomes) attaching one keratinocyte to another, which give the cells serrated edges (Burge, 1994). The stratum granulosum contains the uppermost two or three viable cell layers and is the region in which keratinocytes make the most rapid and dramatic transformation. The cells flatten markedly and a simultaneous disintegration of the nucleus occurs. Stratum granulosum cells contain a large number of small granular deposits that give them a distinctive speckled appearance. The outermost layer, the stratum corneum, begins with the appearance of closely packed layers of corneocytes. Corneocytes are thin, proteinaceous and roughly polygonal with a homogeneous internal structure. They remain essentially unchanged throughout the stratum corneum and are intact when released from the skin surface. Several in vitro and in vivo experiments, and the study of diseases that impair stratum corneum formation, have consistently indicated
that this layer provides most of the permation barrier (Scheuplein and Blank, 1971 and Lavrijsen et al., 1993). On the palms of the hands, soles of the feet, and wherever the skin is subjected to a large amount of mechanical stress, the stratum corneum can contain 30 or more corneocyte layers and be thicker than the viable epidermis. However, for most of the body, the stratum corneum is much thinner and is composed of 5-20 corneocyte layers.

When the epidermis is studied on the submicron scale, it is clear that the formation of the stratum corneum is a process that begins several cell layers beneath the first discernable corneocytes in a light microscope image. At this scale, the subcellular granules, that are numerous in the stratum granulosum, can be resolved into two separate functional entities; keratohyalin granules and lamellar bodies.

Keratohyalin granules (KG) produce the structural proteins that are used in the assembly of the corneocytes. Some of these proteins (principally loricrin and involucrin) are used to create a cornified envelope that forms the corneocyte’s outer surface. The envelope begins to form as a thin layer just inside the apical membranes of keratinocytes in the upper stratum spinosum but thickens rapidly throughout the straum granulosum as transaminase enzymes attach additional layers (Stevens et al., 1990). Other KG generated proteins, principally the keratins K1 and K10, and the keratin aggregating protein filaggrin, are crosslinked across the inside of the cornified envelope to form the corneocyte core (Eckert, 1989). When fully formed, the corneocyte has a sufficient number of internal protein filaments to resist swelling upon contact with water and to resist permeation by other exogenous compounds.
Lamellar bodies (LBs) are the second most prevalent granular organelle found in viable epidermal tissue. Like KGs, LBs are first observable in the upper layers of the stratum spinosum and they increase in number within the stratum granulosum. LBs process and store a complex mixture of lipids and lipid-like materials which ultimately control the loss of water from the skin (Roberts et al, 1978). Along with free fatty acids and cholesterol, this lipid matrix contains a number of hydroxylated amide compounds known as ceramides.

Figure 2-2. Structures of the principal ceramides comprising the lipid bilayers of the lamellar bodies.
Electron micrographs reveal that the lipid components in LBs are often arranged in an ordered pattern that resembles compressed and stacked micelles (Fartasch et al, 1993). On the border between the stratum granulosum and the stratum corneum, LBs migrate to the keratinocyte membrane and their lipid components are expelled into the intercellular space where they are reformed into larger stacked sheets. Concurrently, a single layer of lipid components, primarily ceramides 1 and 2, is bound covalently to the surface of the corneocyte (Wertz et al, 1989). In the fully formed stratum corneum, the inter-keratinocyte lipid components produce a characteristic electron micrograph pattern of alternating light and dark bands that run parallel to the surfaces of the keratinocytes. This pattern is generally thought to indicate that final arrangement of the lipid components in the stratum corneum is a nearly continuous series of stacked and planar lipid bilayers.

It is important to note that the composition of the inter-corneocyte lipids is different from the lipid composition of the lamellar bodies. Phospholipids and glucosylated ceramides constitute a significant proportion of the lamellar body lipids but they are essentially absent in the stratum corneum. In fact, while it contains some ionizable components such as free fatty acids, amino acids and a small amount of cholesterol sulfate, the stratum corneum lipid matrix is predominantly composed of neutral molecules (Lampe et al, 1983). The principle polar functional groups of the matrix are the ceramide hydroxyl and amide groups. They are capable of hydrogen bonding but there are only a small number of these groups per molecule. The polar functional groups of the ceramides do associate to form hydrophilic planes within the lipid lamella but these planes are thin compared to the alkyl regions. It is a reasonable speculation that removal of the highly acidic phosphate and the poly-hydroxylated sugar moieties is necessary to
limit the water permeability of the stratum corneum and to remove groups that would disrupt the cohesiveness of the lipid lamella.

Studies of model systems suggest that the specific molecular arrangement or phase of the lipids in the lamellae influences permeability as much as the chemical composition (Xiang et al, 1998). As such, there has been a fair amount of effort expended in the attempt to experimentally determine the arrangement of lipids in the stratum corneum. X-ray diffraction, NMR, FTIR and electron microscopy have all been used to gather data from the stratum corneum and compare it to the data from model systems, but the results have been difficult to interpret and sometimes contradictory (Hsueh et al, 2004 and Pilgram and Bouwstra, 2004). Many variations of the bilayer lipid arrangement have been observed in the model systems, but they can generally be classified into one of three broad phases (Sparr and Engstrom, 2004). Lipids in a solid crystalline phase (characterized by an orthorhombic or triclinic packing) have the highest cohesive forces, the least freedom of movement and the lowest permeability. Lipids in a gel phase (characterized by a hexagonal packing) have greater rotational freedom and a higher permeability. Lipids in a liquid crystal phase lack a discrete arrangement and have the highest permeability. Many researchers have reported that the stratum corneum matrix contains or can adopt many phases depending upon environmental factors such as temperature, humidity or pH. Others have suggested that the high amount of cholesterol precludes the existence of the more ordered phases and that a single-phase model is more appropriate (Norlén, 2001). Given the number of components contained in the lipid matrix and the changes in water content and temperature between the viable epidermis and the skin surface, it seems unlikely that a single phase can exist throughout the stratum
corneum. For the purpose of predicting dermal delivery, such discussions, while interesting, are perhaps less germane. Ultimately, the most useful description of the skin will be a functional description derived from the skin’s interaction with other compounds rather than a physical description based upon the skin alone.
CHAPTER 3
DEVELOPMENT OF THE PREDICTIVE SOLUBILITY AND FLUX MODELS

Derivation of the Series Specific Organic and Aqueous Solubility Equations

To a large extent, a drug’s solubility properties determine its transdermal flux and much of its other physiological behavior. Unfortunately, since a compound’s solubility is impossible to predict a priori, the only certain means of identifying those prodrugs with optimal solubilities is to synthesize them. In keeping with this limitation, understanding the behavior of a given promoiety has traditionally required the synthesis of many compounds in which the parent drug is joined with many alkyl homologs of the promoiety. Comparing the behavior of two promoieties has only been done once data from several examples of each promoiety has been collected. This approach presents a potential difficulty in estimating the relative performance of the 4-AOC and 4-MOAOC APAP compounds given that only two members of the latter series were synthesized. With such limited data, the relevant question becomes can the behavior of a series be estimated from the performance of two, or even one, of its members? In other words, are there parameters or descriptors, common to each member of a series, which can be used as a means of comparison? The following discussion identifies these parameters and illustrates their use.

The dissolution of a crystalline nonelectrolyte is a complex process that is inaccessible to direct mathematical treatment. However, the free energy of this transformation can be described. Free energy is a state function. Therefore any path or combination of paths (actual or hypothetical) that begins with the crystalline state and
end with the solvated state can be used to calculate free energy for dissolution. With this in mind, it is convenient to separate the dissolution process into two sequential events; the removal of a molecule of solid from the crystal lattice (decrystallization) and the movement of this freed molecule into solution. Both of these events have free energies associated with them and the sum of these energies equals the free energy of the overall process:

$$\Delta G_D + \Delta G_{Mix} = \Delta G_{Dis}$$  \hspace{1cm} (3.1)

where $\Delta G_D$ is the free energy of decrystallization, $\Delta G_{Mix}$ is the free energy of mixing and $\Delta G_{Dis}$ is the free energy of dissolution. In a saturated solution, the crystalline form of the drug is in equilibrium with the solvated form and $\Delta G_{Dis}$ is equal to 0.

$$\Delta G_D + \Delta G_{Mix} = 0$$

$$\Delta G_D = -\Delta G_{Mix}$$  \hspace{1cm} (3.2)

Each side of this equation can be expanded using the Gibbs relationship:

$$\Delta H_D - T\Delta S_D = -\Delta H_{Mix} + T\Delta S_{Mix}$$

The enthalpy ($\Delta H_D$) and entropy ($\Delta S_D$) of decrystallization specifically refers to the energy required to remove a molecule of the drug from the crystal lattice at the solution temperature (T). These are not readily obtained quantities. However, the enthalpy ($\Delta H_F$) and entropy ($\Delta S_F$) of fusion can be used in their place if the heat capacities of the crystalline and molten forms of the drug are equal to one another and reasonably constant over from the solution temperature to the melting point. With this substitution,

$$\Delta H_F - T\Delta S_F = -\Delta H_{Mix} + T\Delta S_{Mix}$$
Since $\Delta H_F = T_M \Delta S_F$ (where $T_M$ is the melting point of the drug), this can be rewritten as

$$\Delta S_F (T_M - T) = -\Delta H_{\text{Mix}} + T \Delta S_{\text{Mix}} \quad (3.3)$$

According to ideal solution theory, $\Delta S_{\text{Mix}}$ is related to the mole fraction of the components in solution by the equation:

$$\Delta S_{\text{Mix}} = -(n_1 R \ln x_1 + n_2 R \ln x_2) \quad (3.4)$$

where $n_1$ and $x_1$ are the amount and mole fraction of the drug in solution and $n_2$ and $x_2$ are the amount and mole fraction of solvent. If there is no substantial loss of entropy upon mixing due to factors such as solvent ordering, then the ideal expression for $\Delta S_{\text{Mix}}$ can be substituted into equation 3.3.

$$\Delta S_F (T_M - T) = -\Delta H_{\text{Mix}} - T(n_1 R \ln x_1 + n_2 R \ln x_2) \quad (3.5)$$

This expression can be further simplified if the solubility of the drug in the solvent is low (< 1%). In this case, the mole fraction of the solvent is approximately equal to 1 and equation 3.5 reduces to

$$\Delta S_F (T_M - T) = -\Delta H_{\text{Mix}} - RTn \ln x$$

$$\frac{\Delta S_F (T_M - T) + \Delta H_{\text{Mix}}}{RTn} = -\ln x \quad (3.6)$$

where $n$ and $x$ now refer only to the amount and mole fraction of drug respectively. If the $\Delta S_F$ and $\Delta H_{\text{Mix}}$ are presumed to be molar quantities, then the amount of drug in solution, $n$, disappears from the equation.

$$-\left(\frac{\Delta S_F (T_M - T) + \Delta H_{\text{Mix}}}{RT}\right) = \ln x \quad (3.7)$$

To convert equation 3.7 into an expression for solubility as amount per unit volume, a dilute solution assumption is again used:
\[ x = \frac{n_1}{n_1 + n_2} \approx \frac{n_1}{n_2} \]

With this assumption, the volume of the solution (V_S) is equal to the molar volume of the solvent (V_2) multiplied by the amount of solvent (n_2). Therefore:

\[ S = \frac{n_1}{V_s} = \frac{n_1}{n_2 V_2} = \frac{x}{V_2} \]

\[ \ln S = \ln x - \ln V_2 \]

Substituting this into equation 3.7 yields:

\[ -\ln V_2 - \frac{\Delta H_{\text{Mix}}}{RT} - \frac{\Delta S_F}{RT} (T_M - T) = \ln S \]

If both sides of the equation are divided by 2.303, the equation is converted from the natural log to the more common base ten log:

\[ -\frac{RT \ln V_2 - \Delta H_{\text{Mix}}}{2.303RT} - \frac{\Delta S_F}{2.303RT} (T_M - T) = \log S \quad (3.8) \]

Equation 3.8 is an expression for solubility in what Hildebrand referred to as a regular solution; i.e. a solution that possesses a non-zero enthalpy of mixing and a nearly ideal entropy of mixing (Hildebrand et al, 1970). This behavior is more likely to occur in organic solutions and it should be followed by saturated IPM solutions of our prodrugs.

According to regular solution theory, the enthalpy of mixing arises from the breaking and reforming of intermolecular bonds that occur when the drug molecule enters solution. Typically, the strength of these intermolecular bonds is determined by the presence of functional groups on the drug that possess unpaired electrons, significant dipole moments or polarizable electron clouds. In a homologous alkyl series, the number and type of
these interacting functional groups remain the same in each series member. Therefore, to a good approximation, $\Delta H_{\text{Mix}}$ is a constant that is characteristic to a given series. If the solubility of each series member is taken at the same temperature, then all quantities preceding the melting point term can be grouped into a single constant that is also characteristic to the series:

$$A_s - \frac{\Delta S_f}{2.303RT}(T_m - T) = \log S$$  \hspace{1cm} (3.9)

Walden’s rule states that the entropy of fusion for most rigid small molecules is approximately equal to 56 J mol\(^{-1}\)°K\(^{-1}\). If the database compounds follow this rule, and if the temperature at which solubility is determined is the same for all compounds, then the terms of the melting point coefficient can be grouped into a second constant that should be independent of series and solvent.

$$A_o - B_o (T_m - T) = \log S_o$$  \hspace{1cm} (3.10)

This is the general equation that relates melting point to solubility for homologs in a regular (organic) solution.

An analogous equation for the aqueous solubility across a homologous series was derived using a method that parallels the derivation of Yalkowsky’s general solubility equation (Yalkowsky and Valvani, 1980). Yalkowsky suggested that the partition coefficient was a simple means of transforming an organic solubility equation to an aqueous solubility if the partition coefficient is assumed to approximately equal to the ratio of the drug’s organic solubility to its aqueous solubility.

$$K_{O,AQ} \approx \frac{S_o}{S_{AQ}}$$
Substituting equation 3.10 into equation 3.11 yields:

\[ \log S_{AQ} = A_0 - B_0 (T_M - T) - \log K_{O:AQ} \]  

(3.12)

The partition coefficients of alkyl homologs are related to one another through the equation:

\[ \log K_{O:AQ} = \log K^0_{O:AQ} + CMW \]  

(3.13)

where \( \log K_{O:AQ} \) is the log of the partition coefficient for a given homolog, \( \log K^0_{O:AQ} \) is a series specific constant, and CMW is a constant (C) multiplied by the homolog’s molecular weight (MW) (Hansch and Leo, 1971). The left side of this equation can be used to replace \( \log K_{O:AQ} \) in equation 3-12.

\[ \log S_{AQ} = A_0 - B_0 (T_M - T) - \log K^0_{O:AQ} - CMW \]

By combining \( A_0 \) and \( \log K^0_{O:AQ} \) into a new constant, \( A_{AQ} \), the final form of the general aqueous solubility equation is obtained.

\[ \log S_{AQ} = A_{AQ} - B_0 (T_M - T) - CMW \]  

(3.14)

In the final form, the aqueous solubility equation is essentially the organic solubility equation with an additional correction term for molecular weight. This is a reasonable result given that the dissolution of a non-electrolyte in aqueous solution is similar in many aspects to its’ dissolution in an organic solution with one principle difference. Creating an aqueous cavity large enough to accommodate a solute molecule disrupts a number of hydrogen bonds between water molecules; an energy expenditure that has no counterpart in most organic solvents. The amount of energy required to open the cavity is proportional to the number of hydrogen bonds that must be broken in its’
formation, which, in turn, is proportional to the cavity’s size. Therefore, the amount of energy required by this process should be related directly to the size of the solute molecule and reasonably proportional to its’ molecular weight. In addition, since the only parameter that affects the number of hydrogen bonds lost during solvation is the size of the solute, the molecular weight coefficient should be independent of the solute.

The parameters that appear in the solubility equations can be characterized as independent or universal (B₀ and C, respectively), compound specific (T_M and MW), or series specific (A₀ or A_AQ). Since there is only one series specific parameter appearing in each equation, all the coefficients for a given series can be determined once data from any one series member is obtained. As the only series specific parameter, A is a measure of the intrinsic influence on solubility conveyed to the prodrug by the promoiety. In other words, when two different prodrugs posses similar melting points and molecular weights, that compound belonging to the series with the higher A coefficient will have the higher solubility. Of course, to make a proper estimate of A, proper values for B₀ and C must first be determined.

**Derivation of the Potts-Guy Equation**

As with many fundamental flux models, the Potts-Guy equation is developed from Fick's 1st Law of Diffusion. Fick discovered this law in the 1800's by observing the movement of dissolved compounds through permeable membranes separating compartments containing solutions of differing concentration (figure 3.1).
Using heat flow equations as a guide, Fick proposed that the flux per unit area ($J$) through each section of the membrane was proportional to the local concentration gradient:

$$J = D \frac{\partial C}{\partial L}$$

where $D$ is the proportionality constant.

In general, the differential $\partial C/\partial L$ need not be a constant across the entire membrane. However, if the concentrations in each compartment ($C_D$ and $C_R$ respectively) are held constant for a sufficient amount of time, equilibrium concentrations, $C_1$ and $C_2$, are established just inside each exposed face of the membrane and the flux through the membrane reaches steady state. Under these conditions, a homogeneous membrane (or a membrane that behaves in a homogeneous manner) will have a constant concentration gradient across the membrane. For a linear concentration gradient, the partial differential will equal the difference in concentration just inside each
face divided by the thickness of the membrane. Substituting this into equation 3.15 yields:

\[ J = D \frac{(C_1 - C_2)}{L} \]  \hspace{1cm} (3.16)

where L is the thickness of the membrane. For a given membrane, the highest flux (JM) is obtained when the difference between C₁ and C₂ is a maximum. This occurs when C₁ is equal to the compound’s solubility in the membrane (SM) and when C₂ is close to zero (sink conditions). Experimentally, these conditions are obtained by keeping a saturated solution of the test compound in the donor compartment while periodically replenishing the solution in the receptor compartment with clean solvent to keep its concentration under 10% of its solubility in the receptor phase. Under these conditions, (C₁- C₂) = (SM - 0) and equation 3.16 becomes:

\[ J_M = D S_M / L \]  \hspace{1cm} (3.17)

From a practical point of view, solubility in the skin is a difficult quantity to measure directly. However since the membrane is in equilibrium with a saturated solution, S_M should equal the solubility of the compound in donor compartment (S_D) multiplied by the partition coefficient between the membrane and the donor solution (K_{M,D}). Substituting this relationship into equation 3.17 yields:

\[ J_M = D S_D K_{M,D} / L \]  \hspace{1cm} (3.18)

From equation 3.18, the maximum steady state flux of a compound is proportional to the solubility of that compound in the donor phase. If flux is divided by this solubility, a new quantity, permeability (P), is obtained

\[ P = D K_{M,D} / L \]  \hspace{1cm} (3.19)
Potts and Guy recognized that each term in equation 3.19 could be estimated from readily available empirical data. Since they were interested in delivery from water and the skin (or at least the stratum corneum) is a lipid rich membrane, they asserted that the skin:water partition coefficient, $K_{M:D}$, could be related to the octanol:water partition coefficient, $K_{OCT}$, through the equation:

$$\log K_{M:D} = f \log K_{OCT} + b$$  \hspace{1cm} (3.20)

Furthermore, previous work had suggested that the diffusion coefficient ($D$) should be related exponentially to the molecular volume of the compound (Cohen and Turnbull, 1959). Since molecular weight correlates well to molecular volume for organic molecules, it is convenient to substitute molecular weight into this equation:

$$D = D^0 e^{-\alpha MW}$$

$$\log D = \log D^0 - (1/\ln 10)\alpha MW$$  \hspace{1cm} (3.21)

Taking the logarithmic form of equation 3.18 and making substitutions using equations 3.20 and 3.21 yields the following result:

$$\log P = \log K_{M:D} + \log D + \log (1/L)$$

$$\log P = f \log K_{OCT} + (1/\ln 10)\alpha MW + \log (D^0/L) + b$$  \hspace{1cm} (3.22)

The final terms of equation 3.22, $\log (D^0/L)$ and $b$, can be collected into a single term for convenience. Similarly, the conversion factor of $1/\ln 10$ can be combined with the $\alpha$ term to create a single coefficient, $\beta$. Doing these two substitutions results in the standard form of the Potts-Guy equation.

$$\log P = f \log K_{OCT} - \beta MW + c$$  \hspace{1cm} (3.23)
Derivation of the Roberts-Sloan Equation

The Potts–Guy equation is useful in certain circumstances, but it does have significant limitations. Flux, not permeability, is the most relevant clinical parameter. Since flux equals permeability multiplied by concentration in the donor phase, there is a tendency to equate increasing permeability with increasing flux. For a homologous series of prodrugs delivered from an aqueous vehicle, log $K_{OCT}$ and log P rise predictably and consistently with increasing alkyl chain length but flux does not. Since the Potts-Guy equation is only concerned with permeability, it does not predict the important experimental observation that the most water-soluble members of homologous series tend to have the highest fluxes (Sloan and Wasdo, 2003). The use of $K_{OCT}$ to estimate $K_{M:D}$ is not applicable to vehicles that are miscible with octanol. This is an important limitation when studying prodrugs, since an aqueous donor is often incompatible with commonly employed labile derivatives. Roberts and Sloan (1999) used the following mathematical manipulation to estimate a partitioning coefficient, $K_{SKIN/IPM}$, between the skin (where $S_{SKIN}$ is solubility in the skin) and isopropyl myristate (IPM):

$$K_{SKIN/IPM} = S_{SKIN}/S_{IPM}$$

$$K_{SKIN/IPM} = (S_{SKIN}/S_{AQ}) / (S_{IPM}/S_{AQ})$$

$$K_{SKIN/IPM} = K_{SKIN:AQ} / K_{IPM:AQ}$$

$$\log K_{SKIN/IPM} = \log K_{SKIN:AQ} - \log K_{IPM:AQ}$$ \hspace{1cm} (3.24)

$K_{IPM:AQ}$ can be used in the same manner as $K_{OCT}$ to estimate $K_{SKIN:AQ}$,

$$\log K_{SKIN:AQ} = f \log K_{IPM:AQ} + b$$

$$\log K_{SKIN/IPM} = f \log K_{IPM:AQ} - \log K_{IPM:AQ} + b$$

$$\log K_{SKIN/IPM} = (f-1) \log K_{IPM:AQ} + b$$ \hspace{1cm} (3.25)
For an IPM vehicle, \((f-1) \log K_{\text{IPM:AQ}} + b\) takes the place of \(f \log K_{\text{OCT}}\) in the Potts-Guy equation:

\[
\log P = (f-1) \log K_{\text{IPM:AQ}} + b - \beta \text{MW} + c
\]

Adding \(\log S_{\text{IPM}}\) to both sides transforms the equation to flux \((J_M)\) instead of \(P\):

\[
\log J_M = f \log S_{\text{IPM}} + (1-f) \log S_{\text{AQ}} - \beta \text{MW} + b + c
\]

In the standard representation, \(z\), \(y\) and \(x\) are used respectively to replace \(\beta\), \(f\) and the combination of \(b + c\):

\[
\log J_M = x + y \log S_{\text{IPM}} + (1-y) \log S_{\text{AQ}} - z \text{MW}
\]

This equation is the Roberts-Sloan (RS) model and it represents our primary method of predicting flux through hairless mouse skin from IPM.

**Modification of the Roberts-Sloan Equation to Include Synthetic Membrane Data**

We also wish to construct a predictive transdermal model that includes the compound's flux through a surrogate membrane as one of the descriptive parameters. Although there are several possible forms for this model (depending principally upon the degree of similarity between the surrogate membrane and skin), they are all derived from the general flux equation (eq. 3.16). The logarithmic form of this equation for flux through skin is shown below.

\[
\log J_S = \log S_S + \log D_S - \log L_S
\]

Flux through an artificial membrane \((X)\) should follow an analogous equation:

\[
\log J_X = \log S_X + \log D_X - \log L_X
\]

To incorporate a \(\log J_X\) term into the skin equation, we must first decide which parameter in equation 3.29 best estimates its counterpart in equation 3.28; the solubility
term or the diffusivity term. Between any membrane and skin, one or both of the following relationships will be valid:

\[
\log S_S = A_S \log S_X + B_S \tag{3.30}
\]

\[
\log D_S = A_D \log D_X + B_D \tag{3.31}
\]

Relationship 3.30 is expected to be valid only in those situations where the surrogate membrane is very similar to skin itself. In general, this is expected only to be true when comparing skin to a similar biological membrane such as mammalian skin from another species. In contrast, equation 3.31 is a more general relationship and should be more widely applicable. Specifically, equation 3.31 will hold for all membranes in which molecular weight and the diffusion coefficient are related as described in equation 3.21. From this we conclude that two models should be investigated; one for comparing fluxes through related biological membranes and one for comparing synthetic to biological membranes.

To derive a model for chemically similar membranes, both equations 3.30 and 3.31 are assumed to be valid. Equation 3.29 can be rewritten to show solubility in the membrane in terms of the other variables.

\[
\log S_X = \log J_X - \log D_X + \log L_X
\]

This relationship can be substituted into equation 3.30:

\[
\log S_S = A_S \log S_X + B_S \tag{3.30}
\]

\[
\log S_S = A_S \log J_X - A_S \log D_X + A_S \log L_X + B_S
\]

This expression for \( \log S_S \) can now be substituted into equation 3.29:

\[
\log J_S = A_S \log J_X - A_S \log D_X + A_S \log L_X + B_S + \log D_S - \log L_S \tag{3.32}
\]

Substituting equation 3.31 into 3.32 removes \( \log D_S \):
\[ \log J_S = A_S \log J_X - A_S \log D_X + A_S \log L_X + B_S + A_D \log D_X + B_D - \log L_S \]

All the terms appearing between the brackets can be collapsed into a single coefficient, \( K_1 \):

\[ \log J_S = A_S \log J_X + (A_D - A_S) \log D_X + (A_D - A_S) (1/\ln 10)\alpha X + K_1 \]

From equation 3.21, \( \log D_X = \log D^0_X - (1/\ln 10)\alpha X \ MW \). If this relationship is substituted into equation 3.32:

\[ \log J_S = A_S \log J_X - (A_D - A_S) (1/\ln 10)\alpha X MW + (A_D - A_S) \log D^0_X + K_1 \]

If the constants are grouped renamed in the following manner:

\[ (A_D - A_S) \log D^0_X + K_1 = a \]
\[ A_S = b \]
\[ (A_D - A_S) (1/\ln 10)\alpha X = c \]

the result is the general form of similar membrane model.

\[ \log J_S = a + b \log J_X + c MW \]

To develop a model to relate the fluxes through two dissimilar membranes, equation 3.29 is rewritten in terms of \( \log D_X \) and then substituted into equation 3.31:

\[ \log D_X = \log J_X - \log S_X + \log L_X \]

\[ \log D_S = A_D \log D_X + B_D \]

\[ \log D_S = A_D \log J_X - A_D \log S_X + A_D \log L_X + B_D \]

\[ \log J_S = A_D \log J_X - A_D \log S_X + A_D \log L_X + B_D + \log S_S - \log L_S \]

\[ \log J_S = A_D \log J_X + \log S_S - A_D \log S_X + A_D \log L_X - \log L_S \] (3.35)
In order to proceed past equation 3.34, some assumptions must be made about the log solubility terms. In the RS equation, log $S_S$ and log $S_X$ are estimated from log $S_{IPM}$ and log $S_{AQ}$ through the following equations:

$$\log S_S = y_S \log S_{IPM} + (1-y_S) \log S_{AQ}$$  \hspace{1cm} (3.36)

$$\log S_X = y_X \log S_{IPM} + (1-y_X) \log S_{AQ}$$  \hspace{1cm} (3.37)

where $y_X$ and $y_S$ are the corresponding $y$ coefficients for skin (S) and the synthetic membrane (X). If these two relationships are substituted into equation 3.35, the result is an expression for log $J_S$ in terms of experimentally determined values:

$$\log J_S = A_D \log J_X + (y_S - A_D y_X) \log S_{IPM} + (1-y_S - A_D + A_D y_X) \log S_{AQ} + A_D \log L_X - \log L_S$$

This expression can be greatly simplified by grouping and renaming constants:

$$A_D \log L_X - \log L_S = a$$

$$A_D = b$$

$$y_S - A_D y_X = c$$

$$\log J_S = a + b \log J_X + c \log S_{IPM} + (1 - b - c) \log S_{AQ}$$  \hspace{1cm} (3.38)

Equation 3.38 will only be predictive when the assumptions of the RS equation are valid. In other words, only when log $K_{M:IPM}$ is related to log $S_{IPM}$ and log $S_{AQ}$ in the following manner:

$$\log K_{M:IPM} = (f-1) \log S_{IPM} - (f-1) \log S_{AQ} + b$$

The success of the RS equation in describing flux though hairless mouse skin from IPM indicates that the above expression is reasonably followed for that system. However, it is not yet known whether or not this can be applied as a general rule. When comparing two widely different systems, it may be necessary to allow unconstrained coefficients for log $S_{IPM}$ and log $S_{AQ}$ to estimate partitioning or solubility. In this case, the coefficients in
equation 25 would no longer be related and a more general form of model 3.38 is produced:

$$\log J_S = a + b \log J_X + c \log S_{PM} + d \log S_{AQ}$$  \hspace{1cm} (3.39)

It will require empirical data to determine which of these two models is most applicable.
CHAPTER 4
EXPERIMENTAL DESIGN

Section I: Synthesis and Characterization of the 4-Alkylxacyarbonyl and 4-Methyloxyalkyloxy Prodrugs of Acetaminophen

Synthesis

Five homologous n-alkyl and two methyloxyalkyl carbonates of 4-hydroxyacetanilide (APAP) have been prepared. The general synthetic method for each carbonate entailed reacting APAP with equimolar amounts of the proper alkylchloroformate in the presence of a poorly nucleophilic base.

\[
\text{APAP} + \text{alkylchloroformate} \rightarrow \text{APAP carbonate}
\]

A well-stirred suspension of APAP (~1.51 g, ~0.01 mol) was prepared in 30 mL of CH\(_2\)Cl\(_2\) containing an equimolar amount of pyridine or triethylamine. To this mixture, a solution of the desired alkylchloroformate (0.01 mol) in ~10 mL CH\(_2\)Cl\(_2\) was added in a drop-wise manner, and the reaction mixture was allowed to react for two hours. The reaction solution was then diluted to approximately 200 mL and extracted sequentially with 10 mL of ~0.6 N HCl and 10 mL deionized water. After the water wash, the CH\(_2\)Cl\(_2\) solution was dried over Na\(_2\)SO\(_4\) for two hours, filtered and concentrated under vacuum until solvent free. The resulting material was purified by recrystallization and, if necessary, column chromatography until a sharp melting point was observed, only one component was discernable by TLC and a clean \(^1\)H-NMR was obtained. In this fashion,
the methyl, ethyl, propyl and butyl carbonates of APAP were prepared from commercially available alkylchloroformates.

For the hexyl and the two methyloxyalkyl carbonates, it was necessary to first synthesize the desired alkylchloroformate. This was accomplished by reacting the proper primary alcohol with triphosgene, a synthetic phosgene equivalent, and a poorly nucleophilic base (triethylamine or pyridine).

![Chemical Reaction]

One molecule of triphosgene rearranges to ultimately yield three molecules of phosgene during the reaction. Therefore a 3:1 molar ratio of alcohol to triphosgene was used to maintain equimolar amounts of phosgene and the alcohol. A solution of triphosgene (~0.0033 mol) in 20 mL CH₂Cl₂ was first prepared. To this solution, a mixture of triethylamine or pyridine (~0.01 mol) and the alcohol (~0.01 mol) in 10 mL CH₂Cl₂ was added at a rate of ~1 mL/min in a drop-wise manner. The ensuing exothermic reaction was allowed to proceed until the solution had once again returned to room temperature. A suspension of APAP and triethylamine or pyridine (0.01 mol each) in 20 mL of CH₂Cl₂ was then added to the alkyl chloroformate solution at a rate of ~2 mL/min. After being allowed to react for at least two hours, the mixture was washed with aqueous acid and dried over Na₂SO₄ as described above. The crude product was purified by recrystallization and column chromatography as necessary to achieve the previously stated criteria of purity. The results and specific conditions for each prodrug’s synthesis are listed below.
2; 4-methyloxycarbonyloxyacetanilide – This compound was prepared in 49 % yield from methyl chloroformate and pyridine in CH$_2$Cl$_2$ after recrystallization from diethyl ether/hexane.

3; 4-ethyloxycarbonyloxyacetanilide – This compound was prepared in 82 % yield from ethyl chloroformate and triethylamine in CH$_2$Cl$_2$ after recrystallization from ethyl acetate/hexane.

4; 4-propyloxycarbonyloxyacetanilide – This compound was prepared in 59% yield from propyl chloroformate and triethylamine in CH$_2$Cl$_2$ after recrystallization from ethyl acetate/hexane.

5; 4-butyloxycarbonyloxyacetanilide – This compound was prepared in 63% yield from butyl chloroformate and triethylamine in CH$_2$Cl$_2$ after recrystallization from ethyl acetate/hexane.

6; 4-hexyloxycarbonyloxyacetanilide – This compound was prepared in 51% yield from hexanol, triphosgene and pyridine in CH$_2$Cl$_2$ after silica gel chromatography in ethyl acetate and recrystallization from ethyl acetate/hexane.

7; 4-(2'-methyloxyethyloxycarbonyloxy)acetanilide - This compound was prepared in 44% yield from 2-methoxyethanol, triphosgene and triethylamine after recrystallization from ethyl acetate/hexane.

8; 4-(1'-methyl-2'-methyloxethyloxycarbonyloxy)acetanilide - This compound was prepared in 29% yield from 1-methyl-2-methoxyethanol, triphosgene and triethylamine after recrystallization from diethyl ether/hexane.
Characterization

Melting point determination and $^1$H-NMR analysis comprised the initial characterization of the 4-AOC and 4-MOAC APAP prodrugs. Melting points were determined using a Meltemp capillary melting point apparatus and were uncorrected. 90 MHz $^1$H-NMR spectra were obtained in CDCl$_3$ using a Varian EM-390 spectrometer.

In addition to the initial characterization, molar absorptivities ($\varepsilon$) in acetonitrile (ACN) and pH 7.1 phosphate buffer containing 0.11% formaldehyde were measured to facilitate quantitation in subsequent solubility and flux experiments. For each compound, three replicate stock solutions were prepared by diluting 10 ±1 mg portions of purified material to 25 mL in ACN. Aliquots of these stock solutions were further diluted with either ACN or buffer and the background-corrected absorbance of these diluted solutions were measured from 400 to 200 nm using a Shimadzu UV 265 Spectrophotometer. Maximum absorbance was observed at 240 nm in both solvents for APAP and each prodrug. In addition, APAP displayed a pronounced shoulder at 280 nm in buffer that was not evident in the carbonate derivatives. The molar absorptivity at these wavelengths for each compound was determined by taking the average of the individual molar absorptivities of the replicate solutions.

Figure 4-1. Regions of the 4-AOCO-ACA prodrug corresponding to the letters given in table 4-1.
Table 4-1. $^1$H NMR Data for the AOC and MOAOC-APAP prodrugs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R^1$</th>
<th>$R^2$</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, APAP</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>- H</td>
<td>- H</td>
<td>s 2.15</td>
<td>d 7.06, d 7.45</td>
<td>s 3.95</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>3</td>
<td>-CH$_3$</td>
<td>- H</td>
<td>s 2.12</td>
<td>d 7.03, d 7.42</td>
<td>q 4.28</td>
<td>t 1.47</td>
<td>----</td>
</tr>
<tr>
<td>4</td>
<td>-CH$_2$CH$_3$</td>
<td>- H</td>
<td>s 2.08</td>
<td>d 7.04, d 7.42</td>
<td>t 4.20</td>
<td>m 1.80, t 1.02</td>
<td>----</td>
</tr>
<tr>
<td>5</td>
<td>-(CH$_2$)$_2$CH$_3$</td>
<td>- H</td>
<td>s 2.14</td>
<td>d 7.08, d 7.45</td>
<td>t 4.28</td>
<td>t 0.95</td>
<td>----</td>
</tr>
<tr>
<td>6</td>
<td>-(CH$_2$)$_2$CH$_3$</td>
<td>- H</td>
<td>s 2.14</td>
<td>d 7.04, d 7.42</td>
<td>t 4.20</td>
<td>Unresolved m</td>
<td>----</td>
</tr>
<tr>
<td>7$^b$</td>
<td>-CH$_2$OCH$_3$</td>
<td>- H</td>
<td>s 2.14</td>
<td>d 7.04, d 7.42</td>
<td>t 4.20</td>
<td>m 1.80, t 1.02</td>
<td>----</td>
</tr>
<tr>
<td>8$^c$</td>
<td>-CH$_2$OCH$_3$</td>
<td>-CH$_3$</td>
<td>s 2.08</td>
<td>d 7.04, d 7.42</td>
<td>m 5.00</td>
<td>d 3.50, s 3.40</td>
<td>d 1.34</td>
</tr>
</tbody>
</table>

$^a$ Obtained in CDCl$_3$ with TMS as an internal standard
$^b$ Elemental analysis for C$_{12}$H$_{15}$NO$_{5}$, Found (Expected): C = 56.84 (56.91), H = 5.98 (5.97), N = 5.53 (5.53).
$^c$ Elemental analysis for C$_{13}$H$_{18}$NO$_{5}$, Found (Expected): C = 58.43 (58.42), H = 6.43 (6.41), N = 5.27 (5.24).

Table 4-2. Melting point and absorptivity values for APAP, the AOC-APAP and MOAOC APAP prodrugs.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Melting Point (°C)</th>
<th>$\varepsilon_{240}^a$ in ACN</th>
<th>$\varepsilon_{240}^a$ in Buffer$^b$</th>
<th>$\varepsilon_{280}^a$ in Buffer$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, APAP</td>
<td>167-170</td>
<td>1.36</td>
<td>0.981</td>
<td>0.191</td>
</tr>
<tr>
<td>2</td>
<td>112-115 (115.5-116.5)$^c$</td>
<td>1.67</td>
<td>1.25</td>
<td>0.0560</td>
</tr>
<tr>
<td>3</td>
<td>120-122 (121-122)$^c$</td>
<td>1.64</td>
<td>1.24</td>
<td>0.0483</td>
</tr>
<tr>
<td>4</td>
<td>104-106 (105-108)$^d$</td>
<td>1.63</td>
<td>1.28</td>
<td>0.0560</td>
</tr>
<tr>
<td>5</td>
<td>118-120 (119-121)$^c$</td>
<td>1.75</td>
<td>1.24</td>
<td>0.0376</td>
</tr>
<tr>
<td>6</td>
<td>108-110 (112.5-113.5)$^c$</td>
<td>1.79</td>
<td>1.12</td>
<td>0.0623</td>
</tr>
<tr>
<td>7</td>
<td>78-81</td>
<td>1.74</td>
<td>1.26</td>
<td>0.0623</td>
</tr>
<tr>
<td>8</td>
<td>120-123</td>
<td>1.60</td>
<td>1.28</td>
<td>0.0520</td>
</tr>
</tbody>
</table>

$^a$ units of M$^{-1}$ x 10$^{4}$.
$^b$ pH 7.1 phosphate buffer with 0.11 % formaldehyde.
$^c$ Literature values in parentheses from Dittert et al, 1963
$^d$ Literature values in parentheses from Merck, 1897.

Section II: Determination of IPM and Aqueous Solubilities

IPM solubilities ($S_{IPM}$) were determined directly by UV analysis on filtered IPM suspensions of prodrugs that were subsequently diluted with ACN. To prepare a suspension, a three mL volume of IPM was initially combined in a 15 mL test tube with approximately twice the amount of APAP or prodrug needed to just saturate the IPM.
The resulting mixture was then stirred continuously at room temperature (23±1 °C) for 72 hours. After stirring, the suspension was rapidly filtered through a 0.25 µm nylon syringe filter and an aliquot of the filtrate (~ 0.100 mL) was diluted to at least 10.0 mL with ACN. The final solution was analyzed by UV to determine its absorbance at 240 nm. The molar absorptivity of each compound in the diluted filtrate was assumed to be equivalent to its' corresponding molar absorptivity in pure ACN. This assumption allowed each compound’s IPM solubility to be determined from the relationship:

\[
S_{IPM} = \left( \frac{V_{\text{final}}}{V_{\text{aliquot}}} \right) A_{240}/\varepsilon_{240}
\]  

where \(V_{\text{aliquot}}\) is the volume of the saturated filtrate aliquot, \(V_{\text{final}}\) is the final diluted sample volume, \(A\) is the sample absorbance at 240 nm and \(\varepsilon_{240}\) is the compound’s molar absorptivity in ACN at 240 nm. Three replicate suspensions were prepared and analyzed for each compound. The average of the three values was reported as \(S_{IPM}\).

Aqueous solubilities \((S_{AQ})\) were estimated using two methods: a direct dissolution in water and a calculation using the compound's IPM:water partition coefficient. For the direct measurements, suspensions of each compound in unbuffered deionized water were prepared in the same manner as the IPM suspensions. However, to be consistent with the preparation of the suspensions used in the diffusion cell experiments and to limit the extent of hydrolysis during analysis, aqueous suspensions were stirred at room temperature for only one hour before filtration and dilution in ACN. As with the IPM solutions, the ACN diluted filtrates were analyzed by UV and the concentration of each compound was calculated using equation 4.1.

To measure each compound’s IPM:water partition coefficient \((K_{IPM:4.0})\), a measured volume of the prodrug suspension in IPM was placed in a 10 mL test tube along with
four milliliters of 0.01 M acetate buffer (pH 4.0). The test tube was capped, shaken vigorously for 10 seconds and allowed to stand until the two phases separated. An aliquot of the IPM layer was removed, diluted in ACN and analyzed by UV using the same protocol followed for the saturated IPM solutions. $K_{IPM:4.0}$ was calculated from the concentration of prodrug remaining in the IPM phase after partitioning ($C_F$), the initial saturated concentration ($S_{IPM}$) and the ratio of IPM and buffer volume used in the partitioning.

$$K_{IPM:4.0} = \frac{V_{AQ}/V_{IPM}} {S_{IPM} - C_F} \frac{C_F} {V_{IPM} - C_F}$$  \hspace{1cm} (4.2)

Within reasonable accuracy, $K_{IPM:AQ}$ is equal to the ratio of $S_{IPM}$ to the prodrug’s solubility in water ($S_{4.0}$). Therefore, aqueous solubility is estimated from the relationship:

$$S_{4.0} = S_{IPM} / K_{IPM:AQ}$$  \hspace{1cm} (4.3)

It is important to note that, while this process does not provide a rigorously measured solubility, it does provide a value that has been shown to be consistent with the directly measured solubility (Taylor and Sloan, 1998). More importantly, it is a method that can be used to estimate the solubility of prodrugs that are too unstable to permit a direct solubility and, as such, can be used to compare compounds regardless of their intrinsic stability.

**Section III: Determination of Flux through Hairless Mouse Skin and Polydimethylsiloxane Membranes**

**Preparation of the Membranes and Assembly of the Diffusion cells**

Adult female hairless mice were rendered unconscious by CO$_2$ and quickly sacrificed by cervical dislocation. Whole thickness skin from the entire region was immediately removed from each mouse by blunt dissection. Sections of this separated skin were cut to a proper size and immediately mounted on the diffusion cell. For the
PDMS membrane, properly sized and shaped sections were trimmed from larger sheets using the diffusion cell donor compartment as a guide. Just prior to mounting, the trimmed sections were rinsed with water and MeOH and then blotted dry to remove accumulated dust.

Shown in vertical profile by the figure below, a Franz cell consists of two separate compartments. The upper or donor compartment (A) is essentially a glass cylinder with a flared and grooved lower edge. The lower or receptor compartment (B) is cylindrical reservoir with a grooved upper edge that mirrors the donor compartment. In addition, the receptor reservoir is equipped with a temperature controlling water jacket and a side arm that allows filling and access to the receptor fluid.

To assemble the cell for analysis, the donor side compartment (A) was inverted and a section of membrane was placed over the opening. Hairless mouse skin sections were placed with the epidermal side facing the donor compartment and gently stretched into place until they completely covered the entire lower opening and edge of the donor compartment without sagging. PDMS membrane sections were placed over the donor compartment opening without further adjustment. Once the membrane section was in position, a rubber o-ring was placed over it and aligned with the groove on the donor cylinder. The receptor section (B) was then inverted and carefully aligned with the donor section. The two sections were then clamped together with a screw locked spring clamp and the assembled cell was returned to an upright position. After assembly, the receptor compartment was completely filled with 0.5 M, pH 7.1 phosphate buffer containing 0.1% formaldehyde by weight as an anti-microbial agent. This concentration of formaldehyde
is essential for maintaining membrane integrity and will prevent membrane degradation for more than 144 hours (Sloan et al, 1991). After filling the receptor compartment, care was taken to ensure that no air bubbles remained adhered to the bottom of the membrane and the fluid level in the side arm was adjusted to the same height as the membrane to prevent any increased hydrostatic pressure. A magnetic stir bar was added through the side arm and the cell suspended over a magnetic stir plate to continuously stir the receptor buffer throughout the experiment. The water circulating through the insulating jacket was set to 32° C. The cells were kept in contact with the receptor fluid for 48 hours prior to the application of the donor phase to leech out any UV active components and to allow the membranes to equilibrate with the receptor phase. Twice during this 48-hour conditioning period, the receptor phase was completely withdrawn and replaced with fresh receptor buffer.
Preparation and Application of Donor Phases

IPM and aqueous suspensions of each compound were prepared. IPM suspensions were prepared by stirring 0.6 to 1.0 mmol of test compound with 3 mL of IPM for 24 hours at room temperature. For each compound, the final suspension concentration exceeded the compound’s solubility in IPM by at least threefold. For aqueous suspensions, similar mmol quantities of material were stirred with 4 mL of deionized water, but the suspensions were stirred for only one hour prior to application to limit hydrolysis of the prodrugs. In addition, new aqueous suspensions were prepared every 24 hours. For either vehicle, application of the donor suspensions occurred immediately after preparation.

Just prior to application of the donor suspension, the entire receptor phase was removed and replaced with fresh buffer solution. Either a 0.5 mL aliquot of well-stirred IPM suspension or a 1.0 mL aliquot of well-stirred aqueous suspension was evenly applied to the conditioned membrane surface as shown in figure 4-2. When working with the aqueous suspensions, the donor compartments were covered with parafilm to prevent excessive loss of water to evaporation. After removal from the diffusion cells, all prodrug donor suspensions were analyzed by $^1$H-NMR to ensure the prodrug had not hydrolyzed in the vehicle.

Sampling of the Diffusion Cells for Flux and Residual Skin Samples

To obtain a sample of the receptor phase, a 3 to 4 mL aliquot of receptor buffer was removed from the sidearm by Pasteur pipette and placed in a test tube for subsequent quantitation. To maintain sink conditions during the experiment, the remaining receptor fluid was removed after each sampling and the entire receptor compartment refilled with fresh buffer. The frequency with which samples were taken was determined by the rate
at which analyte accumulated in the receptor phase and it differed for each membrane-
vehicle combination. The table below outlines the sampling times for APAP and each
prodrug.

Table 4-3: Sampling times for APAP and the AOCA prodrug diffusion experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>Membrane/Vehicle</th>
<th>Sampling Times&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP</td>
<td>HMS / IPM</td>
<td>8.5, 19, 22, 25, 28, 31, 34,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>11, 24, 36, 52</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 36, 48</td>
</tr>
<tr>
<td>C1 AOCA</td>
<td>HMS / IPM</td>
<td>8, 19, 22, 25, 28, 31, 34, 43,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>10, 24, 27, 30, 33, 36, 39, 48</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 36, 48</td>
</tr>
<tr>
<td>C2 AOCA</td>
<td>HMS / IPM</td>
<td>8, 19, 22, 25, 28, 31, 34, 44,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>14.5, 24, 27, 30, 33, 36, 39,</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 30, 36, 48</td>
</tr>
<tr>
<td>C3 AOCA</td>
<td>HMS / IPM</td>
<td>8, 19, 22, 25, 28, 31, 34, 43,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>14.5, 24, 27, 30, 33, 36, 39,</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 30, 36, 48</td>
</tr>
<tr>
<td>C4 AOCA</td>
<td>HMS / IPM</td>
<td>7, 20, 22, 25, 28, 31, 34, 43,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>10, 24, 27, 30, 33, 36, 39, 48</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 36, 48</td>
</tr>
<tr>
<td>C6 AOCA</td>
<td>HMS / IPM</td>
<td>7, 20, 22, 25, 28, 31, 34, 43,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>11, 24, 36, 52</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 36, 48</td>
</tr>
<tr>
<td>C2 MOACA</td>
<td>HMS / IPM</td>
<td>8.5, 19, 22, 25, 28, 31, 34,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>12, 24, 27, 30, 33, 36, 39, 48</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 36, 48</td>
</tr>
<tr>
<td>Ci3 MOACA</td>
<td>HMS / IPM</td>
<td>8, 19, 22, 25, 28, 31, 34, 44,</td>
</tr>
<tr>
<td></td>
<td>HMS / Water</td>
<td>12, 24, 36, 48</td>
</tr>
<tr>
<td></td>
<td>PMDS / Water</td>
<td>24, 36, 48</td>
</tr>
</tbody>
</table>

<sup>a</sup> Time in hours after initial application of donor suspension

Receptor phase samples were analyzed by UV analysis within an hour of
collection. Just prior to analysis, a background correction from 350 to 200 nm was
performed on the instrument using matched quartz cuvettes containing blank receptor
buffer. After this correction, the spectrum of each recently collected receptor sample was
taken over the same wavelength range.
Following the last sampling and refilling of the receptor compartment, the cells were allowed to sit for 24 hours. For IPM treated hairless mouse skin, this period was sufficient to leach any test compound remaining within the skin. As such, the concentration of compound found in the receptor phase after these 24 hours was used to determine the total amount of material that had been absorbed by the skin (the residual skin amount). For compounds delivered from water through hairless mouse skin and PDMS membrane, an additional 24-hour leeching period was necessary and the residual skin amount was determined by totaling the amount of material leached after the first and second 24 hour periods.

**Evaluation of Membrane Integrity**

After the leaching period, a standard suspension of theophylline in propylene glycol (400 mg/mL) was applied to the membranes to determine their general integrity. Following the final residual membrane sample, the remaining receptor buffer was removed, replaced with fresh solution and a 0.5 mL aliquot of the theophylline suspension was applied evenly to the membrane surface. Samples of the receptor compartment were taken at regular intervals using the same protocol as for a test compound. A sufficient number of samples were taken over the subsequent 24 hours to determine the flux of theophylline through the membrane.

**Determination of Analyte Concentration and Extent of Hydrolysis**

For any wavelength, the absorbance of each sample was assumed to result solely from a combination of the absorbances of APAP and any intact prodrug. Using Beer’s law, this assumption can be stated mathematically as:

$$A_{\lambda} = C_{PEP_{\lambda}} + C_{DDE_{D\lambda}}$$  \(\text{(4.4)}\)
A\lambda \text{ represents the absorbance at wavelength } \lambda, \ C_P \text{ and } C_D \text{ are the respective concentrations of the prodrug and drug, and } \varepsilon_P, \text{ and } \varepsilon_D, \text{ are the respective molar absorptivities for the prodrug and APAP, respectively, at wavelength } \lambda. \text{ By measuring the absorbance of a sample at two wavelengths (240 nm and 280 nm for APAP and its prodrugs), it was possible to determine the values of } C_P \text{ and } C_D \text{ by simultaneously solving the resulting two Beer’s Law equations.}

\begin{align*}
A_{280} &= C_P \varepsilon_{P280} + C_D \varepsilon_{D280} \\
A_{240} &= C_P \varepsilon_{P240} + C_D \varepsilon_{D240}
\end{align*}

\begin{align*}
C_P &= \frac{(A_{240} \varepsilon_{D280} - A_{280} \varepsilon_{D240})}{(\varepsilon_{P240} \varepsilon_{D280} - \varepsilon_{P280} \varepsilon_{D240})} \quad (4.5) \\
C_D &= \frac{(A_{240} - C_P \varepsilon_{P240})}{\varepsilon_{D240}} \quad (4.6)
\end{align*}

\( C_P \) \text{ and } \( C_D \) \text{ were added to determine the total concentration of APAP species present in the sample. The total amount of APAP species present was then determined by multiplying total concentration by the volume of the receptor phase.}

**Determination of Maximum Flux (J_M)**

Maximum flux was determined from a plot of the cumulative amount of APAP species delivered through the membrane versus time. Since the receptor fluid was replaced after every sampling with clean buffer, the amount of compound found in each sample was indicative of only that amount which had passed through the membrane between sampling times. As such, the cumulative amount delivered was calculated by sequentially adding these sampled amounts. A typical graph of cumulative amount plotted against sample time is shown below.
Figure 4-3. Plot of cumulative amount of prodrug delivered versus time for compound 3 from IPM through hairless mouse skin.

Linear regression was performed on data in the steady-state region of the plot (usually data collected after 24 hours). The slope of this best-fit line was divided by the cross sectional area of the diffusion cell to yield the compound’s maximum flux, $J_M$. 

$y = 1.7012x - 26.715$

$R^2 = 0.9995$
CHAPTER 5
RESULTS

Melting Point Behavior of the APAP Carbonates

As noted in chapter 1, nonionic compounds, in general, display a negative correlation between melting point and solubility. As such, compounds with lower melting points tend to have higher solubilities in lipid and water which favor higher transdermal flux. It was also noted that the relatively high melting points of APAP and of other phenol containing compounds are likely a consequence of their ability to form strong intermolecular hydrogen bonds. It was anticipated that masking the phenolic moiety of APAP would disrupt this ability and produce derivatives with consistently lower melting points. Table 5.1 shows that this is indeed the case. All of the carbonate prodrugs have melting points that are substantially lower then that of APAP. In particular, the two highest melting prodrugs (3 and 8) melt 50ºC lower then APAP while the lowest melting compound (7) melts approximately 90ºC lower.

Table 5-1: Melting points (ºC), log partition coefficients (log $K_{IPM:AQ}$), log solubility ratio (log $SR_{IPM:AQ}$), log solubilities in IPM (log $S_{IPM}$) and log solubilities in water (log $S_{AQ}$) for APAP and the 4-AOC and 4-MOAOC prodrugs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting Point (ºC)</th>
<th>log $S_{IPM}$</th>
<th>Direct log $S_{AQ}$</th>
<th>log $K_{IPM:4.0}$</th>
<th>Estimated log $S_{4.0}$</th>
<th>log $SR_{IPM:AQ}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP, 1</td>
<td>167-170</td>
<td>0.278</td>
<td>2.000</td>
<td>-0.174</td>
<td>-0.174</td>
<td>-0.174</td>
</tr>
<tr>
<td>2</td>
<td>112-115</td>
<td>1.076</td>
<td>1.314</td>
<td>-0.156</td>
<td>1.232</td>
<td>-0.238</td>
</tr>
<tr>
<td>3</td>
<td>120-122</td>
<td>0.968</td>
<td>0.578</td>
<td>0.315</td>
<td>0.653</td>
<td>0.390</td>
</tr>
<tr>
<td>4</td>
<td>104-106</td>
<td>1.374</td>
<td>0.427</td>
<td>0.897</td>
<td>0.477</td>
<td>0.947</td>
</tr>
<tr>
<td>5</td>
<td>118-120</td>
<td>1.143</td>
<td>-0.372</td>
<td>1.497</td>
<td>-0.354</td>
<td>1.515</td>
</tr>
<tr>
<td>6</td>
<td>108-110</td>
<td>1.219</td>
<td>-1.324</td>
<td>2.706</td>
<td>-1.487</td>
<td>2.543</td>
</tr>
<tr>
<td>7</td>
<td>78-81</td>
<td>1.014</td>
<td>1.536</td>
<td>-0.300</td>
<td>1.314</td>
<td>-0.522</td>
</tr>
<tr>
<td>8</td>
<td>120-123</td>
<td>0.529</td>
<td>0.516</td>
<td>0.132</td>
<td>0.386</td>
<td>0.013</td>
</tr>
</tbody>
</table>

$^a$ In units of mM at 23ºC  
$^b$ Using pH 4.0 buffer at 23ºC.
However, the drop in melting point does not follow any simple trend among the prodrugs. The 4-AOC-APAP compounds demonstrate an odd-even alteration in their melting points as the number of methylene groups in the promoiety increases. This effect, where the melting points of homologs will alternately rise and fall with the addition of each methylene group to the alkyl chain of the promoiety, has been observed in several series of homologous series (Chikos and Nichols, 2001), but has not been seen in the compounds of the 5-FU and 6-MP series (Roberts and Sloan, 1999).

Since the addition of each CH$_2$ has a proportionally larger effect upon the entire molecule when the promoiety is small, this behavior is generally more pronounced with the first few members of a series. In the 4-AOC-APAP series, there is an average alternating fluctuation of 12$^\circ$ C between the first four members. Perhaps more surprising, the addition of a single methyl side chain to the ethoxymethyl promoiety of compound 7 resulted in a 40$^\circ$C increase in melting point and produced the highest melting prodrug of either type (i.e. 8). Such erratic behavior highlights the difficulty in predicting melting points a priori without empirical evidence.

**Direct IPM and Aqueous Solubility**

With the exception of compound 7, the melting points of the APAP carbonates fall within a relatively small range (~20$^\circ$C). Given the relationship between melting point and IPM solubility, a correspondingly small range in IPM solubility was expected and consequently observed. The most IPM soluble derivative (4) is only eight times more soluble than the least soluble compound (8). However, all of the prodrugs were more soluble in IPM then APAP itself. Compound 2 showed a 6-fold improvement in IPM solubility over APAP and even compound 8 was 1.8 times as soluble. This range of solubility is similar to that of the 3-AC 5-FU series and the maximum IPM solubility of
this series ranks as forth among the nine homologous series so far studied (Sloan and Wasdo, 2003).

Unlike the IPM solubility values, the direct aqueous solubilities of compounds 2 through 8 were substantially less than that of APAP. As previously noted, all of the APAP prodrugs possessed lower melting points than APAP, which indicates a decrease in the lattice energy of these compounds relative to APAP. This lower lattice energy is most likely the result of masking the phenolic moiety. However, masking this moiety in the prodrugs also removes some of their ability to form beneficial hydrogen bonds when in aqueous solution. It is apparent from the consistent decrease in aqueous solubility exhibited by the APAP prodrugs that the loss of hydrogen bond stabilization in solution caused by masking the phenol, combined with the extra energy required to create a larger cavity in solution to accommodate their larger molecular size, outweighs the benefits of lower lattice energy for these compounds.

In terms of relative solubility, compound 2 had an aqueous solubility of approximately 21% that of APAP, whereas compound 6 possessed only 0.04% of APAP’s water solubility. Compound 7 had highest water solubility of all prodrugs and was 34% as soluble in water as APAP, while the other MOAOC derivative, compound 8, had a water solubility of approximately 3% that of APAP. When compared to the entire database (Sloan and Wasdo, 2003), compounds 2 and 7 have water solubilities greater than two thirds of the characterized compounds. However, five out of the other nine series have at least one member with water solubility as good or better than either compound 2 or 7. The remaining APAP produgs rank only within the lower 50% of the database’s values. Therefore, the performance of these prodrugs is somewhat mediocre.
Partition Coefficients and Solubility Ratios

As discussed in chapter 3, there is a linear relationship in a homologous alkyl series between the log of the partition coefficient and the molecular weight of the homolog (eq. 3-13). Since molecular weight increases proportionally with the number of methylene units in the promoiety, a plot of log K versus methylene number will also yield a linear relationship. The slope of this line, the $\pi$ value, should be independent of the series’ parent molecule and, therefore, should be the same for all homologous series. Empirically, this prediction has been supported. For the series comprising our database, $\pi$ values are very consistent with an average value of 0.58 and a standard deviation of 0.03. This consistency makes the $\pi$ value a robust indicator of homologous series behavior.

Linear regression on compounds 2 through 6 yields a $\pi$ value of 0.58, which closely agrees with the database average (Sloan and Wasdo, 2003). This is evidence that the physical properties of this series are both internally consistent and behaving in a theoretically predicted manner. Since the other series have displayed similar properties and since this consistency is fundamental in determining comparable estimated water solubility ($S_{4.0}$), we can anticipate that the estimated water solubility for these compounds should be compatible with the other estimated database values.

The two MOAOC derivatives differ by the addition of a methyl group as a side chain rather then the insertion of a CH$_2$ group into the existing alkyl chain. However, due to its similar size, chemical composition and polarity, the addition of the methyl group should have a similar effect upon partitioning as a methylene group. If the basic theory of partitioning holds true, the slightly larger size of the methyl group should have resulted
in larger drop in log $K$ between compounds 7 and 8 than what was measured for the AOC-APAP compounds.

However, this was not the case. Using the difference in log $K_{IPM:4.0}$ as an approximation, a $\pi$ value of 0.44 was obtained. This is significantly smaller than expected and only 75% of the $\pi$ value for the AOC series. To develop a possible explanation for these results, we must first examine the behavior of the actual IPM:water solubility ratios ($SR_{IPM:AQ} = S_{IPM} /$ directly measured $S_{AQ}$) as a point of comparison.

By design, many members of the current database are unstable to hydrolysis even at neutral pH. In contrast, the APAP carbonate prodrugs are much more stable and afforded an opportunity to directly measure aqueous solubility and subsequently determine $SR_{IPM:AQ}$. If log $SR_{IPM:AQ}$ is plotted against methylene number instead of log $K_{IPM:4.0}$, a $\pi$ value of 0.55 is obtained for the AOC compounds. As predicted, this is in close agreement with the $\pi$ value obtained from the log $K_{IPM:AQ}$ plot. The agreement is so close that a difference of less than 0.1 log units was measured between estimated and direct solubility for compounds 2 through 5. Even for compound 6, whose low solubility values have the highest associated uncertainty, a discrepancy of less than 0.2 log units was measured.

For the 4-MOAOC-APAP derivatives, the change in log $SR_{IPM:AQ}$ between compound 7 and 8 was 0.53. While still lower than expected, this is 0.1 log units larger than the corresponding change in log $K_{IPM:4.0}$ and closer to the log $K_{IPM:4.0}$ $\pi$ value for the AOC-APAP prodrugs. There is too little experimental evidence to ascertain the reason for this difference, however the higher log SR relative to log K does suggest that unexpected partitioning behavior is responsible rather than an intrinsic attribute of the MOAOC promoiety.
Permeability Coefficient Behavior

The permeability coefficient is the most often used parameter appearing in models of topical delivery. As discussed in chapter 3, many authors have reported that the log of the permeability coefficient correlates positively to the log of octanol:water partition coefficient (log \(K_{OW}\)) and this relationship was used by Potts and Guy (1992) in the development of their permeability model.

\[
\log P = f \log K_{OCT} + \beta MW + c
\]  

(3.23)

It is this positive association with \(\log K_{OW}\) that has led in part to the misleading principle that greater lipophiliciry is absolutely beneficial to topical delivery.

Table 5-2: Log permeability values for the APAP prodrugs from IPM through hairless mouse skin (log \(P_{MIPM}\)), from water through hairless mouse skin (log \(P_{MAQ}\)) and from water through PDMS membrane (log \(P_{PAQ}\)).

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\log P_{MIPM}^a)</th>
<th>(\log P_{MAQ}^a)</th>
<th>(\log P_{PAQ}^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-1.08</td>
<td>-2.69</td>
<td>-2.74</td>
</tr>
<tr>
<td>3</td>
<td>-1.73</td>
<td>-2.27</td>
<td>-2.39</td>
</tr>
<tr>
<td>4</td>
<td>-1.82</td>
<td>-2.04</td>
<td>-1.92</td>
</tr>
<tr>
<td>5</td>
<td>-2.15</td>
<td>-1.82</td>
<td>-1.43</td>
</tr>
<tr>
<td>6</td>
<td>-2.71</td>
<td>-0.79</td>
<td>-0.68</td>
</tr>
<tr>
<td>7</td>
<td>-1.12</td>
<td>-2.77</td>
<td>-3.16</td>
</tr>
<tr>
<td>8</td>
<td>-1.59</td>
<td>-2.77</td>
<td>-2.79</td>
</tr>
</tbody>
</table>

\(^a\) In units of cm h\(^{-1}\).

For the AOC-APAP prodrugs, \(\log P_{MAQ}\) increases linearly with increasing size of the promoetiy in a manner similar to \(\log K_{IPM:4.0}\) and if \(\log K_{IPM:4.0}\) is plotted against \(\log P_{MAQ}\), the following best-fit linear relationship is found:

\[
\log P_{MAQ} = 0.652 \log K_{IPM:4.0} - 2.64 (r^2 = 0.963)
\]  

(5.1)

A plot of \(\log P_{PAQ}\) versus \(\log K_{IPM:4.0}\) yields a similar correlation.

\[
\log P_{PAQ} = 0.805 \log K_{IPM:4.0} - 2.45 (r^2 = 0.974)
\]  

(5.2)
Figure 5-1. Plots of log $K_{IPM:4.0}$ versus log $P$ for compounds 2 through 8 using flux data from the three membrane/vehicle systems.

As expected, the permeability coefficient has a positive correlation to the IPM-water partition coefficient for both membranes. However, a quite different picture emerges if log $P_{MIPM}$ is plotted against log $K_{IPM:AQ}$:

$$\log P_{MIPM} = -0.517 K_{IPM:AQ} - 1.36 \quad (r^2 = 0.940) \quad (5.3)$$

When the delivery vehicle is changed from water to IPM, the correlation between permeability and partition coefficient becomes negative. These are unusual findings for they suggest that mouse skin behaves as a lipophilic membrane for one system and as a hydrophilic membrane in another. One can reconcile this behavior by realizing that IPM and water lie at opposing ends of the polarity spectrum. Therefore, the data more accurately shows that, relative to water, mouse skin is a lipophilic membrane and, relative to IPM, mouse skin is a hydrophilic membrane.
Conversion to the Parent Drug

As mentioned earlier, simple carbonates undergo chemical hydrolysis at a much slower rate than do simple esters and are expected to be relatively stable in neutral aqueous solutions. Dittert et al (1963) studied the hydrolysis of compounds 2, 3, 5 and 6 in pH 7.4 buffer and found them to have half-lives in excess of 150 hours. Hydrolysis studies were not performed on the novel members of the APAP series (4, 7 and 8), but aqueous donor phases from all diffusion cell experiments were collected after use, allowed to evaporate overnight and were subsequently analyzed by ¹H-NMR and melting point. Even after more than 48 hours of exposure to an aqueous environment and recrystallization from water, no evidence of hydrolysis was detectable for any member of the series. This data indicates that chemical hydrolysis would not be responsible for a significant release of parent drug during the diffusion cell experiments.

By comparison to chemical hydrolysis, carbonates are far more susceptible to enzymatic hydrolysis. When Dittert and Swintoski (1968) exposed carbonates 2, 3, 5 and 6 to a 2% solution of human plasma, the most stable compound, 2, had a half-life of three hours; a 50 fold increase in the rate of hydrolysis over that in buffer. They also found the rate of enzymatic hydrolysis increased with the size of the alkyl side chain. By compound 6 (C6), the half-life in human plasma had fallen to only 11 minutes compared to its half-life in buffer of 22,800 minutes; a 200 fold increase.

The percentage of intact prodrug appearing in the receptor phase the carbonate series is consistent with these findings and indicative of enzymatic conversion back to APAP, especially for the IPM data. Compounds 5 and 6, the most enzymatically labile derivatives, were completely hydrolyzed during the experiment. In contrast, the most enzymatically stable compound, 2, showed the highest percentage of intact prodrug when
delivered from IPM. There is also a general correlation between higher flux and a higher percentage of intact prodrug. Two mechanisms could explain this. A higher flux may result in a shorter residence time in the membrane and reduce the chance for interaction between a prodrug molecule and hydrolytic enzymes in the skin. Alternatively, higher flux also correlates with a higher concentration of prodrug in the skin, which could overwhelm the enzyme system and allow a higher percentage of intact molecules through the membrane. Regardless, the best evidence for the enzymatic role in hydrolysis comes from the high percentage of intact prodrug appearing in the receptor phase of the enzyme-free polymer membrane system. With the exception of compound 2, which was 70% hydrolyzed, greater than 90% of each alkyl carbonate was recovered intact from the receptor phase after passing through the PDMS membrane.

**Flux of APAP and its AOC and MOAOC Prodrugs through Hairless Mouse Skin**

The maximum flux, $J_M$, of each compound through hairless mouse skin and PDMS membrane is presented in table 5-3. The flux through hairless mouse skin from a saturated solution of IPM is designated $J_{\text{MIPM}}$, the flux through hairless mouse skin from a saturated aqueous solution is designated $J_{\text{MAQ}}$, and the flux through PDMS membrane from a saturated aqueous solution is designated $J_{\text{PAQ}}$. For convenience, these values are shown in their logarithmic form. The table also contains the second application flux of theophylline ($J_J$) corresponding to each compound and experimental condition.

When delivered from IPM, APAP permeates mouse skin more rapidly than 5-FU, 6-MP or theophylline. It also demonstrates a maximum flux through mouse skin higher than half the prodrugs in the IPM database. In contrast, the carbonate derivatives of APAP perform worse than this on average. Only two of the carbonate prodrugs, 2 and
Table 5-3. Maximum steady-state flux and second application flux of the AOC-APAP and MOAOC-APAP prodrugs through hairless mouse skin and PDMS membrane.

<table>
<thead>
<tr>
<th>Compound</th>
<th>log $J_{\text{MIPM}}$</th>
<th>$J_{\text{MIPM}}$</th>
<th>log $J_{\text{MAQ}}$</th>
<th>$J_{\text{MAQ}}$</th>
<th>log $J_{\text{PAQ}}$</th>
<th>$J_{\text{PAQ}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP, 1</td>
<td>-0.29</td>
<td>0.74</td>
<td>-1.73</td>
<td>0.015</td>
<td>-2.68</td>
<td>0.0013</td>
</tr>
<tr>
<td>2</td>
<td>-0.00</td>
<td>1.12</td>
<td>-1.46</td>
<td>0.034</td>
<td>-1.51</td>
<td>0.0013</td>
</tr>
<tr>
<td>3</td>
<td>-0.76</td>
<td>0.64</td>
<td>-1.62</td>
<td>0.078</td>
<td>-1.74</td>
<td>0.0017</td>
</tr>
<tr>
<td>4</td>
<td>-0.45</td>
<td>1.14</td>
<td>-1.57</td>
<td>0.072</td>
<td>-1.44</td>
<td>0.0018</td>
</tr>
<tr>
<td>5</td>
<td>-1.01</td>
<td>0.85</td>
<td>-2.17</td>
<td>0.051</td>
<td>-1.79</td>
<td>0.0013</td>
</tr>
<tr>
<td>6</td>
<td>-1.49</td>
<td>0.76</td>
<td>-2.28</td>
<td>0.018</td>
<td>-2.16</td>
<td>0.0017</td>
</tr>
<tr>
<td>7</td>
<td>-0.11</td>
<td>0.98</td>
<td>-1.45</td>
<td>0.033</td>
<td>-1.85</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>-1.06</td>
<td>0.94</td>
<td>-2.38</td>
<td>0.022</td>
<td>-2.41</td>
<td>NA</td>
</tr>
</tbody>
</table>

*In units of $\mu$mol cm$^{-2}$ h$^{-1}$

Figure 5-2. Correlation between solubility and flux for APAP, its’ AOC derivatives and its’ MOAOC derivatives.

7, give maximum flux values from IPM that are higher then APAP itself. Compound 2 delivered 1.95 times the amount of APAP through mouse skin as the parent drug and compound 7 delivered 1.5 times as much APAP as the parent. Of the remaining series members, compound 4 performed the best (maximum flux 70% that of APAP) and compound 6 performed the worst (maximum flux 6% that of APAP).
From water, the flux through mouse skin of all compounds is substantially reduced. APAP does not perform quite as well from water and has a lower aqueous flux than four of the carbonate prodrugs. As with flux from IPM, compounds 2 and 7 deliver the highest amounts of APAP, but compound 7 out performs compound 2 when delivered from water (1.90 and 1.68 times APAP flux respectively). Compounds 3 and 4 produced slightly higher aqueous flux values than APAP but, with flux values of only 1.16 and 1.09 times that of APAP, these differences lack significance. Compounds 6 and 8 are the worst performing carbonates with compound 8 giving the lowest aqueous flux. It is worth noting, that while the relative performance among APAP, the AOC compounds and the MOAOC compounds changes depending upon the nature of the vehicle, the order of performance for each type remains nearly the same.

The fluxes of the APAP prodrugs through PDMS membrane from water were similar in magnitude to their fluxes from water through hairless mouse skin, but the order of their performance was not the same. Through PDMS, compound 4 was the best performing prodrug and it possessed a flux similar to that of compound 2. This is a significant change from the flux through mouse skin where the flux of compound 2 was triple that of compound 4 when delivered from IPM and 50 % higher when delivered from water. Compound 5, the third most lipophilic prodrug, has a PDMS flux nearly equal to that of compound 3 and a PDMS flux higher then that of compound 7. In contrast, compound 5 has only one third the flux of compound 3 and one fifth the flux of compound 5 through mouse skin from the same vehicle. An equally profound change is in the performance of the parent APAP. APAP has the third highest flux through hairless mouse skin when delivered from IPM and fifth highest when delivered from water, but it has the lowest flux through PDMS.
Even though the stratum corneum is widely considered to be primarily a lipophilic membrane, IPM solubility alone does not correlate well with flux through mouse skin for either vehicle. The most IPM soluble prodrug, 5, has only the third highest flux from both vehicles and the second most lipid soluble compound, 6, has the lowest flux from both vehicles. In contrast, the most water soluble prodrug, 2, has the highest flux from IPM and the second most water soluble prodrug, 7, has the highest flux from water. Comparing compounds 3 and 4, which have nearly equal IPM solubility, a decrease in water solubility correlates to a drop in flux. Conversely, when aqueous solubility remains nearly constant and IPM solubility increases (such as between compounds 4 and 5) flux also rises. Even without a strict mathematical treatment of this data, it is apparent that flux through mouse skin is benefited by a increasing both lipid and aqueous solubility, regardless of the vehicle used.

The situation is very different for the purely lipophilic PDMS membrane. Although the mouse skin and PDMS flux plots shown in figure 5-3 appear similar in many respects, flux through PDMS shows a much greater dependence on lipophilicity and a decreased dependence on hydrophilicity than flux through mouse skin. As stated previously, the most hydrophilic prodrugs, 2 and 7, have the best flux through mouse skin from either vehicle whereas the most lipophilic compound, 4, has only the fourth highest flux. Through PDMS, however, it is compound 4, which has the highest flux and APAP, the compound with the lowest IPM solubility, which has the lowest flux. Compounds 5 and 7 possess similar molecular weights and similar IPM solubilities but differ by an order of magnitude in their water solubilities. Through PDMS membrane, their fluxes are similar with the slightly more lipophilic compound 5 possessing the higher flux. Through hairless mouse skin, it is the more hydrophilic compound 7 which has 7.9 times
the flux of compound 5 when delivered from IPM and 5 times the flux of compound 5 when delivered from water.

Application of the Roberts-Sloan equation to this and other data in the next chapter will allow us to quantify the relative effects of IPM solubility, water solubility and molecular weight on flux through PDMS membrane. However, even without a strict analysis, it is obvious that, without some modification, flux through PDMS does not correlate to flux through mouse skin. The physical basis for this effect is still a matter of debate, but the favorable influence of aqueous and IPM solubility has been observed in all series comprising the current databases. Determining the relative effect of log $S_{IPM}$ and log $S_{AQ}$ will require a more rigorous model and will be addressed in chapter 6 when this and the other mouse skin data are fit to the Roberts-Sloan equation.

**Effect of the Vehicle on Flux through Hairless Mouse Skin**

It is clear that the vehicle has a profound effect on flux through hairless mouse skin. Flux values from IPM were nearly 10 times higher than flux values from water no matter which compound was present in the vehicle. A similarly consistent 10-fold increase was seen for the second application fluxes through IPM exposed mouse skin compared to those through water exposed mouse skin. This is especially significant because the same vehicle, propylene glycol, was always used to deliver theophylline. The persistence of the increased flux through the IPM exposed mouse skin, even after removal of the initial vehicle, supports the conclusion that exposure to IPM alters hairless mouse skin in an irreversible way and permanently decreases its resistance to penetration.

The relative effects of IPM and water upon the permeation barrier of mouse skin have been well characterized. In 2003, we compared the delivery of chemically stable 5-FU and 6-MP prodrugs through hairless mouse skin from water and IPM (Sloan et al
There were a sufficient number of compounds included in this study to prepare a predictive flux model for each vehicle. The conclusions of these experiments will be covered in more detail in the next chapter; however, an important finding was a nearly constant ten-fold increase in the flux of compounds delivered from IPM. From this and from the APAP prodrug data, it would appear that the influence of IPM on the permeability of hairless mouse skin is consistent and predictable.

**Residual Membrane Amounts**

If the assumptions of the Roberts-Sloan model hold true and if mouse skin is reasonably consistent between individuals, the amount of drug or prodrug remaining in the skin after the diffusion cell experiment should be proportional to the solubility of the prodrug in the skin. Furthermore, since higher solubility in the skin corresponds to higher flux, a correlation should be observed between higher flux and higher residual skin amount. This should be especially true for the lower weight prodrugs whose smaller molecular size has a lesser effect on flux.

Table 5-4. Average residual amounts (± Std. Dev.) of APAP and its prodrugs remaining in hairless mouse skin (HMS) and PDMS membrane after the flux experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HMS/IPM&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>HMS/Aq&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>PDMS/Aq&lt;sup&gt;a,b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP, 1</td>
<td>2.74 (±0.70)</td>
<td>0.90 (±0.30)</td>
<td>0.18 (±0.04)</td>
</tr>
<tr>
<td>2</td>
<td>5.45 (±1.57)</td>
<td>0.95 (±0.15)</td>
<td>0.88 (±0.31)</td>
</tr>
<tr>
<td>3</td>
<td>1.08 (±0.13)</td>
<td>0.76 (±0.13)</td>
<td>0.38 (±0.06)</td>
</tr>
<tr>
<td>4</td>
<td>2.84 (±1.44)</td>
<td>0.95 (±0.22)</td>
<td>0.63 (±0.05)</td>
</tr>
<tr>
<td>5</td>
<td>1.91 (±0.08)</td>
<td>0.25 (±0.05)</td>
<td>0.13 (±0.03)</td>
</tr>
<tr>
<td>6</td>
<td>1.79 (±0.43)</td>
<td>0.40 (±0.14)</td>
<td>0.12 (±0.02)</td>
</tr>
<tr>
<td>7</td>
<td>3.75 (±0.74)</td>
<td>1.56 (±0.22)</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>0.64 (±0.12)</td>
<td>0.34 (±0.07)</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Membrane/Vehicle.

<sup>b</sup> In units of µmols.

<sup>c</sup> Not measured.
The data does show a reasonable agreement correlation between the residual membrane amount and the measured flux values for hairless mouse skin. From both water and IPM, those compounds with the highest flux and next to highest flux (I and VI) also delivered the highest amounts of APAP to the skin. For the remaining APAP carbonates, the relative position of each compound, when ranked by flux, is within one, or at most two positions, of its value when ranked by residual skin amount.

This correlation is acceptable but it does not take into account the effect of molecular weight on flux. Flux and residual skin data can be log transformed and fit to a simplified version of the Roberts-Sloan model with the residual skin amount being used as a surrogate for skin solubility:

\[ \log J_{MV} = x + \log \text{(Residual Skin Amount)} - z \text{ MW} \]

The best fit equations for flux from water and IPM through hairless mouse skin and from water through PDMS are:

\[ \log J_{MIPM} = 0.650 + \log \text{(Residual Skin Amount)} - 0.00748 \text{ MW} \quad (r^2 = 0.797) \quad (5.4) \]
\[ \log J_{MAQ} = -1.38 + \log \text{(Residual Skin Amount)} - 0.00113 \text{ MW} \quad (r^2 = 0.820) \quad (5.5) \]
\[ \log J_{PAQ} = -2.86 + \log \text{(Residual Skin Amount)} + 0.0069 \text{ MW} \quad (r^2 = 0.851) \quad (5.6) \]

Although the correlation coefficients are not particularly high, it must be remembered that residual skin amount is more sensitive to minute differences in the membrane then is maximum flux. Even for the highly homogenous PDMS membrane, which showed very consistent flux values, residual membrane amounts had an average relative standard deviation of 19% which is only slightly smaller then the same values for IPM and water treated mouse skin (23% and 22% respectively). Given the inherent variability in the amount of compound absorbed into the membrane, equations 5-4, 5-5
and 5-6 are consistent with the homogenous membrane assumption for hairless mouse skin.
CHAPTER 6
PREDICTIVE MODELS OF SOLUBILITY AND FLUX

Determination of the Coefficients of the General Solubility Equations

In theory, the melting point coefficient found in equations 3-10 and 3-14, $B_O$, can be calculated directly by using the Walden’s Rule, which states that $\Delta S_F$ for most small nonelectrolytes is approximately $56.6 \text{ J K}^{-1} \text{ mol}^{-1}$. However, an empirical approach based primarily upon our own solubility data was expected to give a better representative value. It was also necessary to use our solubility data to determine a value for the molecular weight coefficient, $C$. Using our 5-FU and 6-MP prodrugs and an additional set of ACOM phenytoin (ACOM-PhT) prodrugs from Stella et al. (1999), aqueous and IPM solubility, melting point and molecular weight data was analyzed using nonlinear multiple regression to generate best-fit coefficients to equations 3.10 and 3.14 for each series. The results of this analysis are summarized in tables 6-1 and 6-2.

Table 6-1: Series specific best fit coefficients to equation 3.10 using IPM solubility data and physical properties from the 5-FU and 6-MP prodrugs.

<table>
<thead>
<tr>
<th>Series ID</th>
<th>$A_{IPM}$</th>
<th>$B_O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-ACOM-5-FU</td>
<td>2.41</td>
<td>0.0188</td>
</tr>
<tr>
<td>1-AOC-5-FU</td>
<td>2.98</td>
<td>0.0190</td>
</tr>
<tr>
<td>1-AC-5-FU</td>
<td>2.99</td>
<td>0.0144</td>
</tr>
<tr>
<td>1-AAC-5-FU</td>
<td>3.65</td>
<td>0.0217</td>
</tr>
<tr>
<td>6-ACOM-6-MP</td>
<td>2.19</td>
<td>0.0129</td>
</tr>
<tr>
<td>6,9-ACOM-6-MP</td>
<td>2.86</td>
<td>0.0225</td>
</tr>
<tr>
<td>3-ACOM-5-FU</td>
<td>2.37</td>
<td>0.0165</td>
</tr>
<tr>
<td>Average (±Std. Dev)</td>
<td>2.74 (±0.50)</td>
<td>0.0177 (±0.0034)</td>
</tr>
</tbody>
</table>
Table 6-2: Series specific best-fit coefficients to equation 3.14 using aqueous solubility data and physical properties from the 5-FU, 6-MP and phenytoin (PhT) prodrugs.

<table>
<thead>
<tr>
<th>Series ID</th>
<th>$A_{AQ}$</th>
<th>$B_O$</th>
<th>$C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-ACOM-5-FU</td>
<td>12.74</td>
<td>0.0167</td>
<td>0.0433</td>
</tr>
<tr>
<td>1-AOC-5-FU</td>
<td>12.16</td>
<td>0.0160</td>
<td>0.0416</td>
</tr>
<tr>
<td>1-AC-5-FU</td>
<td>10.58</td>
<td>0.0125</td>
<td>0.0412</td>
</tr>
<tr>
<td>1-AAC-5-FU</td>
<td>13.65</td>
<td>0.0238</td>
<td>0.0458</td>
</tr>
<tr>
<td>6-ACOM-6-MP</td>
<td>10.44</td>
<td>0.0106</td>
<td>0.0348</td>
</tr>
<tr>
<td>6,9ACOM-6-MP</td>
<td>14.23</td>
<td>0.0218</td>
<td>0.0395</td>
</tr>
<tr>
<td>3-ACOM-5-FU</td>
<td>12.00</td>
<td>0.0154</td>
<td>0.0403</td>
</tr>
<tr>
<td>3-ACOM-PhT</td>
<td>20.54</td>
<td>0.0230</td>
<td>0.0568</td>
</tr>
<tr>
<td><strong>Average (±Std. Dev)</strong></td>
<td><strong>13.29 (±3.21)</strong></td>
<td><strong>0.0175 (±0.0049)</strong></td>
<td><strong>0.0429 (±0.0064)</strong></td>
</tr>
</tbody>
</table>

The estimate for the molecular weight coefficient, $C$, appears to be the most robust. The individual series values for $C$ possess a lower variability than the corresponding series values used to estimate the other coefficients and have a relative standard deviation of 14%. The average $C$ value of 0.0429 agrees very well with the molecular volume effect on aqueous solubility of 0.0437 MV reported by Huibers and Katritzky (1998) in their model for predicting the aqueous solubility of hydrocarbons and halogenated hydrocarbons. Given that their model (which is based upon a dataset of 241 compounds) and the prodrug series model are both attempting to measure the effect of what is essentially pure steric bulk on aqueous solubility, it is encouraging that they are in such close agreement.

When derived from IPM solubility data, the best-fit $B_O$ values have a relative standard deviation that is close to the relative standard deviation for the best-fit $A$ values (19% compared to 18%, respectively). When derived from water solubility data, the relative standard deviations of the best-fit $B_O$ and $A$ coefficients increase to 28% and
24%, respectively. For both water and IPM data, the A coefficient estimates have less relative variability than the B₀ estimates. This somewhat undermines the supposition that B₀ should be more conserved between different series than A. While it is possible that for a given solvent both A and B₀ may be equally independent of the nature of the solute, it is also possible that some of the consistency in the A value estimates is a consequence of the homogeneity in the series used to generate the estimates. Of the seven series used to estimate coefficients from IPM solubility (table 6-1), five of them have 5-FU as the parent compound. Similarly, five of the eight series used to determine the A and B₀ coefficients from water solubility are based on 5-FU (table 6-2). Since the A coefficient is, in theory, determined by a molecule's specific structure and since the same parent moiety appears in all the members of the various 5-FU series, it is reasonable that the small variation in the A coefficient estimates is a result of the large proportion of 5-FU compounds comprising the two data sets.

In addition to the preceding arguments, there are two observations, which lend support to B₀ being considered a universal value for prediction. First, despite the somewhat large variability among the individual series values, the average IPM derived B₀ value is nearly identical to the water derived value. This is consistent with the expectation that the B₀ would be the same for all solvents. Second, when the average A value for a given solvent is used to calculate potential B₀ values for the members of a series, these calculated B₀ values are erratic across the series. Conversely, if the average B₀ value for a given solvent is used to calculate A values for series members, the A values are more consistent across the series. Therefore, the predictive strength of equations 3.10 and 3.14 was tested using the average values of C and B₀.
The intent of developing the solubility equations was to enable data from a single series member to predict the behavior of the other series members. To establish the ability of these equations to accomplish this, solubility data from the first member of each series appearing in tables 6-1 and 6-2 were used to determine $A_{IPM}$ and $A_{AQ}$ coefficient values for their respective series. The one exception to this was the ACOM-Th in which the first series member appears to be an outlier so the second series member was used as a replacement. In turn, these $A_{IPM}$ and $A_{AQ}$ values were used to calculate IPM and aqueous solubility values for all remaining compounds covered in the tables. Figures 6-2 and 6-3 show the correlations between predicted and experimental solubilities in IPM and water.

![Figure 6-1. Calculated versus experimental log IPM solubility using equation 3.10 and A coefficients determined from the smallest series members.](image-url)
Figure 6-2. Calculated versus experimental log $S_{AQ}$ using equation 3.14 and A coefficients determined from the smallest series members.

The average absolute error for predicting solubility in both solvents is approximately the same with 0.18 log units for the IPM model and 0.21 log units for the aqueous model. This is approximately half that of the 0.41 log unit average absolute error reported by Ran, Jain and Yalkowsky (2001) with their modified form of the general solubility equation. The largest outlier is the C1-ACOM-Theophylline compound with an absolute average error of approximately one log unit for both models; twice the magnitude of the next largest errors in both models. Since the IPM and aqueous solubility of this compound are consistent with its’ flux and with its’ expected partitioning coefficient, the most likely explanation for its’ poor adherence to the solubility models lies with its’ melting point. It is quite possible that the original method for purifying the C1-ACOM theophylline isolated a low melting point polymorph that was converted to a more stable (and less soluble) form during solubility determination.
The C1-ACOM-Theophylline notwithstanding, the low average absolute errors of both models indicate that they are capable of making useful predictions and some useful generalizations

**Solubility Behavior of the 4-AOC and 4-MOAOC-APAP Prodrugs**

The 4-AOC-APAP prodrugs exhibit solubility behavior that is typical of the other homologous prodrug series. When the solubility data from compounds 2-6 were fit to the general organic and aqueous solubility models (eq. 3.10 and 3.14), the following best-fit equations were obtained:

\[
\log S_{IPM} = 2.921 - 0.0200 (T_M - T) \quad (r^2 = 0.765) \quad (6.1)
\]

\[
\log S_{AQ} = 11.183 - 0.0169 (T_M - T) - 0.0400 \text{MW} \quad (r^2 = 0.990) \quad (6.2)
\]

The agreement between calculated and experimental solubility values generated from these equations is very close for both IPM and aqueous solubility. For IPM solubility, the average absolute error is only 0.059 log units and, for aqueous solubility, it is 0.085 log units. The accuracy of the calculated IPM solubility values indicates that the relatively poor correlation coefficient for the IPM solubility equation should not be interpreted as a failure of the model. The poor correlation coefficient of equation 6.1 is actually a consequence of the small range in \( \log S_{IPM} \) values possessed by the AOC-APAP prodrugs. Since the difference in IPM solubility between the most and least soluble prodrug in this series is only 0.406 log units, even the small observed deviation from the model is sufficient to produce a poor \( r^2 \).

The series independent coefficients, \( B_O \) and \( C \), determined from the AOC-APAP prodrug data are close to the corresponding average values obtained from the eight other prodrug series. The melting point coefficient obtained from AOC-APAP IPM solubility data is only 0.0023 from the combined database average \( B_O \) value, while the coefficient
obtained from aqueous solubility data is only 0.0008 from the database average. Similarly, the molecular weight coefficient of the AOC-APAP series differs from the database average C value by 0.0029. As with the other series, these observations support the relative independence of these coefficients from series-specific influences.

The series specific solubility parameter for the IPM solubility, $A_{IPM}$, ranged from 2.19 to 3.65 for the series comprising the database. The $A_{IPM}$ value for compounds 2-6 lies precisely in the center of this range. In direct comparison, this $A_{IPM}$ value is very close to the corresponding values of the 1-AOC-5-FU, 1-AC-5-FU and 6,9-ACOM-6-MP series. Of these three series, the 1-AOC-5-FU series also has a $B_O$ value of 0.0190, which is close to the 0.0200 $B_O$ value of the AOC APAP series. Therefore, the AOC-APAP series has an inherent organic solubility equivalent to that of 1-AOC-5-FU series. In other words, for a given molecular weight and melting point, a 4-AOC-APAP prodrug will have the approximately same IPM solubility as a 1-AOC-5-FU prodrug.

The situation is quite different for aqueous solubility. The AOC-APAP compounds have the third smallest $A_{AQ}$ parameter of the homologous series yet examined. Compared to the 1-AOC-5-FU series, $A_{AQ}$ for the 4-AOC-APAP series is smaller by 0.98. With such a decrease in $A_{AQ}$, a 4-AOC-APAP prodrug will therefore be approximately one-tenth as water soluble as a 1-AOC-5-FU prodrug with similar molecular weight and melting point. Such a comparison can be made between compound 2 and the C2 member of the 1-AOC-5-FU series. Both compounds have similar molecular weights (209 and 202 amu respectively) but a 15ºC difference in melting point. As predicted, despite having the higher melting point, the C2-AOC-5-FU compound has a ten-fold higher water solubility. It is this systematically weak ability of the 4-AOC-
APAP prodrugs to interact favorably in aqueous solution, which, along with their only average IPM solubility, ultimately results in their modest flux.

In Chapter 3, it was theorized that $A_{IPM}$ and $A_{AQ}$ for a given homologous series could be estimated from a single series member so long as $B_0$ and $C$ were sufficiently constant for all series. Using solubility data from the first member of each homologous series in the database, IPM and water solubility for the remaining series members were predicted from melting point and molecular weight with reasonable accuracy. This same process was applied to the members of the 4-AOC APAP series. Using the IPM and water solubility of compound 2, and using the average Bo and C parameters obtained from the IPM flux database, the following $A_{IPM}$ and $A_{AQ}$ were obtained.

\[
\log S_{IPM} = A_{IPM} - 0.0176 (T_M - T)
\]
\[
A_{IPM} = \log S_{IPM} + 0.0176 (T_M - T)
\]
\[
A_{IPM} = 1.076 - 0.0176 (113.5-25) = 2.63
\]

\[
\log S_{AQ} = A_{AQ} - 0.0176 (T_M - T) - 0.0429 \text{ MW}
\]
\[
A_{AQ} = \log S_{AQ} + 0.0176 (T_M - T) + 0.0429 \text{ MW}
\]
\[
A_{AQ} = 1.232 + 0.0176 (113.5-25) + 0.0429 (209) = 11.76
\]

Using these values for $A_{IPM}$ and $A_{AQ}$, calculated IPM and water solubilities were predicted for compounds 3-6.

Using an $A_{IPM}$ of 2.63, IPM solubility was very well predicted for the 4-AOC series yielding an average prediction error of 0.10 log units. Using an $A_{AQ}$ value of 11.76, water solubility is less well predicted, although, with an average prediction error of 0.24
Table 6-3. Predicted IPM and water solubilities for compounds 3-6.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exp. Log S_{IPM}</th>
<th>Pred. Log S_{IPM}</th>
<th>Exp. Log S_{AQ}</th>
<th>Pred. Log S_{AQ}</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.968</td>
<td>0.944</td>
<td>0.653</td>
<td>0.499</td>
</tr>
<tr>
<td>4</td>
<td>1.37</td>
<td>1.23</td>
<td>0.477</td>
<td>0.180</td>
</tr>
<tr>
<td>5</td>
<td>1.14</td>
<td>0.979</td>
<td>-0.354</td>
<td>-0.666</td>
</tr>
<tr>
<td>6</td>
<td>1.22</td>
<td>1.16</td>
<td>-1.49</td>
<td>-1.69</td>
</tr>
</tbody>
</table>

a In Units of mM.

log units, the model is still reasonably accurate. It is important to note that the predicted water solubilities are consistently lower than the experimental values. This suggests that the data from compound 2 did not estimate a value for A_{AQ}, which truly reflected the series value. If compound 3 had been used to estimate A_{AQ} instead of compound 2, then the average error of prediction for log S_{AQ} would have been 0.08 log units. This is a problem intrinsic to this type of analysis and it highlights how care must be taken in the interpretation of predicted results.

In addition to predicting solubility behavior across a series, the series specific IPM and water solubility equations were developed to quantify the effects of various physical parameters on solubility. Accounting for melting point and molecular weight influences on solubility allows the inherent solubilizing effect of different promoieties to be compared. Compounds 7 and 8 can be used to estimate the series specific A_{IPM} and A_{AQ} values for ether containing carbonate promoities by following the same procedure that was used to estimate these parameters from compound 2. Since the 4-AOC-APAP prodrugs and the 4-MOAOC-APAP prodrugs differ only in the composition of the promoity, any difference in the A_{IPM} and A_{AQ} values must be due the promoity alone. The results of this analysis are summarized in Table 6-4.
Figure 6-3. Predicted versus experimental IPM solubilities for the 4-AOC-APAP prodrugs ($A_{IPM} = 2.63$).

Figure 6-4. Predicted versus experimental aqueous solubilities for the 4-AOC-APAP prodrugs ($A_{AQ} = 11.76$).
Table 6-4. Estimated $A_{IPM}$ and $A_{AQ}$ using data from compounds 7 and 8.

<table>
<thead>
<tr>
<th>Data Source</th>
<th>Est. $A_{IPM}$</th>
<th>Est. $A_{AQ}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp. 7</td>
<td>1.96</td>
<td>13.12</td>
</tr>
<tr>
<td>Comp. 8</td>
<td>2.24</td>
<td>13.55</td>
</tr>
<tr>
<td>4-AOC-APAP Average$^a$</td>
<td>2.71</td>
<td>11.95</td>
</tr>
</tbody>
</table>

$^a$ Average of estimated parameters using data from compounds 1-5.

The two 4-MOAOC APAP compounds generate series specific parameters that are consistent with one another. Both compounds indicate that ether containing carbonate promoieties are less effective at increasing IPM solubility than alkyl carbonates, but are better at improving water solubility. When matched for melting point and molecular weight, a prodrug of the MOAOC type will have approximately one fourth the IPM solubility of an AOC type prodrug ($\log S_{IPM}$ lower by 0.6 log units), but approximately 24 times the water solubility ($\log S_{AQ}$ higher by 1.38 log units). Given the roughly equal effect of IPM and aqueous solubility on flux, ether-containing promoieties have a higher inherent potential to improve topical delivery then do n-alkyl promoieties.

**Modeling the Flux of the 4-AOC and 4-MOAOC APAP Prodrugs through Hairless Mouse Skin from IPM and Water**

To examine the flux behavior of the APAP prodrugs relative to the flux behavior of the heterocyclic prodrugs, the Roberts-Sloan equation was applied to several data sets. The first attempt to predict the fluxes of the APAP prodrugs was performed using the Roberts-Sloan model derived from the original 42-compound database Roberts and Sloan, 1999).
log $J_{\text{MIPM}} = -0.216 + 0.534 \log S_{\text{IPM}} + (1-0.534) \log S_{\text{AQ}} – 0.00361 \text{ MW}$ \hspace{1em} (r^2 = 0.951) \hspace{1em} (6.3)

Following publication of the first Roberts-Sloan equation, three additional series of 5-FU prodrugs and the C5 member of the 6,9-bis-ACOM 6-MP series were synthesized, characterized and added to the IPM/Hairless mouse skin database. The inclusion of these thirteen compounds revised the coefficients of the model as shown in equation 6.4.

log $J_{\text{MIPM}} = -0.287 + 0.535 \log S_{\text{IPM}} + (1-0.535) \log S_{\text{AQ}} – 0.00341 \text{ MW}$ \hspace{1em} (r^2 = 0.937) \hspace{1em} (6.4)

Finally, compounds 1 through 8 were added to the database to give the final form of the model.

Log $J_{\text{MIPM}} = -0.501 + 0.517 \log S_{\text{IPM}} + (1-0.517) \log S_{\text{AQ}} – 0.00266 \text{ MW}$ \hspace{1em} (r^2 = 0.912) \hspace{1em} (6.5)

Each model was used to calculate expected IPM flux values for the APAP prodrugs and these values are presented and compared to the experimental flux values in table 6-5.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental log $J_{\text{MIPM}}$</th>
<th>Predicted log $J_{\text{MIPM}}$ a \hspace{1em} Eq. 6.3</th>
<th>Predicted log $J_{\text{MIPM}}$ a \hspace{1em} Eq. 6.4</th>
<th>Predicted log $J_{\text{MIPM}}$ a \hspace{1em} Eq. 6.5</th>
<th>$\Delta$ log $J_{\text{MIPM}}$ Eq. 6.3</th>
<th>$\Delta$ log $J_{\text{MIPM}}$ Eq. 6.4</th>
<th>$\Delta$ log $J_{\text{MIPM}}$ Eq. 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, APAP</td>
<td>-0.29</td>
<td>0.32</td>
<td>-0.61</td>
<td>0.28</td>
<td>-0.57</td>
<td>0.21</td>
<td>-0.50</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>0.18</td>
<td>-0.18</td>
<td>0.15</td>
<td>-0.15</td>
<td>0.09</td>
<td>-0.10</td>
</tr>
<tr>
<td>3</td>
<td>-0.76</td>
<td>-0.20</td>
<td>-0.56</td>
<td>-0.23</td>
<td>-0.53</td>
<td>-0.28</td>
<td>-0.48</td>
</tr>
<tr>
<td>4</td>
<td>-0.45</td>
<td>-0.12</td>
<td>-0.33</td>
<td>-0.14</td>
<td>-0.31</td>
<td>-0.19</td>
<td>-0.26</td>
</tr>
<tr>
<td>5</td>
<td>-1.01</td>
<td>-0.68</td>
<td>-0.33</td>
<td>-0.70</td>
<td>-0.31</td>
<td>-0.75</td>
<td>-0.26</td>
</tr>
<tr>
<td>6</td>
<td>-1.49</td>
<td>-1.27</td>
<td>-0.22</td>
<td>-1.28</td>
<td>-0.21</td>
<td>-1.33</td>
<td>-0.16</td>
</tr>
<tr>
<td>7</td>
<td>-0.11</td>
<td>0.02</td>
<td>-0.13</td>
<td>0.00</td>
<td>-0.11</td>
<td>-0.02</td>
<td>-0.09</td>
</tr>
<tr>
<td>8</td>
<td>-1.06</td>
<td>-0.72</td>
<td>-0.34</td>
<td>-0.73</td>
<td>-0.33</td>
<td>-0.75</td>
<td>-0.31</td>
</tr>
</tbody>
</table>

In units of $\mu$mol cm$^{-2}$ h$^{-1}$. 

Table 6-5. Predicted and experimental flux values for compounds 1-8 through hairless mouse skin from IPM.
Figure 6-5. Experimental versus calculated log maximum flux values through hairless mouse skin from IPM using equation 6.5.

The revised coefficients of equation 6.5 are close to those obtained from the smaller database and are not significantly different from the original coefficients of equation 6.3. In addition, the average difference between the calculated and the experimental log $J_{MIPM}$ values for members of both databases is identical (~0.16 log units). These observations indicate that the behavior of the additional heterocyclic produgs is similar to that of the original database members and their addition to the database does not significantly alter the model.

APAP and all of its produgs gave lower flux then anticipated, regardless of the model used. Compounds 1 through 8 under performed by an average of 0.34 log units according to equation 6.3 and by an average of 0.32 log units according to equation 6.4.
Even when the APAP prodrugs are included in the model (eq. 6.5), they still underperform by an average of 0.27 log units according to equation 6.4; approximately twice the average difference of the remaining members of the database. In addition, three of the series members, compounds 1, 3 and 8, have the largest estimation error of the entire database. With no other APAP series or series based upon molecules similar to APAP in the database to serve as a comparison, it is impossible to determine whether the lower than expected performance is due to a unpredicted aspect of APAP or due to the model being influenced disproportionately by the other heterocyclic compounds. In other words, it is not clear how the addition of other types of prodrugs to the database will ultimately affect the IPM model and it is possible that their addition will mitigate the apparent underperformance of the APAP prodrugs. In any case, the performance of the APAP series is not likely a result of the carbonate promoiety since APAP itself also underperforms.

Although the IPM model does not predict the behavior of the APAP compounds as well as the other prodrug series, it still provides a more then adequate estimation of their flux. Along with predicting topical delivery, flux models are used to determine those members of a series that are most likely to perform best. Therefore, while accurate flux prediction is the target, it is also important for a flux model to identify the relative order of performance for the members of a given series. All the models correctly identified the best performing prodrugs of the 4-AOC and 4-MOAOC APAP series and they correctly ranked the performance of all the remaining prodrugs.

The Roberts-Sloan equation was initially derived to predict flux from IPM. However, its final form is independent of the vehicle used. Therefore, it can be used
without modification to predict the delivery of compounds from water, or any other vehicle, in a manner analogous to the prediction of flux from IPM. In the heterocyclic database, only three series, the 3-ACOM 5-FU, 6-ACOM 6-MP and bis-6,9-ACOM 6-MP prodrugs, were sufficiently stable to hydrolysis to allow their delivery from water in mouse skin diffusion cell experiments. The flux of these compounds through hairless mouse skin from saturated aqueous solutions was measured and, when combined, the members of these series produced a database of 18 compounds (Sloan et al, 2003). These data were used to generate a Roberts-Sloan model for aqueous flux:

\[
\log J_{MAQ} = -1.497 + 0.660 \log S_{IPM} + (1-0.660) \log S_{AQ} - 0.00468 \text{MW} \quad (r^2 = 0.765) \quad (6.6)
\]

Although the aqueous Roberts-Sloan model does not have as high a regression coefficient as the IPM model, its average error of prediction is only slightly larger, 0.18 log units for flux from water as opposed to 0.14 log units for flux from IPM. In contrast to their delivery from IPM, the APAP prodrugs conform to the aqueous model more closely then any other database series. When equation 6.6 was used to predict the flux for compounds 1 through 8, the average error of prediction was 0.15 log units; close to the error for the IPM model. It is consistent with this good fit to model 6.6 that inclusion of the APAP prodrugs into the database only slightly affects the coefficients of the model and gives a slight improvement to the model’s overall fit (equation 6.7).

\[
\log J_{MAQ} = -1.665 + 0.657 \log S_{IPM} + (1-0.657) \log S_{AQ} - 0.00409 \text{MW} \quad (r^2 = 0.774) \quad (6.7)
\]

Equation 6.7 generates an average absolute error of prediction of 0.16 log units for the entire database and 0.13 log units for the APAP prodrugs.
Table 6-6. Predicted and experimental flux values for compounds 1-8 through hairless mouse skin from water.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental log $J_{MAQ}$</th>
<th>Predicted log $J_{MAQ}$ a Eq. 6-6</th>
<th>$\Delta$ log $J_{MAQ}$ Eq. 6-6</th>
<th>Predicted log $J_{MAQ}$ a Eq. 6-7</th>
<th>$\Delta$ log $J_{MAQ}$ Eq. 6-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, APAP</td>
<td>-1.73</td>
<td>-1.34</td>
<td>-0.39</td>
<td>-1.41</td>
<td>-0.32</td>
</tr>
<tr>
<td>2</td>
<td>-1.46</td>
<td>-1.35</td>
<td>-0.12</td>
<td>-1.39</td>
<td>-0.07</td>
</tr>
<tr>
<td>3</td>
<td>-1.62</td>
<td>-1.68</td>
<td>0.06</td>
<td>-1.72</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>-1.57</td>
<td>-1.54</td>
<td>-0.03</td>
<td>-1.57</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>-2.17</td>
<td>-2.04</td>
<td>-0.13</td>
<td>-2.06</td>
<td>-0.11</td>
</tr>
<tr>
<td>6</td>
<td>-2.28</td>
<td>-2.50</td>
<td>0.23</td>
<td>-2.52</td>
<td>0.24</td>
</tr>
<tr>
<td>7</td>
<td>-1.45</td>
<td>-1.57</td>
<td>0.11</td>
<td>-1.58</td>
<td>0.13</td>
</tr>
<tr>
<td>8</td>
<td>-2.38</td>
<td>-2.27</td>
<td>-0.11</td>
<td>-2.28</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

a In units of $\mu$mol cm$^{-2}$ h$^{-1}$.

Figure 6-6. Calculated versus experimental log maximum flux values through hairless mouse skin from water using equation 6-7.
The flux of 6-MP from IPM demonstrates the largest deviation from the Roberts-Sloan model of any compound yet measured ($\Delta \log J_{\text{IPM}} = 0.69$). A similarly large deviation from the model is seen when 6-MP is delivered from water. Since the low flux of 6-MP is independent of the vehicle, it is reasonable to speculate that 6-MP exhibits unusual behavior in solution. Sloan hypothesized that the ability of 6-MP molecules to stack efficiently with one another might lead to a strong self-association in solution. This association would limit the amount of monomeric 6-MP available to enter the skin and reduce the observed flux. Such a situation does not exist with the APAP prodrugs. The close agreement between the predicted and experimental flux values for the water delivered APAP prodrugs suggests that, unlike 6-MP, these compounds do not possess inherent properties that cause them to deviate from behavior predicted by the model.

Prediction of flux is not the only information that can be gained from the various Roberts-Sloan models. The theoretical development of the Roberts-Sloan model, like the Potts-Guy model, makes as one of its fundamental assumptions that the skin behaves like a homogeneous membrane with regard to diffusion. Recalling the equation for maximum flux through a homogeneous membrane and comparing this to the Roberts-Sloan equation, it is possible for the coefficients and solubility terms of the flux models can be correlated to the properties of the skin. Therefore given the two equations,

$$\log J_M = \log D_0 - \log L + \log S_M - \beta MW$$

$$\log J_M = x + y \log S_{\text{IPM}} + (1-y) \log S_{\text{AQ}} - z MW$$

the following correlations can be made:

$$x = \log D_0 - \log L$$

$$\log S_M = y \log S_{\text{IPM}} + (1-y) \log S_{\text{AQ}}$$

(6.8)

(6.9)
Equation 6.8 indicates that the $x$ coefficient is a combination of the membrane’s intrinsic resistance to diffusion, $D_0$, and the effective thickness or path length through the membrane ($L$). Whether the skin is exposed to IPM or an aqueous vehicle, the conditioning process ensures that the skin is near maximum hydration before the application of the drug suspensions and has undergone a similar amount of swelling. Therefore, the thickness of the skin is approximately the same regardless of the vehicle used. Given a near constant thickness, the $x$ coefficient becomes a reflection of the intrinsic diffusivity of the skin and the difference between the $x$ coefficients the two models is equal to the log of the $D_0$ ratio. Recalling the theoretical development from chapter 2, the factor $D_0$ has units of h$^{-1}$ and it indicates the apparent average velocity with which a hypothetical entity of negligible volume moves through the skin. Since a higher apparent velocity corresponds to a lower energy requirement for movement, $D_0$ is an inverse indicator of the work required to move through the membrane.

The difference between $x$ terms in the IPM model and the aqueous model is 1.15, which converts to an anti-log value of approximately 14 for the ratio of $D_{0\text{IPM}}/D_{0\text{AQ}}$. In other words, for a given compound, the flux through IPM damaged skin is expected to be 14 times higher than through water damaged skin as a result of the decrease in the energy cost required to move through the skin. This is consistent with the observed approximate tenfold lower flux of compounds delivered from water as compared to those delivered from IPM (Sloan et al, 2003).

Unfortunately, the models are unable to assign an underlying physical cause to the decrease in membrane integrity. However, it is well established that the high degree of
organization in the components of the stratum corneum is responsible for the barrier of
the skin. The substantial amount of these components is kept in this organization without
covalent bonding to relatively immobile structures such as the corneocytes. While IPM
does not readily enter undamaged stratum corneum, it is possible that IPM is capable of
leaching from the stratum corneum some of its lipophilic components. For example,
octanol has been shown to remove enzymes from the stratum corneum that are capable of
hydrolyzing prodrugs in the vehicle (Waranis and Sloan, 1987). The loss of these
components may disrupt inter-corneocyte lipid organization and lead to a loss in barrier
function. The inability of water to similarly leach these components may account for its
less damaging effect.

Although the intercorneocyte lipids have a lamellar structure of alternating polar
and non-polar regions, the results of the Roberts-Sloan treatments demonstrate that a
series description is not required for accurate prediction of flux. From a functional point
of view, the barrier of the skin can be viewed as a single phase and the properties of this
phase can be inferred from the $y$ coefficient. As indicated by equation 6.9, a compound’s
solubility in the skin can be estimated from its solubility in IPM and water. Beginning
with the fundamental work of Hildebrand (1936), many researchers have made attempts
to predict or explain solubility behavior from the physical properties of the solute and
solvent. Just as solubility can be predicted from physical parameters, physical parameters
can be conversely predicted from solubility. Of the solubility models available, the
amended solvation energy relationship of Abraham (Abraham and Le, 1999) provides the
most convenient method of examining the physical properties of skin from solubility.
The general form of Abraham’s model states that any physical property (i.e., solubility, partition coefficient or permeability) can be predicted from parameters that reflect the various molecular interactions of the solute and solvent:

$$\log SP = c + rR_2 + s\pi_2^H + a\Sigma\sigma_2^H + b\Sigma\beta_2^H + vV_x$$  \hspace{1cm} (6.11)

Each term of the equation contains a parameter derived from the solute ($R_2$, $\pi_2$, $\alpha$, $\beta$ and $V_x$) and a corresponding coefficient characteristic of the solvent ($r$, $s$, $a$, $b$ and $v$). $R$ is the excess molar refraction, $\pi_2$ is the polarizability, $\alpha$ is the hydrogen bond acidity, $\beta$ is hydrogen bond basicity and $V_x$ is the molecular volume. An equation corresponding to 6.9 may be written for both IPM and water solubility:

$$\log S_{IPM} = c + r_{IPM}R_2 + s_{IPM}\pi_2^H + a_{IPM}\Sigma\sigma_2^H + b_{IPM}\Sigma\beta_2^H + v_{IPM}V_x$$

$$\log S_{AQ} = c + r_{AQ}R_2 + s_{AQ}\pi_2^H + a_{AQ}\Sigma\sigma_2^H + b_{AQ}\Sigma\beta_2^H + v_{AQ}V_x$$

Substituting the above two equations into equation 6.11 results in an Abraham equation for solubility in the skin:

$$\log S_{MS} = y \log S_{IPM} + (1-y) \log S_{AQ}$$

$$y \log S_{IPM} = y c_{IPM} + y r_{IPM}R_2 + y s_{IPM}\pi_2^H + y a_{IPM}\Sigma\sigma_2^H + y b_{IPM}\Sigma\beta_2^H + y v_{IPM}V_x$$

$$(1-y) \log S_{AQ} = (1-y) c_{AQ} + (1-y) r_{AQ}R_2 + (1-y) s_{AQ}\pi_2^H + (1-y) a_{AQ}\Sigma\sigma_2^H + (1-y)b_{AQ}\Sigma\beta_2^H$$

$$+ (1-y) v_{AQ}V_x$$

$$\log S_{MS} = [y c_{IPM} + (1-y) c_{AQ}] + [y r_{IPM} + (1-y) r_{AQ}]R_2 + [y s_{IPM} + (1-y) s_{AQ}]\pi_2^H$$

$$+ [y a_{IPM} + (1-y) a_{AQ}]\Sigma\sigma_2^H + [y b_{IPM} + (1-y) b_{AQ}]\Sigma\beta_2^H$$  \hspace{1cm} (6.12)

From equation 6.12, each coefficient that is characteristic to the skin is equivalent to a sum of the corresponding Abraham terms for water and IPM scaled by $y$ coefficient from the Roberts-Sloan model.
Water solubility is greatly influenced by the ability to form hydrogen bonds. The hydrogen bond acidity and basicity terms for water solubility ($a_{AQ}$ and $b_{AQ}$) were reported by Abraham and Le to be 0.646 and 3.279, respectively. Corresponding model coefficients for IPM have not been yet reported, but Abraham has used the same model to predict partitioning between water and various organic solvents. Using $a$ and $b$ values from the partitioning models for organic solvents similar to IPM, we can infer that $a_{IPM}$ and $b_{IPM}$ are either small compared to $a_{AQ}$ and $b_{AQ}$ or somewhat negative. Assuming that $a_{IPM}$ and $b_{IPM}$ are small compared to and $a_{AQ}$ and $b_{AQ}$, the hydrogen bond values for mouse skin, $a_{MS}$ and $b_{MS}$, can be approximated as:

$$a_{MS} \approx (1-y) \, a_{AQ} \approx 0.483 \, a_{AQ}$$

$$b_{MS} \approx (1-y) \, b_{AQ} \approx 0.483 \, b_{AQ}$$

With these assumptions, IPM-treated mouse skin has approximately one half the ability to interact favorably with a drug through hydrogen bonding as does water. However, even if the assumptions are not true, hydrogen-bonding capacity of mouse skin still increases linearly with decreasing $y$ and this factor can be used to compare skin sections exposed to different vehicles. It follows that compounds, which take advantage of these interactions, will more readily penetrate into the skin and the parallel between water solubility and the ability to form hydrogen bonds underlies the positive correlation between water solubility and flux.

The $y$ coefficient of mouse skin exposed to IPM is 0.14 lower than the $y$ value for mouse skin exposed to water. This indicates that mouse skin damaged by IPM has a higher capacity to form hydrogen bonds than does water damaged skin. Given that the matrix of the stratum corneum is a complex, multi-component mixture, a relative increase
in the capacity to hydrogen bond is most likely the result of an increase in the concentration of hydrogen bonding components. This result is consistent with the theory that IPM is capable of leaching lipophilic components from the stratum corneum. As lipophilic components are removed from the inter-corneocyte matrix and the organization of the matrix disrupted, water from the dermis will diffuse into the stratum corneum. Since the prodrug saturated IPM solution covering the epidermis prevents the loss of this water through evaporation, the resulting equilibrium concentration of water in the stratum corneum is increased. In turn, the greater percentage of water in the stratum corneum is observed as a decrease in the y coefficient of the Roberts-Sloan model. Ultimately, the observed effect on flux caused by IPM results from a simultaneous change in the lipid organization and composition of the stratum corneum.

**Modeling the Flux of the 4-AOC and 4-MOAOC APAP Prodrugs through PDMS Polymer Membrane from Water**

The initial attempts to model the flux of the water stable prodrugs through PDMS membrane were unexpectedly disappointing. Since PDMS membrane is very homogeneous and given the highly consistent results from the diffusion cells using polymer membranes, it was anticipated that the flux through the polymer would show the best correlation to the Roberts-Sloan model. Unfortunately, the model generated from the entire dataset displayed an unusually poor correlation:

\[
\log J_{PAQ} = -2.131 + 0.841 \log S_{IPM} + (1-0.841) \log S_{AQ} - 0.00364 \text{MW} \ (r^2 = 0.653) \ (6.13)
\]

In spite of the poor fit, equation 6.13 still possessed coefficients that were in agreement with known properties of PDMS membrane. The higher y coefficient,
indicating a greater dependence of flux on IPM solubility, was consistent with the more lipophilic nature of the polymer membrane relative to mouse skin. The lower intrinsic diffusivity of the polymer membrane was also reflected by a relative decrease in $x$. The
reasonable coefficients of the model suggested that the poor correlation may be due to the unusual behavior of select database members, but an examination of the delta log $J_{PAQ}$ values failed to identify any specific outliers. To further investigate the behavior of the polymer membrane, alternate models for predicting flux were used.

$$\log J_{PAQ} = -2.131 + 0.841 \log S_{IPM} + (1-0.841) \log S_{AQ} - 0.00364 \text{MW}$$

Figure 6-7. Calculated versus experimental log maximum flux values through PDMS from water by Equation 6.13.

In the development of their flux model, Kastings, Smith and Cooper (1987) assumed that the stratum corneum was lipophilic enough to use octanol solubility as a surrogate to estimate stratum corneum solubility. While this assumption may be debatable for stratum corneum, it is likely to apply to the highly lipophilic PDMS membrane. By assuming that the log of IPM solubility is proportional to the log of polymer solubility ($y \log S_{IPM} \approx \log S_{PDMS}$) and by keeping the other assumptions
inherent in the Roberts-Sloan model, a simplified model for flux through PDMS can be derived:

\[
\log J_{PAQ} = x + y \log S_{IPM} - z MW \quad (6.14)
\]

Performing multiple linear regression on the flux through PDMS data using equation 6.14 resulted in the following best fit coefficients:

\[
\log J_{PAQ} = -1.206 + 1.00 \log S_{IPM} - 0.00758 MW \quad (r^2 = 0.617) \quad (6.15)
\]

Although the fit of this model is no better, equation 6-15 justifies the assumption that IPM solubility and polymer solubility mirror one another. A y coefficient of unity indicates that the best estimate of polymer flux is obtained if it is presumed that \(S_{IPM} \approx S_{PDMS}\). Therefore, if molecular weight effects are reasonably small, a plot of \(\log S_{IPM}\) versus \(\log J_{PAQ}\) should produce a linear relationship.

Figure 6-8 demonstrates the clear linear correlation between flux and solubility in IPM. Even without a correction for molecular weight, all but two of the prodrugs lie within +/-0.5 log units of the best-fit regression line shown. This is in spite of the fact that the molecular weights of these compounds range from 151 to 352 amu. In comparison, the two outliers, the C4 and C5 bis-6,9-6 MP prodrugs, have molecular weights of 380 and 408, an increase of only 28 and 56 amu over the largest remaining database member, and yet they under perform by over 1.5 log units. Excluding the C4 and C5 bis 6,9-6-MP prodrugs from the database, improves the fit of the IPM based polymer model (6.15) and the Roberts-Sloan polymer model to approximately that of the aqueous mouse skin model (6-7).

\[
\log J_{PAQ} = -2.323 + 1.013 \log S_{IPM} - 0.00295 MW \quad (r^2 = 0.794) \quad (6.16)
\]

\[
\log J_{PAQ} = -2.855 + 0.902 \log S_{IPM} + (1-0.902) \log S_{AQ} - 0.00064 MW \quad (r^2 = 0.814) \quad (6.17)
\]
Figure 6-8. Log IPM solubility versus log maximum flux through PDMS membrane from water for the aqueous database

Figure 6-9. Calculated versus experimental log maximum flux values through PDMS from water by Equation 6.17.
Table 6-8. Calculated maximum log flux values through PDMS from water and errors of calculation for the chemically stable prodrug series.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>Exp. log ( J_{PAQ} )a (eq. 6.16)</th>
<th>Calc. log ( J_{PAQ} )a (eq. 6.16)</th>
<th>( \Delta ) log ( J_{PAQ} )a (eq 6.17)</th>
<th>Calc. log ( J_{PAQ} )a (eq 6.17)</th>
<th>( \Delta ) log ( J_{PAQ} )a (eq 6.17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-ACOM-5-FU</td>
<td>-2.64</td>
<td>-2.83</td>
<td>-0.19</td>
<td>-2.73</td>
<td>-0.09</td>
</tr>
<tr>
<td>C2</td>
<td>-1.78</td>
<td>-1.74</td>
<td>0.04</td>
<td>-1.69</td>
<td>0.09</td>
</tr>
<tr>
<td>C3</td>
<td>-1.6</td>
<td>-1.56</td>
<td>0.04</td>
<td>-1.53</td>
<td>0.07</td>
</tr>
<tr>
<td>C4</td>
<td>-1.7</td>
<td>-1.55</td>
<td>0.15</td>
<td>-1.55</td>
<td>0.15</td>
</tr>
<tr>
<td>C5</td>
<td>-1.58</td>
<td>-1.43</td>
<td>0.15</td>
<td>-1.46</td>
<td>0.12</td>
</tr>
<tr>
<td>C7</td>
<td>-1.82</td>
<td>-1.54</td>
<td>0.28</td>
<td>-1.62</td>
<td>0.21</td>
</tr>
<tr>
<td>C1-6-ACOM-6-MP</td>
<td>-3.32</td>
<td>-2.96</td>
<td>0.36</td>
<td>-2.89</td>
<td>0.42</td>
</tr>
<tr>
<td>C2</td>
<td>-2.82</td>
<td>-2.66</td>
<td>0.17</td>
<td>-2.62</td>
<td>0.20</td>
</tr>
<tr>
<td>C3</td>
<td>-2.67</td>
<td>-2.54</td>
<td>0.13</td>
<td>-2.52</td>
<td>0.15</td>
</tr>
<tr>
<td>C4</td>
<td>-2.65</td>
<td>-2.47</td>
<td>0.18</td>
<td>-2.47</td>
<td>0.18</td>
</tr>
<tr>
<td>C5</td>
<td>-2.73</td>
<td>-2.57</td>
<td>0.16</td>
<td>-2.59</td>
<td>0.14</td>
</tr>
<tr>
<td>C1-6,9ACOM-6-MP</td>
<td>-1.92</td>
<td>-2.46</td>
<td>-0.54</td>
<td>-2.35</td>
<td>-0.42</td>
</tr>
<tr>
<td>C2</td>
<td>-1.36</td>
<td>-1.73</td>
<td>-0.37</td>
<td>-1.66</td>
<td>-0.30</td>
</tr>
<tr>
<td>C3</td>
<td>-1.68</td>
<td>-1.38</td>
<td>0.30</td>
<td>-1.38</td>
<td>0.29</td>
</tr>
<tr>
<td>C4</td>
<td>-2.4</td>
<td>-1.17</td>
<td>1.23</td>
<td>-1.21</td>
<td>1.19</td>
</tr>
<tr>
<td>C5</td>
<td>-3.29</td>
<td>-1.80</td>
<td>1.49</td>
<td>-1.87</td>
<td>1.42</td>
</tr>
<tr>
<td>APAP, 1</td>
<td>-2.69</td>
<td>-2.48</td>
<td>0.20</td>
<td>-2.50</td>
<td>0.18</td>
</tr>
<tr>
<td>C1, 2</td>
<td>-1.5</td>
<td>-1.85</td>
<td>-0.34</td>
<td>-1.90</td>
<td>-0.39</td>
</tr>
<tr>
<td>C2, 3</td>
<td>-1.74</td>
<td>-2.00</td>
<td>-0.26</td>
<td>-2.06</td>
<td>-0.32</td>
</tr>
<tr>
<td>C3, 4</td>
<td>-1.43</td>
<td>-1.63</td>
<td>-0.19</td>
<td>-1.72</td>
<td>-0.28</td>
</tr>
<tr>
<td>C4, 5</td>
<td>-1.79</td>
<td>-1.91</td>
<td>-0.12</td>
<td>-2.02</td>
<td>-0.23</td>
</tr>
<tr>
<td>C6, 6</td>
<td>-2.18</td>
<td>-1.91</td>
<td>0.25</td>
<td>-2.08</td>
<td>0.08</td>
</tr>
<tr>
<td>MeO-C2, 7</td>
<td>-1.85</td>
<td>-2.04</td>
<td>-0.19</td>
<td>-1.97</td>
<td>-0.13</td>
</tr>
<tr>
<td>MeO-C3i, 8</td>
<td>-2.38</td>
<td>-2.57</td>
<td>-0.17</td>
<td>-2.51</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

\( a \) In units of µmol cm\(^{-2}\) h\(^{-1}\)

If the two largest outliers are excluded from database, the average absolute error of calculation for the remaining compounds is 0.28 log units and 0.22 log units for the APAP prodrugs when calculated by equation 6.16. If equation 6.17 is used instead, the
average absolute error of calculation for the whole database and the APAP prodrug series is 0.21 log units. It is important to note that while the Roberts-Sloan equation is capable of adequately predicting flux through polymer membrane, a reasonable prediction is possible without considering water solubility; even though water was used as the vehicle. This is quite different from flux through mouse and human skin where accurate predictions require adequate consideration of water solubility. This is further evidence that the influence of aqueous solubility on flux is not a fictitious effect derived from an aqueous vehicle, but an attribute of mammalian skin.

Unfortunately, it is still uncertain why a flux through homogeneous membrane like PDMS, which in theory should conform to the simplifying assumptions of the model far more closely than mouse skin, is not more accurately predicted. Central to this uncertainty is why the two largest prodrugs displayed such divergent flux behavior, although there are numerous mechanisms that would account for these observations. One possibility is that the dissolution rate of these compounds is much slower then the rate with which they diffuse into the polymer (dissolution-rate control). It is also possible (as suggested by Sloan to explain the unusual behavior of 6-MP) that these compounds may associate so strongly in solution that only a small the amount of monomer is present for diffusion. Yet another mechanism is that of static diffusion layer control. Flynn and Yalkowsky discussed the possibility of such a mechanism when attempting to explain the fluxes of large (>C5) n-alkyl-para-aminobenzoic acid (PABA) esters through silicone membrane. They reported that the time required by these compounds to reach steady state flux in diffusion cells was disproportionately high relative to the smaller series members. In addition, for the C6 PABA ester, flux through silicone membrane was
independent of the membrane thickness. Given the low aqueous solubility of large PABA esters and their relatively high solubility in silicone membrane, Flynn and Yalkowky argued that it was possible for the region of saturated solution nearest to the silicone membrane to become depleted of PABA ester. They hypothesized that this layer of depleted solution acted effectively as a second “membrane” which controlled flux through the silicone polymer.

Although Flynn and Yalkowsky developed several equations to relate flux to partition coefficient and alkyl chain length, they failed to investigate the most fundamental relationships between solubility, molecular weight and flux. Flynn and Yalkowski measured and reported the solubilities of the PABA esters in both silicone oil and hexane and considered these solubilities to be indicative of solubility in PDMS polymer. But, they did not adequately account for the effect of molecular weight on flux and in doing so may have drawn an erroneous conclusion.

Table 6-9. Solubility, molecular weight and flux data for the PABA esters

<table>
<thead>
<tr>
<th>PABA Ester</th>
<th>MW</th>
<th>log $S_{SO}$</th>
<th>log $S_{HEX}$</th>
<th>log $J_{PDMS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>202</td>
<td>0.72</td>
<td>0.75</td>
<td>-2.61</td>
</tr>
<tr>
<td>C2</td>
<td>216</td>
<td>0.92</td>
<td>1.11</td>
<td>-2.20</td>
</tr>
<tr>
<td>C3</td>
<td>230</td>
<td>1.11</td>
<td>1.39</td>
<td>-2.11</td>
</tr>
<tr>
<td>C4</td>
<td>244</td>
<td>1.25</td>
<td>1.66</td>
<td>-2.14</td>
</tr>
<tr>
<td>C5</td>
<td>258</td>
<td>1.25</td>
<td>1.80</td>
<td>-2.50</td>
</tr>
<tr>
<td>C6</td>
<td>286</td>
<td>1.05</td>
<td>1.60</td>
<td>-2.96</td>
</tr>
<tr>
<td>C7</td>
<td>224</td>
<td>1.00</td>
<td>1.58</td>
<td>-3.59</td>
</tr>
</tbody>
</table>

* In units of mM.

By using this data, a Kastings type flux model can be developed for the first seven PABA esters.
\[
\log J_{PDMS} = -1.196 + 2.27 \log S_{SO} - 0.0195 \text{ MW} \quad (r^2 = 0.958) \quad (6.18)
\]
\[
\log J_{PDMS} = 0.648 + 1.73 \log S_{HEX} - 0.0295 \text{ MW} \quad (r^2 = 0.953) \quad (6.19)
\]

Regardless of whether silicone oil or hexane is used in the model, the model can accurately calculate the flux of the PABA esters. Such close agreement to a single membrane model and the strong dependence of flux through PDMS upon organic solubility indicates that a static diffusion layer is not needed to describe the behavior of this series.

All of these previously described mechanisms limit diffusion independently of the membrane. Therefore, if any of these mechanisms are responsible for the poor fit of the largest 6-MP prodrugs to the polymer model, then a similar deviation in their flux through hairless mouse skin should also be observed. However, the C4 and C5 bis-6,9-ACOM-6-MP compounds only under perform from the aqueous mouse skin model by 0.23 log units. This is larger than the average absolute error for the database but smaller or equal to five other series members. Hence, it seems likely that an alternative mechanism is required.

The most direct membrane based mechanism involves a disproportional increase in the energy requirement for the opening of accessible volumes of space within the polymer for larger molecules. The structure of PDMS is generally accepted as an amorphous polymer that has no regular lattice or ordered spacing between the polymer chains. Unlike cavity formation in a small molecular liquid, which generally requires the translational movement of several molecules, diffusion in a polymer occurs when rotation of the polymer chains opens holes into which the drug molecules can move. The rate at which these rotations occur governs the rate of diffusion. There is certainly a limit to
the cavity size that can result from the rotation of a single polymer chain. It may be that molecules above ~360 amu exceed this limit and require the movement of more then one chain or require flexing as well as rotation to achieve a sufficiently large volume of open space.

**Prediction of Flux through Hairless Mouse Skin from Flux through PDMS**

The traditional view of skin permeation presumes that the principle barrier of the skin is purely lipophilic. This view has led some researchers to expect a direct correlation between flux through hydrophobic polymers and flux through skin. The success of these attempts was usually disappointing but a similar attempt with our database gave results that were unexpectedly good. Using the equation 3.34 for relating flux through similar membranes, the following model was obtained:

\[
\log J_{MAQ} = 0.396 + 0.526 \log J_{PAQ} - 0.00459 \text{MW} \quad (r^2 = 0.825) \quad (6.20)
\]

Even though this is an overly simple model, the average absolute \( \Delta \log J_{MAQ} \) was 0.17. This is a slight improvement over the results of the Roberts-Sloan model, but the improvement is too small to be considered significant. However, even though the database error is comparable, the average error in calculation of the APAP series is 0.25; more then twice that of the Roberts-Sloan model.

In Chapter 3, it was asserted that a model which uses the flux through a synthetic membrane to estimate the diffusivity term (eq. 3.38) would be the most useful means to incorporate flux data from a synthetic membrane into a predictive model for biological membranes. The equation of this form which best fits the aqueous database is shown in equation 6.21.
\[ \log J_{\text{M AQ}} = -1.841 + 0.305 \log S_{\text{IP M}} + 0.367 \log J_{\text{PAQ}} + (1 - 0.305 - 0.367) \log S_{\text{AQ}} \]

\[ (r^2 = 0.830) \] (6.21)

The correlation improves slightly over that of the equation 6.20, but again the improvement is minimal. With an average prediction error of 0.16, model 6.21 performs only as well as model 6.20, though its ability to predict the behavior of the APAP compounds is much better. Unfortunately this improvement in prediction for compounds 1 through 8 is balanced by poor prediction for both 6-MP based series, which have average prediction errors of 0.21.

It is only when unconstrained coefficients are allowed into the model that the predictability improves above mediocre:

\[ \log J_{\text{M AQ}} = -1.471 + 0.115 \log S_{\text{IP M}} + 0.323 \log J_{\text{PAQ}} + 0.289 \log S_{\text{AQ}} \] \[ (r^2 = 0.894) \] (6.22)

With equation 6.22, the average absolute error of prediction drops to 0.13; which translates to an absolute prediction error of about 35%. Given the inherent 30% variability in mouse skin, this level of uncertainty is as low as is realistically obtainable. All series in the database are well predicted with the bis-6,9-ACOM-6 MP series having the largest error (0.16) and 3-ACOM-5 FU series having the smallest error (0.10). The model even predicts the fluxes of C4 and C5 bis-6,9-ACOM-6 MP with errors of 0.12 and 0.11, respectively.

As stated in chapter 3, the effects of solubility and molecular weight on flux, while related, will in general vary by different proportions. Therefore, flux through a surrogate membrane can typically be used to substitute for either solubility or molecular weight effects, but not both. However, with the current database it is possible for IPM solubility to be removed from the model without substantially affecting its performance:
\[
\log J_{MAQ} = -1.156 + 0.409 \log J_{PAQ} + 0.245 \log S_{AQ} \quad (r^2 = 0.887) \quad (6.23)
\]

Equation 6.23 is the most efficient model for predicting flux from water through hairless mouse skin from flux through a polymer membrane. The average absolute prediction error for the database is 0.13 and the individual series average errors range from 0.11 to 0.16.

![Graph](image)

Figure 6-6. Calculated versus experimental log maximum flux values through hairless mouse skin from water by equation 6.23.

Yet again, the common result of equations 6.20 through 6.23 is that good biphasic solubility is the best indicator of high transdermal flux. Even with equation 6.23, which is well removed from the Roberts-Sloan equation, flux is nearly equally improved by water and lipid solubility. While a molecular weight term is not essential, it is clear that aqueous solubility cannot be excluded.
CHAPTER 7
CONCLUSIONS AND FUTURE WORK

There were three major objectives to this work. The first objective was to synthesize and characterize a series of 4-alkyloxycarbonyl prodrugs of acetaminophen and examine the effectiveness of these compounds at improving the delivery of acetaminophen through hairless mouse skin from water and IPM. The second objective was to combine this new data with similar data collected from previously synthesized prodrug series of 5-FU, 6-MP and theophylline, and use the resulting expanded databases to modify predictive models for topical delivery from both IPM and water. The final objective was to create a new database from flux measurements through silicone membrane of the hydrolytically stable prodrugs and use this data to develop a model to predict flux through mouse skin from flux through silicon membrane. Each of these objectives was achieved.

Only one of the alkyloxycarbonyl prodrugs of acetaminophen produced higher transdermal flux than acetaminophen itself. However, this is still encouraging. As with other hydrogen bonding functional groups, masking the phenolic moiety on a drug with the proper promoiety can result in a compound whose solubility characteristics produce greater topical delivery. The fact that this was accomplished with acetaminophen, a compound whose relatively high water and lipid solubility are already conducive to topical delivery and therefore more difficult to improve, suggests that applying this approach to a polyphenolic compound will result in a greater relative improvement in flux.
What remains to be demonstrated is whether masking only selected phenolic moieties on the polyphenol will be more beneficial than masking all phenolic groups. It has been well established that compounds, which have good water and lipid solubility permeate the skin most effectively. In general, masking hydrogen-bonding groups on the parent drug reduces the crystal lattice energy and results in higher lipid solubility for the prodrug. However, for water solubility to simultaneously increase, this drop in lattice energy must be higher than the increase in aqueous solvation energy resulting from any loss of favorable hydrogen bonding potential and the prodrug’s larger molecular volume.

Using the general aqueous solubility equation presented in Chapter 3 (eq. 3.13), one can estimate the minimum loss in crystal lattice energy that is required to compensate for the increase in molecular size in order to maintain aqueous solubility. For two consecutive members of a hypothetical homologous series (X and Y), separate aqueous solubility equations can be written:

\[
\log S_{AQX} = A - B_0 \left( T_{MX} - T \right) - C MW_X \quad (7.1)
\]

\[
\log S_{AQY} = A - B_0 \left( T_{MY} - T \right) - C MW_Y \quad (7.2)
\]

If X and Y have equal aqueous solubilities, then the following rearrangement of equations 7.1 from 7.2 can be performed.

\[
A - B_0 \left( T_{MX} - T \right) - C MW_X = A - B_0 \left( T_{MY} - T \right) - C MW_Y
\]

\[
-B_0 \left( T_{MX} - T \right) - C MW_X = -B_0 \left( T_{MY} - T \right) - C MW_Y
\]

\[
B_0 \left( T_{MY} - T \right) - B_0 \left( T_{MX} - T \right) = C MW_X - C MW_Y
\]

\[
B_0 (T_{MY} - T_{MX}) = C (MW_X - MW_Y) = -C(MW_Y - MW_X)
\]

\[
B_0 \Delta T_M = -C \Delta MW
\]

\[
\Delta T_M = -\left( C/ B_0 \right) \Delta MW
\]
If the values for C and B₀, 0.0429 and 0.0176 respectively, are inserted then the result is

\[ \Delta T_M = -2.44 \Delta MW \]  \hspace{1cm} (7.3)

Since X and Y are sequential homologs, DMW equals 14.

\[ \Delta T_M = -2.44 \times (14) = -34^\circ C \]

In other words, for any pair of sequential alkyl homologs, the larger member will possess greater water solubility then the smaller member only if the melting point of the larger member is lower then that of the smaller member by at least 34\(^\circ\)C.

This prediction is supported by an investigation of the database compounds. In the database, there are numerous examples of homologs having increased water solubility after the addition of a methylene group to the promoiety. In each case, the larger homolog has a melting point at least 32\(^\circ\) lower then the smaller homolog.

For a tri-substituted prodrug, such as naringenin triacetate, the addition of only one methylene group to each promoiety would result in an overall increase of 42 amu in molecular weight. By equation 7.3, such an increase in molecular weight would require a drop in melting point of over 100\(^\circ\)C for water solubility to increase. It is therefore not surprising that even though the triacetate of naringenin has a melting point of ~78\(^\circ\), which is ~170\(^\circ\)C lower then the melting point of naringenin, it still has poor water solubility. Producing polyphenolic prodrugs with the highest topical delivery will most likely be accomplished by masking as few phenolic groups as possible to reduce crystal lattice energy.

It was hypothesized that replacing an alkyl functional group with an ether in the promoiety would create more water-soluble prodrugs. Even though only a small amount
of data was gathered from these types of promoieties, the experimental results do support this hypothesis. The methoxyethyl carbonate of APAP outperformed all other APAP prodrugs when delivered from water and all but the methyl carbonate when delivered from IPM. After accounting for the influence of molecular weight and melting point using eq. 3.14, the 4-MOAOC-APAP compounds demonstrated a greater intrinsic capacity for water solubility and a roughly equivalent capacity for lipid solubility than the AOC-APAP compounds, which should result in higher flux. While it is still not certain whether these promoieties will consistently produce better performing prodrugs, they have performed well enough to warrant further study.

The addition of APAP and its prodrugs to the IPM database reinforced the conclusions of the previous Roberts-Sloan model. After final regression of the expanded IPM database, flux from IPM remained equally dependent upon IPM and water solubilities. The flux of the APAP compounds were not predicted as well as other series in the database, but well enough to demonstrate that they do conform to the model.

A similar result was obtained from the expanded aqueous database. The relative importance of water and IPM solubility on flux from water remained essentially unchanged after the addition of the APAP compounds. As with flux from IPM, flux from water was positively influenced by both aqueous and IPM solubility. However a slightly greater positive influence of lipid solubility and a slightly reduced positive influence of water solubility were observed for aqueous delivery. In contrast to IPM model, the aqueous model predicted the flux of the APAP compounds more accurately than any other series in the database.
For a compound to have optimal transdermal flux, it must also have good biphasic solubility. The data of the expanded IPM and aqueous databases have given this principle increased experimental support. In addition, it is now certain that the importance of biphasic solubility is independent of the vehicle used and, therefore, is an attribute of the skin itself. As such, the coefficients can be interpreted as representing the chemical nature of the skin barrier. It is noteworthy that in spite of the increased damage caused by IPM to the skin and the resultant > 10-fold increase in flux when IPM is used a vehicle compared to water, the corresponding coefficients of the two models remain close to one another. This is an indication that IPM alters the organization of the skin barrier more so than its composition. It would be interesting to see how other vehicles alter these coefficients.

The Roberts-Sloan equation has been successful in predicting the behavior of homologous series. The next challenge will be to apply this treatment to a database of heterogeneous compounds. This work is currently underway.

By itself, PDMS membrane proved to be a poor experimental surrogate for mouse skin, which suggests it would be a poor surrogate for mammalian skin in general. Although polymer flux can yield a fair approximation of the mechanical aspects of diffusion and estimate the effect of molecular weight on skin flux, solubility in the purely lipophilic polymer does not conform to the solubility in the skin. The simple addition of an aqueous solubility term, however, overcame this deficiency and greatly improved the model’s predictability. For this limited database, the predictability of the polymer model was better than the aqueous Roberts-Sloan model. However, it will require a larger
database to determine whether this improvement actually indicates a better performing model.

Both the polymer model and the Roberts-Sloan model provide evidence that, despite the skin’s complexity, homogeneous membrane models can be effective at predicting flux through skin. The next stage in model development will be to investigate more complicated models that mimic the true construction of skin more closely. Roberts and Sloan have already published a series/parallel model, which describes skin permeation as occurring through multiple paths that mirror the anatomical structures found in the skin. In this model, diffusion can occur through lipid paths, aqueous paths or across alternating lipid and aqueous layers. This model was developed from 41 of the IPM database compounds and it successfully predicted flux through mouse skin. Future modeling of the current IPM database and, when expanded, the aqueous database will involve applying these more sophisticated models.
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BIOGRAPHICAL SKETCH

Scott C. Wasdo was born in Waterbury, Connecticut, on July 4, 1965. At the age of five, he and his family moved to Rochester, New York, where he lived until his graduation from Penfield High School in June of 1983. He attended the University of Hawaii at Manoa until January of 1985, when he transferred to the University of Florida. After graduating with a B.S. degree in chemistry in 1987, he worked as an analytical chemist until 1998, when he returned to graduate school in the Department of Medicinal Chemistry.