DRUG TRANSPORT PATHWAYS IN RABBIT ORAL MUCOSA

by

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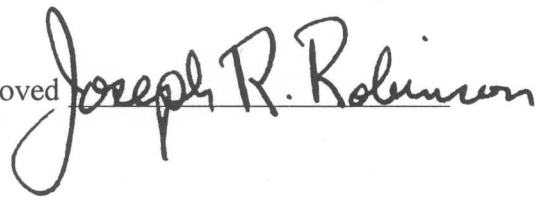
ABSTRACT

The pathways of drug transport across the oral mucosa are fundamental to an understanding of the drug absorption phenomena. Due to the complexity of this issue, drug absorption via the oral mucosa has traditionally been simplified to be a passive diffusion process across a hydrophobic membrane, i.e., a one-pathway model. However, the cellular structure of the oral mucosa shows that it is not a uniform hydrophobic barrier but rather consists of relatively hydrophobic cell membranes and hydrophilic intercellular spaces. The entire oral epithelium is a mosaic of hydrophilic and lipophilic regions, where hydrophilic intercellular spaces are connected to form a network and lipophilic cell membranes are isolated like islands in a sea of hydrophilic regions. Based on this information, a two-pathway model, i.e., a hydrophilic paracellular pathway and a lipophilic transcellular pathway, is developed to describe the drug transport process through oral mucosa. From both theoretical and experimental points of view, this dissertation addresses the relationship between oral mucosal permeability, the diffusant lipophilicity (partition coefficient), and the bathing solution pH. An understanding of these relationships can help explain many

complicated and sometimes controversial issues, e.g., the effect of pH on the permeability coefficient, dependency of permeability coefficient on diffusant partition coefficient, drug accumulation in the oral mucosa, and the compatibility between *in vivo* and *in vitro* studies.

Approved 

To my parents, Chun You Zhang and Shu Fang Liu,

my brothers, Jie Zhang and Bo Liu

and my fiancé Eva Huang

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CHAPTER 1: INTRODUCTION

I. History of Oral Mucosal Drug Delivery

The oral mucosa has been used as a drug delivery route since Sobrero discovered the systemic effect of nitroglycerin via the oral mucosa in 1847 (Sobrero, 1847). This drug delivery route was firmly established in 1879 when William Murrell introduced nitroglycerin drops under the tongue for the treatment of angina pectoris (Murrell, 1879). Since then, many studies about absorption of drugs through the oral mucosa have been published. Substantial review articles of this object have been written by many scientists in this field (Gibaldi, 1965; Squier, 1975a; Wertz, 1991; Harris, 1992; De Vries, 1991a).

Compared to other drug delivery routes, the oral mucosa has unique characteristics and advantages. The onset time of this route is shorter than oral (gastrointestinal tract) and transdermal (skin) routes. Moreover, it is more accessible and has better patient compliance than the other alternative drug delivery routes, such as the nasal, rectal and vaginal areas. It also avoids the harsh acidic and enzymatic environment in the stomach and first pass metabolism in the liver. More importantly, the drug dose can be easily administered and somewhat controlled by patients.

Certainly, there are limitations to oral mucosal drug delivery. The total surface area of an adult's oral mucosa is around 200 cm^2 (Collins, 1987). It is relatively small

compared to more than 350,000 cm^2 of gastrointestinal tract (Squier, 1993) and 20,000 cm^2 of skin (Jacob, 1970). The limited surface area will surely limit the total amount of drug that can be delivered per unit time. In addition, the oral mucosa is relatively impermeable to many drugs, especially large water soluble drugs. This feature will also limit the number of drugs that can be delivered via the oral mucosa. However, unlike the surface area, which is unchangeable, the permeability of the oral mucosa can be temporarily altered to a certain extent to become more permeable. This has been the focus of many studies attempting to find suitable permeation enhancers for oral mucosal drug delivery

Early oral mucosal drug delivery studies were focused on the phenomenon that drugs can be absorbed via the oral mucosa (Walton, 1935; Walton, 1944; Beckett, 1971; Moffat, 1971). This interest has diminished with the finding that only a few drugs are clinically needed and practically feasible to be delivered via the oral mucosal route. Recent attention is on understanding the biological and physicochemical nature of the oral mucosa drug absorption process and the mechanism of drug penetration across the oral mucosa at the cellular and molecular level (Wertz, 1991; De Vries, 1991a). The long term goal is to find a way to temporarily alter the permeability of the oral mucosa for drug delivery purposes to expand the number of drugs that can be delivered via this route. This will open a new era of using the oral mucosa as a route to deliver many water soluble drugs, especially peptide drugs, that would not penetrate the oral mucosa without the help of penetration enhancers.

Meanwhile, a number of attempts have been made to develop oral mucosal drug delivery systems. Recently the FDA approved an oral transmucosal fentanyl citrate lollipop (OTFC®). Other companies are exploring the use of buccal patches.

Nevertheless, this is still an early stage of using the oral mucosa as a drug delivery route. More mechanistic studies about the permeability characteristics of the oral mucosa and its correlation with physicochemical properties of diffusants and delivery vehicle are necessary.

II. Anatomy, Structure and Function of the Oral Mucosal Tissues

The oral cavity can be divided into two regions, the outer oral vestibule and the oral cavity proper. The outer oral vestibule consists of the mucosa between the lips and dental arches, including the lips, buccal and gingiva tissues. The oral cavity proper consists of the mucosa inside the dental arches, including the hard palate, soft palate, tongue, gingiva and floor of the mouth (Fig. I-1).

Tissues in various regions have different structures and functions. Based on cellular structure, the oral mucosa can be classified into three categories, the masticatory, lining and specialized mucosa (Collins, 1987). The masticatory mucosa occupies about 24% of the total surface area of the oral mucosa, and includes the gingiva and

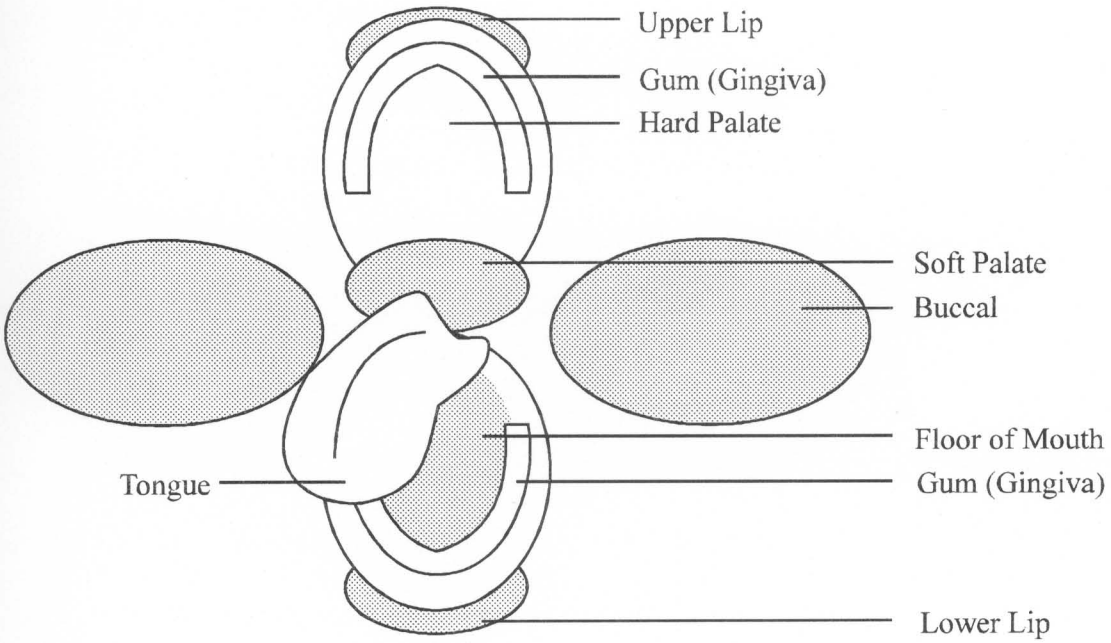


Fig. I-1. Schematic diagram of an "open" oral cavity, showing keratinized (white) and nonkeratinized (gray) regions. (Modified from De Vries, 1991a)

hard palate. In these regions, the mucosa is subject to the mechanical force of mastication. In order to protect the tissue against the shearing and tearing forces of mastication, a keratin layer is present on the outmost layer of the tissue. The lining mucosa, the mucosal tissues in the cheeks (buccal), floor of mouth (sublingual) and ventral surface of the tongue, accounts for about 60% of the oral mucosa. These tissues are stretched or compressed during speech and mastication. In these regions, the flexibility of the tissue is required for its function. Therefore, the tissue is not covered by a keratin layer. The specialized mucosa is the dorsal surface of the tongue, which has special structural configurations for taste purposes. The mucosal tissue of interest in drug delivery is the lining mucosa, more specifically, the buccal and sublingual tissue because these tissues are not keratinized, and, thus, are more permeable than tissues in the other regions.

The general cellular structure of the oral mucosa consists of a superficial layer of stratified squamous epithelium, a basal lamina (basement membrane), and an underlying connective tissue (Fig. I-2) (Squier, 1976a). Connective tissue may contain lamina propria alone or lamina propria and submucosa, depending on the location in the oral cavity. Submucosa is present only in the lining mucosa, where flexibility is needed. Without the submucosa, the tissue is less flexible and fixed in position for better support from the underlying bones. Regional differences of the oral mucosa are shown in Table I-1.

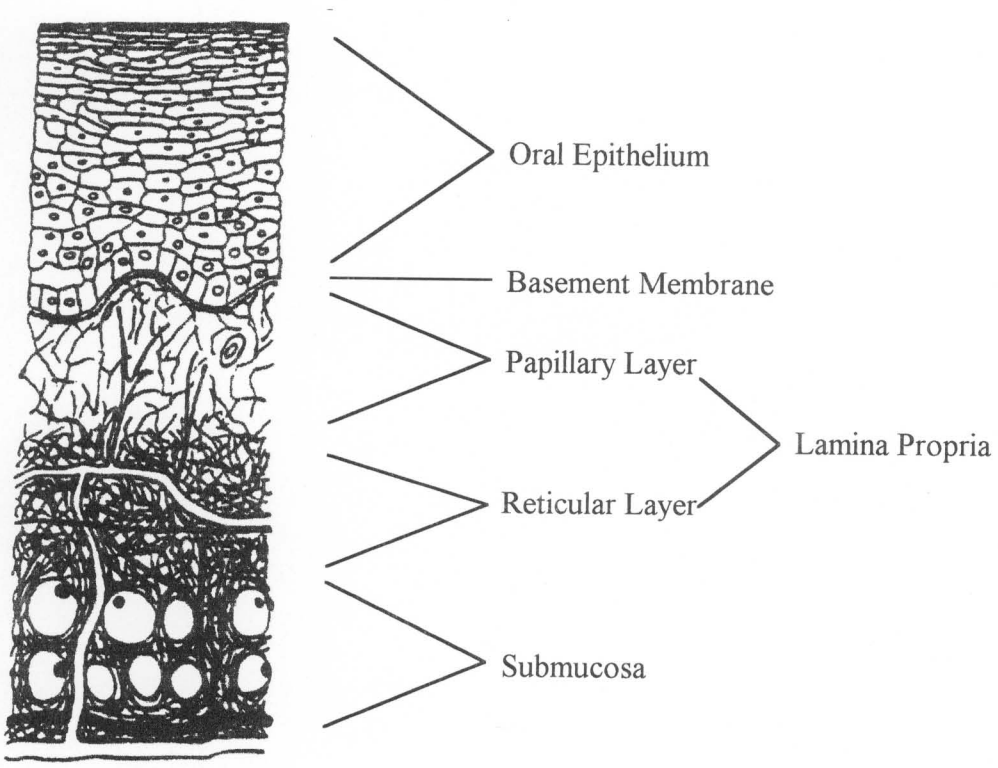


Fig. I-2. General structure of the oral mucosa. All the main tissue components can be identified under light microscope (Taken from Squier, 1976a).

Table I-1. Mucosal tissue characteristics for various regions of the oral cavity.

Region	Turnover Time (Day)[1]	Epithelial Thickness[2] (μm)	Blood Flow [3] (ml/min/100cm²)*	Keratinization[4]
Hard Palate	24	250	0.89	yes
Gingiva	68	200	1.47	yes
Buccal	14	500-600	2.40	no
Sublingual	20	100-200	0.97	no

*Rhesus monkeys were used to measure the blood flow rate.

[1] Squier, 1975b

[2] Chen, 1984

[3] Squier, 1985a

[4] Squier, 1976a

The primary function of the oral epithelial is to protect the underlying tissue against fluid loss and potential harmful agents in the oral environment (Squier, 1975b). The closely compacted epithelial cells on the top quarter or one third of the epithelial tissue forms the main penetration barrier (Squier, 1973; Squier, 1976b; Dowty, 1992). The lipophilic cell membrane forms a diffusion barrier for water soluble compounds, and the intercellular space is hydrophilic to form a barrier for lipophilic compounds. The presence of these two barriers eliminates most of the potential toxic agents from ingested food and beverages. Unfortunately, this is also a barrier for drug delivery.

III. Experimental Approaches for Studying Oral Mucosal Drug Penetration

There are many ways to study the properties of oral mucosal drug penetration. Each approach has its advantages and limitations. The choice of the experimental method depends on the specific purpose of the study. However, it is important to remember the assumptions and limitations of the system before designing and proceeding with an experiment.

A. *In Vivo* System

1. The Buccal Absorption Test

This method was first introduced in 1967 by Beckett and Triggs (Beckett, 1967), and has been used to determine the potential of drug absorption via the oral mucosa

(Beckett, 1968; Beckett, 1969a; Beckett, 1969b; Beckett, 1970; Evered, 1980; Evered, 1981; Evered, 1983). In brief, a drug solution with known volume and concentration was introduced into the oral cavity of a test subject, and was swirled in the mouth for a measured period of time, usually 5 to 10 minutes. The solution was then expelled and the concentration of remaining drug measured. The difference of drug amount before and after the oral test was a measure of drug absorption. The advantages of this method are that it is easy to perform and relatively close to real conditions for oral mucosal drug delivery. However, there are several major experimental limitations that make this test unacceptable for studying the mechanism of oral mucosal drug penetration. For example, the area of the tested oral mucosa is unknown and the different regions of the oral cavity are not distinguished. Drug absorption is measured by the disappearance of drug from the donor solution rather than drug on the receiver side, i.e., plasma concentration, in this case. There may be a difference between drug disappearance and appearance because drug may have been metabolized (Dowty, 1992) or simply deposited somewhere in the epithelial tissue (Davis, 1979; Henry, 1980; Zhang, 1991b). In addition, the time period in the study is usually too short for diffusion to reach steady state (lag time). Due to these limitations, this method is not a first choice for studying mechanisms of oral mucosal drug penetration.

2. Perfusion Study

An *In vivo* perfusion study is really a modified version of the Buccal Absorption Test (Barsuhn, 1988; Yamahara, 1990; Veillard, 1987; Zhang, 1989; Zhang, 1992). The major improvements are that the area and the region of the study are known, and the duration of the study is typically much longer so that steady state can be reached. However, as far as using disappearances of the drug from the perfusing solution to measure drug absorption, there is the question of metabolism and deposition. There are two ways to overcome this problem. If the biological effect of the drug can be measured, e.g., drop of blood glucose concentration by administration of insulin, the effect of a perfusion experiment can be compared with the effect of an I.V. infusion experiment to obtain the flux and permeability coefficient of the drug across the oral mucosa into the circulation system (Zhang, 1993). The limitation is that the drug must have a measurable biological effect which is proportional to the drug concentration. The other way is to measure the plasma drug concentration. However, for many drugs that have low oral mucosal permeability and high elimination rate, the plasma concentration is often too low to be measured.

B. *In Vitro* System

1. Diffusion Study

In vitro diffusion systems have been used extensively for studying the mechanisms of

oral mucosal drug delivery (Dowty, 1992; Longer, 1988; Siegel, 1981a; Siegel, 1981b; Tolo, 1975; Le Brun, 1989). This method is also called an *ex vivo* diffusion study.

The advantages of the *in vitro* diffusion study are that the experiment is performed in a controlled environment and the conditions of the study are well defined. Receiver side drug concentration is usually measured as a function of time and the drug permeability coefficient computed. The drug permeability coefficient is a measure of drug permeation across the tissue and is independent of donor concentration. It is recommended that permeability coefficients be used as a measure of drug permeation because it is easy to compare the permeability coefficients for different drugs and different tissues.

The drawback of this system is that the tissue is isolated and taken out of its natural environment. Although Dowty & Robinson have shown that rabbit buccal tissue maintains its ATP level and barrier function for up to six hours after removal from the animal (Dowty, 1992), it is difficult to verify that the permeation property of the tissue is identical to that of a live animal. Nevertheless, this is an excellent method to study the mechanism of drug permeation across oral tissue.

Although a variety of diffusion cells are commercially available, such as the Franz® cells used by Eggerth (Eggerth, 1987), small volume diffusion cells used by Grass and Sweetana (Grass, 1988) and Dowty *et al* (Dowty, 1992) (Precision Instrument Design, Los Altos, CA), most people use homemade modified Ussing chambers (Ussing,

1949). We prefer to use the Grass cells because they minimize the stagnant diffusion layer, i.e., gas is bubbled close to the membrane surface, and electrical leads are present to continuously monitor electrical resistance, which gives a direct measure of tissue integrity. The key points are that agitation must be sufficient to minimize the stagnant layer and the seals between tissue and diffusion cells are patent to prevent leakage. Diffusion cells are usually made of glass, Teflon® or plastics. If plastic or Teflon® diffusion cells are used, surface adsorption of lipophilic drug onto the diffusion cell should be tested. Of course, the same concern applies to the Teflon® coated stirring bar. Pre-moisturized oxygen or oxygen/carbon dioxide mixture (95%/5%) gases are often used to supply aeration and agitation for the buffer solution. Experimentalists should be aware that pH shifts do occur for small volume diffusion cells during lengthy experiments if carbogen gas is employed. Because there is 5% carbon dioxide in carbogen gas, the pH may drop caused by dissolving carbon dioxide to form carbonic acid.

2. Diffusion Study with Cell Cultured Membranes

Using an epithelial or endothelial cell cultured membrane to study permeability properties is becoming popular(Squier, 1978; Tavakoli-Saberi, 1989a; White, 1969). A cultured buccal epithelial model has been successfully developed and tested for enzyme activity and permeation properties (Tavakoli-Saberi, 1989a). In both categories, the cultured epithelial membrane has similar properties to freshly excised tissues.

The experimental set up is similar to that of a diffusion study except that cell cultured membranes are used instead of tissues from animal. The gain is obvious because cultured epithelial membranes are much easier to handle and individual variation is typically smaller than that of tissues. Diffusion study with cultured membrane can rapidly assess the potential permeability and metabolism of a drug, and evaluate the strategies for achieving optimum drug delivery across the membrane. This method can also provides an alternative to the time consuming and more expensive animal studies. As with other approaches, cultured epithelial membrane study may have certain limitations. The transport and metabolism properties of cultured epithelial cells can vary depending on how the cells were cultured. Many factors may affect the permeability properties of the cultured cells, such as whether the cells are primary cultures, passaged lines, or transformed lines, how many times the cells have been passaged, the ability of the cell line to undergo differentiation, the seeding density, and whether a certain nutrient is present or absent (Audus, 1990). The point is that due to the complexity of the cell culture itself, the use of such a system in studying drug transport must be done in a carefully controlled and defined manner.

IV. Animal Models for In vitro Permeability Measurement

Since human oral mucosa is not widely available, animal oral mucosa is usually used for the *in vitro* experiment. The main concern of choosing an animal model is the

resemblance of the animal mucosa to the oral mucosa of human beings in both ultrastructure and enzyme activity, which represents the physical and metabolic barriers of the oral mucosa. It's important to know there is no animal model that can fully represent humans except humans. The purpose of using an animal model is not to find an imitation that has exactly the same oral mucosa as human, but to find an animal mucosa that has equivalent physical and biochemical properties to human. The differences between animal and human models can be easily assessed by a comparison study. However, an *in vitro* study allows control of numerous variables. Thus, the actual values of the permeability coefficients are not as important as other transport properties, such as the contribution of hydrophilicity of diffusants, the pH of the delivery vehicle, the pH gradient across the oral mucosa, the osmolarity of the buffer solution.

Many lab animals have been tested for *in vitro* oral mucosa permeability studies. The most commonly used animals are dogs (Squier, 1984; Galey, 1976; Bergman, 1969; Tobey, 1988; Siegel, 1985a), rabbits (Dowty, 1992; Siegel, 1986; Siegel, 1981b; Tolo, 1975), pigs (Siegel, 1986; Squier, 1986a; Squier, 1986b; Squier, 1985b; Squier, 1984; Le Brun, 1989; De Vries, 1991b), Rhesus monkeys (Squier, 1984; Mehta, 1991), guinea pigs (Alfano, 1975; Alfano, 1977; Tolo, 1971), rats (Siegel, 1985b; Hussain, 1987; Jepsen, 1974) and hamsters (Eggerth, 1987; Garren, 1989a; Garren, 1989b). Characteristics of oral mucosa from various species and regions are listed in Table II-2.

Table II-2. Characteristics of oral mucosa from various species and regions.

Species	Tissue	Thickness (μm)	Keratinization
Human	Buccal	500-600	No
	Floor of Mouth	100-200	No
Dog	Buccal	767	No
	Lingual Frenulum	149	No
	Floor of Mouth	168	No
Rabbit	Buccal	600	No
	Lingual Frenulum	—	No
Pig	Buccal	772	No
	Floor of Mouth	192	No
Monkey	Buccal	—	No
Rat	Buccal	—	Yes
Hamster	Buccal	—	Yes

The oral mucosa of dogs, pigs, rabbits and Rhesus monkeys are believed to be similar to oral mucosa of humans primarily because the epithelium is non-keratinized. *In vivo* buccal absorption studies of flurbiprofen in the human and dog reveal that the permeability of human and dog buccal mucosa are equivalent (Barsuhn, 1988). In vitro permeability coefficients of tritiated water in human and pig oral mucosa, buccal and floor of mouth, are also similar (Lesch, 1989). Many other comparative studies between these animal models have been reported (Siegel, 1981b; Eggerth, 1987). All these studies demonstrate that dogs, rabbits, pigs and Rhesus monkeys are reasonably good animal models for studying oral mucosal drug absorption. The choice of which animal is mostly dependent on cost and availability.

Rats and hamsters, however, have heavily keratinized oral mucosa which differ from the buccal and sublingual mucosa of humans (Ebert, 1987). The permeability coefficients of short chain alcohols and acids are significantly less in hamsters than in dogs, rabbits and humans (Eggerth, 1987). The permeability of dextromethorphan in the hamster is not only much lower than in humans, but also has a different pH dependency pattern (Eggerth, 1987), which implies that the oral mucosa of hamster is much more lipophilic than that of humans and coincides with the fact that hamster oral mucosa is keratinized. Based on this evidence, the use of rats and hamsters as an animal model should been avoided unless the experiment is designed to study the permeability of keratinized oral mucosa (Jepsen, 1974).

Dogs, rabbits and pigs are the most commonly used animal in studying the drug absorption of oral mucosa *in vitro*. Rabbits are relatively cheap and are easier to handle than dogs and pigs. However, the surface area of rabbit buccal and sublingual tissue is very small and the number of experiments that can be performed in one animal is limited. Dogs and pigs have large mucosal areas so that several studies can be done simultaneously using the same animal.

V. Statement of the Problem

Oral mucosal drug delivery is generally considered as a passive drug diffusion process. To characterize, understand and eventually manipulate this process, many attempts have been made to look for a correlation between permeability and physicochemical properties of the delivery vehicle and drugs. The most commonly sought correlations are permeability coefficient vs. partition coefficient (Beckett, 1969a; Beckett, 1969b; Siegel, 1981a) and permeability coefficient vs. pH for ionizable drugs (Beckett, 1967; Beckett, 1970; Al-Sayed-Omar, 1987; Dowty, 1992; Zhang, 1991a). In many cases, the correlation is either poor or there is no correlation at all. This may be because the oral mucosa is treated as if it is a homogeneous hydrophobic membrane in these studies. The mathematical model used is typically a one-pathway model which is derived directly from Fick's first law. However, the cellular structure of the oral mucosa shows that it is inadequate to consider the whole tissue as a uniform

hydrophobic barrier. It is clear that there are two general pathways in the oral mucosa, a hydrophilic pathway and a lipophilic pathway. However, such a concept has never been developed into a useful mathematical model, nor have the parameters of this model been defined. The objective of this study is to establish an elementary two-pathway drug absorption model for the oral mucosa which will allow a correlation between permeability coefficient and partition coefficient. This model will be tested using morphine and fentanyl as probe drugs. In addition, based on an understanding of the drug pathways, it is also important to know the effect of pH gradient across the oral mucosa on ionizable drug permeation because a pH gradient is almost inevitable in an *in vivo* system. How large the effect will be and how to describe this effect mathematically is another objective of this study.

CHAPTER 2: TWO-PATHWAY MODEL FOR DRUG ABSORPTION ACROSS THE RABBIT ORAL MUCOSAL

I. Introduction

The cellular structure of the oral mucosa clearly demonstrates that the oral mucosa is not a uniform hydrophobic membrane. Rather, the oral epithelium consists of multilayers of stratified squamous epithelial cells. The epithelial cell membrane is composed of a lipid bilayer where the hydrophobic fatty acid tail projects inward to form a hydrophobic interior. Therefore, the cell membrane is a hydrophobic (lipophilic) barrier for many water soluble hydrophilic compounds. The intercellular space, on the other hand, is generally hydrophilic, although there are many lipophilic components secreted from membrane coating granules into the intercellular space (Squier, 1977). The hydrophilic intercellular space forms a permeability barrier for lipophilic compounds. The cooperation of these two barriers limits permeability. At the hydrophilic end, the compounds penetrate through the intercellular space (paracellular route). The limited surface area and the tortuous pathways are the main barrier for this route. At the lipophilic end, although the permeability across the cell membrane is high, the low solubility of these compounds become the major limiting factor and the total flux of the compounds across the oral mucosa actually decreases with a further

increase in lipophilicity. Only compounds that have high lipophilicity and water solubility can penetrate the oral mucosa with high flux. This requirement will limit many compounds from penetrating the oral mucosa.

If the maximum flux is plotted against the lipophilicity (partition coefficient), a bell shaped curve is commonly obtained (McKim, 1985). This is because there is a shift in the rate limiting step. In the low partition coefficient region, the limiting step is permeability across the oral mucosa. Therefore, the permeability coefficient increases with an increase in the partition coefficient. In the high partition coefficient region, however, the limiting step is no longer permeability across the oral mucosa, but is the solubility of the compounds instead. This is the reason that the flux drops in the high partition coefficient region and the flux versus partition coefficient plot is a bell shaped curve. Theoretically, if the permeability coefficient is plotted against the partition coefficient, a sigmoid shaped curve should occur (Hilgers, 1990). The permeability will reach a plateau, not because the permeability reaches a maximum, but because our measuring system fails to measure permeability higher than 10^{-3} cm/sec, above which, the permeability is diffusion layer controlled.

The main focus of the study is to develop a two-pathway model to describe the correlation between permeability coefficient and partition coefficient, and to define the relationship between model parameter and the physicochemical properties of the tissue, diffusants and the delivery vehicle. An understanding of this model can help in

the search for penetration enhancers and design better strategies to increase drug transport across the oral mucosa.

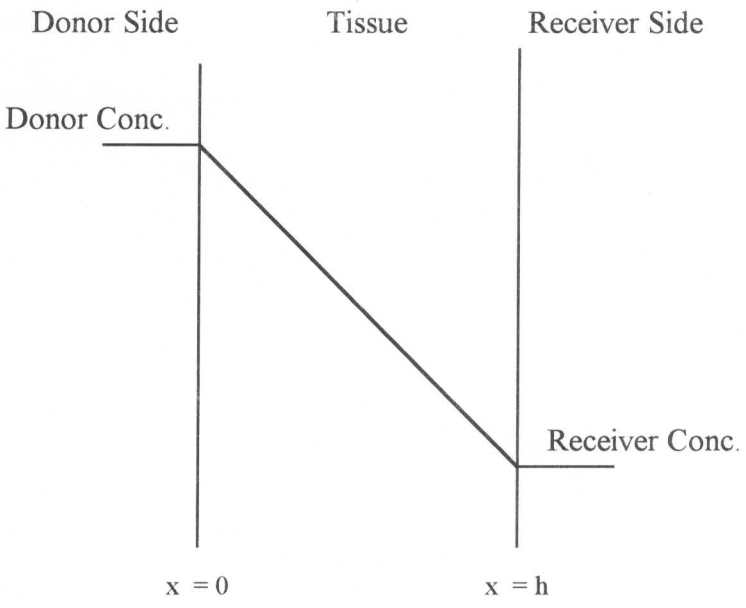
II. Theory

A. Fick's Diffusion Theory

Passive drug diffusion through a membrane can be described by Fick's first law in one dimension (Fig. II-1). The flux across the membrane, which is defined as the amount of drug crossing the membrane per unit area and time, can be expressed as:

$$J = \frac{D}{h} \cdot C_D \qquad \text{(II-1)}$$

where D is the diffusion coefficient, h is thickness of the membrane or pathlength, and C_D is the drug concentration on the donor side. The assumptions of this system are that the membrane is homogeneous, diffusion of the drug reaches steady state, drug concentration on the receiver side is zero (sink condition), and there is no preferential partitioning of the drug into the membrane/donor and membrane/receiver interfaces. The assumed homogeneous membrane will further lead to a linear drug concentration gradient and a constant diffusion coefficient inside the membrane.



$$J = \frac{D}{h} \cdot C_D$$

Fig. II-1. One dimensional Fick's first law under the assumptions that the membrane is homogeneous, diffusion of the drug reaches steady state, drug concentration on receiver side is zero (sink condition), and there is no preferential partitioning of the drug between donor/membrane and membrane/receiver interfaces.

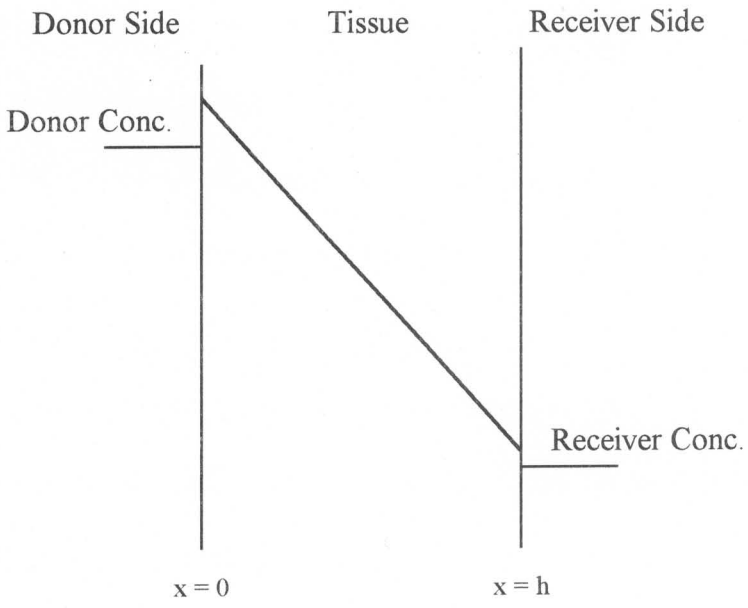
If the membrane is hydrophobic and the donor and receiver solutions are aqueous, preferential partitioning of the drug between the membrane and aqueous solution will occur (Fig. II-2). In this case, a partition coefficient, K_P, is introduced into the system, and the flux of drug diffusion across the membrane can be written as:

$$J = \frac{D \cdot K_P}{h} \cdot C_D \qquad \text{(II-2)}$$

Since D, K_P and h are all constants, they can be combined to become the permeability coefficient:

$$P = \frac{D \cdot K_P}{h} \qquad \text{(II-3)}$$

Use of the permeability coefficient is highly recommended in reporting the results of diffusion studies because it is independent of the diffusant concentration on the donor side, and it is easy to compare for different drugs and different drug concentrations.



$$J = \frac{D \cdot K_P}{h} \cdot C_D$$

Fig. II-2. Fick's first law with preferential partitioning between membrane and buffer solutions.

B. Two-Pathway Model for Oral Mucosal Drug Absorption

The cellular structure of the oral mucosa is not a homogeneous membrane, rather, it is a complicated multi-cell layer tissue. The lipid bilayer of the cell membrane forms a lipophilic barrier for water soluble compounds, and the hydrophilic intercellular space forms a barrier for lipid soluble compounds. The cooperation of these two barriers performs the barrier function for the oral mucosa.

For a drug that passively diffuses through the oral mucosa, there are two general pathways, the lipophilic pathway and the hydrophilic pathway (Fig. II-3). The lipophilic pathway is also called the transcellular pathway where drug diffuses across cell membranes. Hydrophilic pathway is sometimes called the paracellular pathway where the drug diffuses around the cells through the intercellular spaces.

In the transcellular pathway, the diffusant will diffuse across both hydrophilic and lipophilic regions (Fig. II-4). Since the cell membrane has a much higher density and viscosity, it is the rate limiting step of this pathway. Therefore, drug diffusion in the cytoplasm and intercellular space is assumed instant, which means that there is no concentration gradient inside the cytoplasm and intercellular space.

The cell membrane is a hydrophobic membrane. Thus, there is preferential partitioning of the drug between the cell membrane and the buffer solution. The flux of the drug through this pathway is:

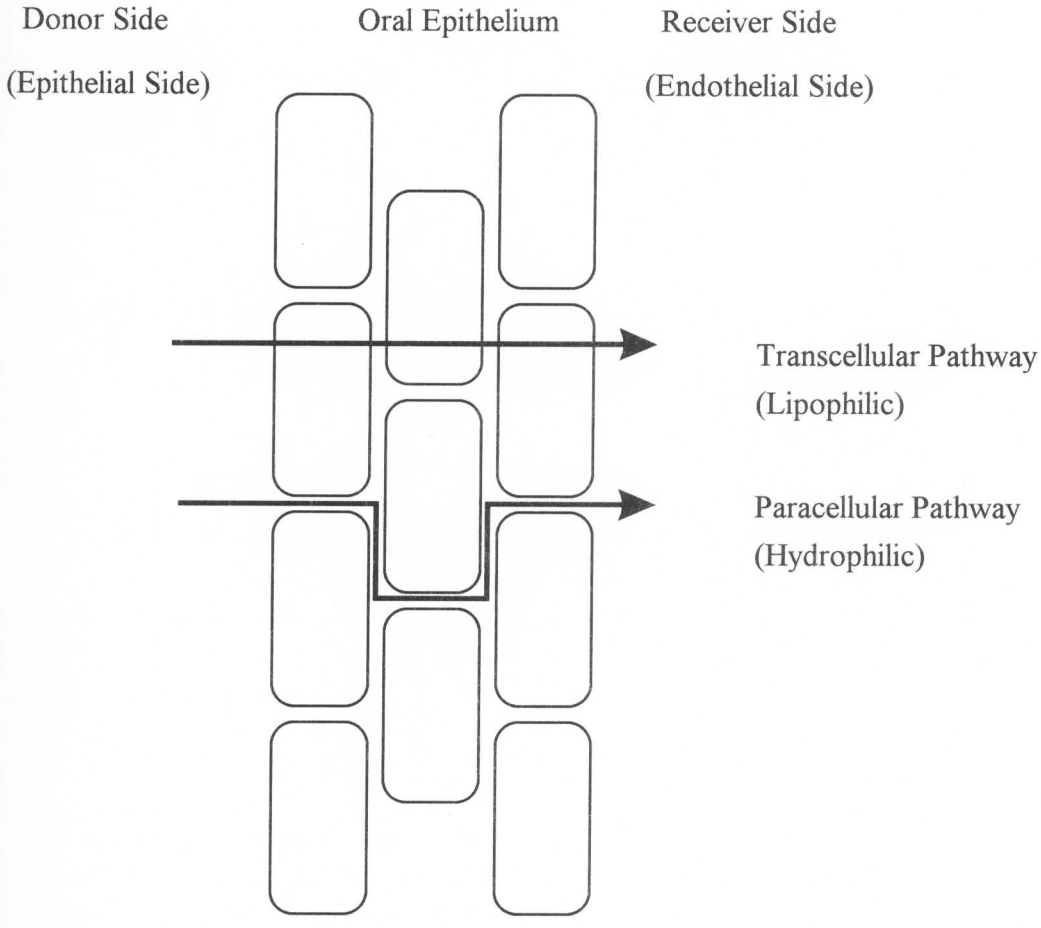


Fig. II-3. Schematic diagram of drug movement across the oral mucosa showing the two-pathway model, the lipophilic transcellular pathway and the hydrophilic paracellular pathway.

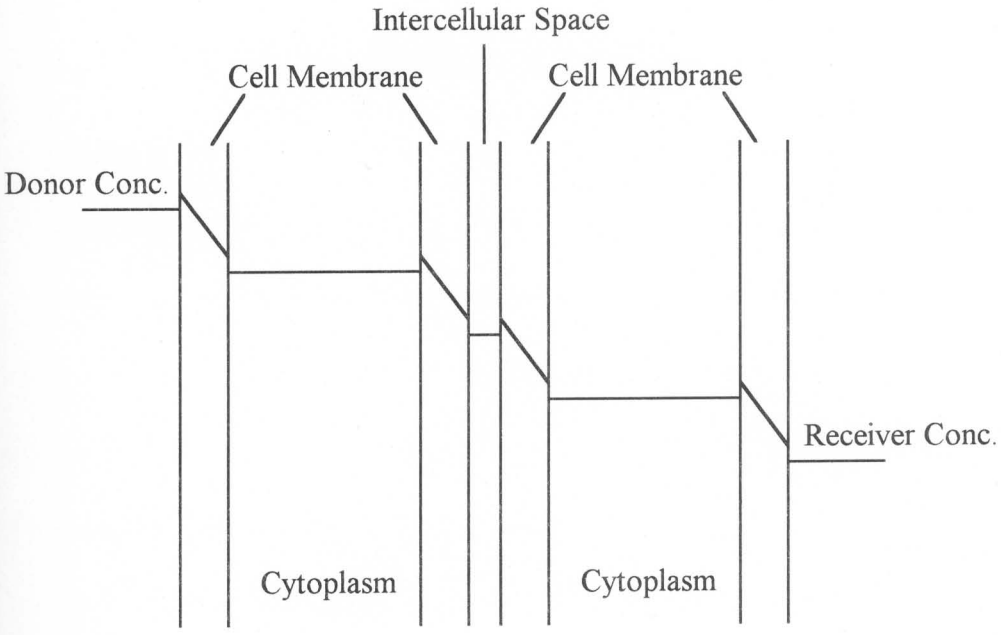


Fig. II-4. Schematic diagram of the transcellular pathway. The drug has to diffuse through both the hydrophilic (cytoplasm and intercellular space) and lipophilic (cell membrane) regions. Notice that there is no concentration gradient in the hydrophilic region because the density and viscosity in the lipophilic region is much higher than that in the hydrophilic region, therefore, drug diffusion in the hydrophilic region is assumed instantaneous.

$$J_L = \frac{D_L \cdot K_{M/B}}{h_L} \cdot C_D \qquad \text{(II-4)}$$

where D_L is the diffusion coefficient in the lipophilic pathway, $K_{M/B}$ is the partition coefficient between the cell membrane and the buffer solution, and h_L is the path length of the lipophilic pathway, which is the sum of all the cell membranes the drug passes through. The partition coefficient between the cell membrane and buffer solution cannot easily be measured experimentally. However, based on the linear free energy relationship, the partition coefficient between octanol and buffer solution, K_P, can be used in the system by introducing a linear free energy coefficient (f). Then the lipophilic pathway flux can be written as:

$$J_L = \frac{D_L \cdot K_P^{\,f}}{h_L} \cdot C_D \qquad \text{(II-5)}$$

The intercellular space, on the other hand, is hydrophilic and the drug can diffuse through this tortuous route without partitioning into a hydrophobic media. In this case, the flux of the hydrophilic pathway is expressed as:

$$J_H = \frac{D_H}{h_H} \cdot C_D \qquad \text{(II-6)}$$

where D_H and h_H are the diffusion coefficient and path length, respectively, of the hydrophilic pathway.

The overall flux of the tissue, J, is the sum of J_L and J_H because these two routes are parallel to each other. To that end, one more parameter, ε, is needed. ε, or porosity, is the fraction of the area devoted to the hydrophilic pathway. Then, the fraction of the lipophilic pathway is (1-ε), and the overall flux of the oral mucosa, J, is:

$$J = \left(\frac{\varepsilon \cdot D_H}{h_H} + \frac{(1-\varepsilon) \cdot D_L \cdot K_P^{\,f}}{h_L} \right) \cdot C_D \qquad \text{(II-7)}$$

and the overall permeability coefficient is:

$$P = \frac{\varepsilon \cdot D_H}{h_H} + \frac{(1-\varepsilon) \cdot D_L \cdot K_P^{\,f}}{h_L} \qquad \text{(II-8)}$$

C. Troublesome Experimental Designs

Equation (II-8) depicts the relationship between permeability coefficient and partition coefficient. A plot of permeability coefficient versus partition coefficient can be constructed using reasonable estimated values for ε, D_H, h_H, D_L, and h_L to show the

relationship between permeability coefficient and partition coefficient graphically (Fig. II-5). In the low partition coefficient region, the permeability coefficient is independent of the partition coefficient, where the paracellular pathway dominates over the transcellular pathway. In the high partition coefficient region, the permeability coefficient is proportional to the partition coefficient, and the transcellular pathway dominates over the paracellular pathway. In the middle, there is a transition region where both pathways are used for drug transport.

Theoretically, it appears that the relationship between the permeability coefficient and the partition coefficient is clear and straight forward. Experimentally, however, it is very difficult to test this relationship using diffusion study data because it is impossible to meet the necessary assumptions in constructing the permeability coefficient versus partition coefficient plot. Although the assumptions in setting up the two-pathway model are easily fulfilled, it is impossible to keep the model parameters, ε, D_H, h_H, D_L, and h_L, constant while varying the partition coefficient to cover the entire partition coefficient spectrum. Common approaches have used a series of compounds with varying partition coefficients (Siegel, 1981b; Beckett, 1969b). In these systems, the molecular weight, shape, and other physicochemical properties are different from one compound to another. As we know, the diffusion coefficient is a function of molecular weight and shape. A series of compounds with different molecular weight and shape will have different diffusion coefficients in both the lipophilic and hydrophilic

pathways. Molecular size and shape will also affect the parameter ε and h_H because size and shape will change the accessibility and pathway of the diffusant. Table II-1 shows the relationship between model parameters and the physicochemical properties of the diffusants.

Table II-1. Effect of diffusant physicochemical properties on model parameters.

	ε	D_L	D_H	h_L	h_H	f	K_P
Molecular Weight	Yes	Yes	Yes	No	Yes	No	Yes
Molecular Shape	Yes	Yes	Yes	No	Yes	No	Yes
# of Charged Group	No	—	Yes	—	Yes	No	Yes
# of H Bonds	No	No	Yes	No	No	No	Yes
Ionization	No	—	No	—	No	No	Yes

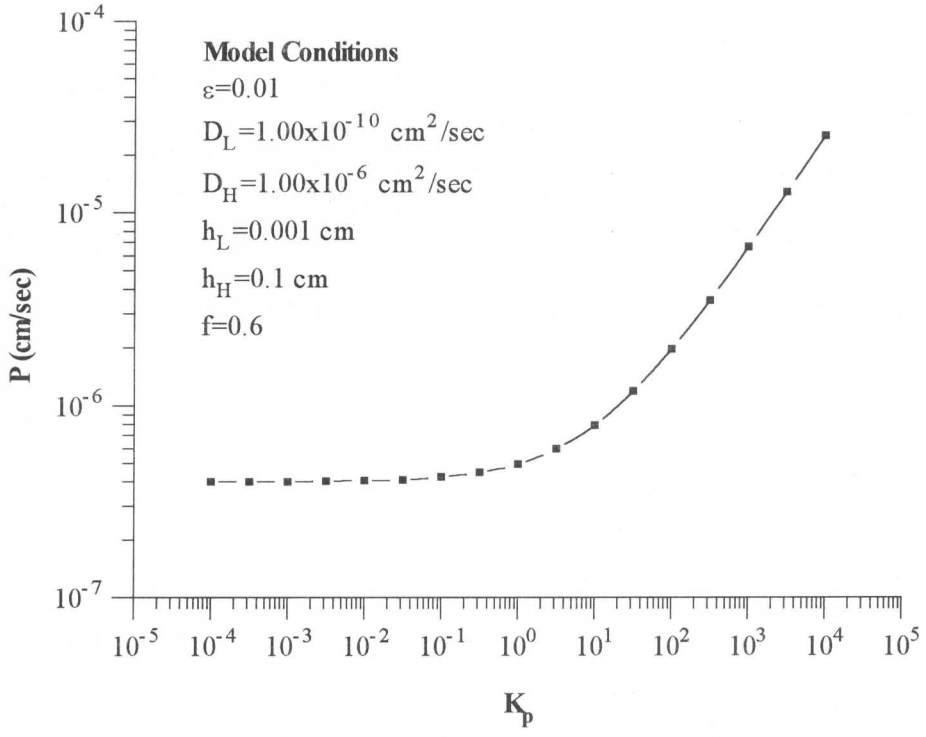


Fig. II-5. A hypothetical plot of permeability coefficient versus partition coefficient using Equation (II-8) and computer generated data employing reasonable parameters.

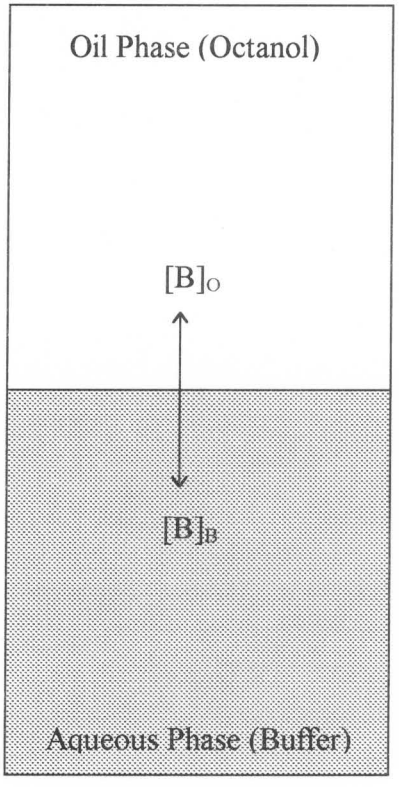
The issue of correlation between permeability coefficient and partition coefficient comes to the point of how to keep everything else constant while changing the partition coefficient. There are two ways to address this problem. One is to carefully select a series of compounds with similar physicochemical properties. This approach has been used in human and hairless rat skin (Morimoto, 1992), and a curve similar to Fig. II-5 has been obtained. The alternative is to use an ionizable compound and change pH of the buffer solution to alter the partition coefficient. Of course, the pH of the solution must be changed in a restricted range so that the pH effect on tissue is not significant.

D. pH Controlled Apparent Partition Coefficient of An Ionizable Compound

Partition coefficient for an unionizable compound is simply the concentration ratio between an organic and aqueous phase (Fig. II-6). For an ionizable compound, however, the apparent partition coefficient is dependent on pH of the solution (Fig. II-7). For example, if the ionizable compound is a weak base which is ionized at low pH and unionized at high pH, the ionization equilibrium is:

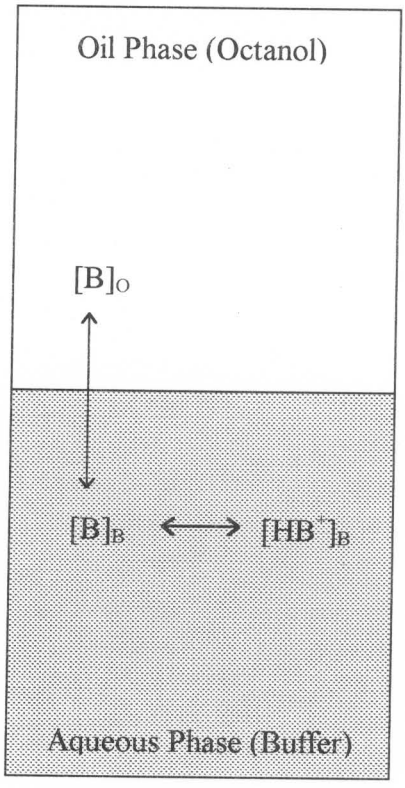
$$HB^{+} \xleftrightarrow{K_a} H^{+} + B \qquad \text{(II-9)}$$

and the ionization constant is:



$$K_P = \frac{[B]_O}{[B]_B}$$

Fig. II-6. Partition coefficient of unionizable drug between organic phase (octanol) and aqueous phase (buffer).



$$K_P = \frac{[B]_O}{[B]_B + [HB^+]_B}$$

Fig. II-7. Apparent partition coefficient of an ionizable compound between organic phase (octanol) and aqueous phase (buffer).

$$K_a = \frac{[H^+]\cdot[B]}{[HB^+]} \qquad \text{(II-10)}$$

When an organic phase is in contact with this aqueous solution, the unionized form, B, will dissolve into the organic phase and reach equilibrium with the unionized form in aqueous buffer phase. The concentration ratio is called the intrinsic partition coefficient.

$$K_I = \frac{[B]_O}{[B]_B} \qquad \text{(II-11)}$$

The overall concentration ratio is called the apparent partition coefficient.

$$K_P = \frac{[B]_O}{[B]_B + [HB^+]_B} \qquad \text{(II-12)}$$

Substituting Equation (II-10) and (II-11) into Equation (II-12), the pH dependency of the apparent partition coefficient is shown in Equation (II-13) or (II-14):

$$K_P = \frac{K_I}{1 + \frac{[H^+]}{K_a}} \qquad \text{(II-13)}$$

or

$$\frac{1}{K_P} = \frac{1}{K_I} + \frac{1}{K_I \cdot K_a} \cdot [H^+] \qquad \text{(II-14)}$$

Equation (II-14) shows that the plot of $\frac{1}{K_P}$ vs. $[H^+]$ is a linear curve with y-axis intercept of $\frac{1}{K_I}$ and slope of $\frac{1}{K_I \cdot K_a}$.

Equation (II-13) shows that the partition coefficient of an ionizable compound can be changed and controlled by pH of the aqueous phase. The major advantage of using pH to control the partition coefficient is that the partition coefficient can be changed while the size and shape remain essentially the same. Therefore, the parameters, ε, D_H, h_H, D_L, and h_L in the two-pathway model will remain unchanged. This will help overcome the problems created by using a series of compounds to study the correlation between permeability coefficient and partition coefficient.

III. Experimental

A. Material and Solution Preparation

All chemicals were used as received without further purification unless otherwise stated. 1,3-bis[tris(hydroxymethyl)methylamino]-propane (Bis-tris propane), morphine sulfate, and fentanyl citrate were all purchased from Sigma Chemical Company (St. Louis, MO). Potassium phosphate monobasic (KH_2PO_4) and sodium chloride (NaCl) were analytical reagent grade and purchased from Mallinckrodt Inc. (Paris, KY). Acetonitrile (for HPLC) was purchased from EM Science (Gibbstown, NJ).

Bis-tris propane buffer was used in this study because of its physiological compatibility and useful pH range. 25 mM bis-tris propane buffer solution was adjusted to the desired pH's with 5 N HCl, and was made isotonic with an appropriate amount of NaCl. The final pH was within 0.1 unit of the desired pH, and the osmolarity was 300 ± 10 mOsm measured on a Wescor 5500 vapor pressure osmometer (Logan, UT).

Morphine or fentanyl solution (~ 0.1 mg/ml) was prepared by dissolving morphine sulfate or fentanyl citrate in bis-tris propane buffer. The pH of the buffer solution was unchanged after adding morphine or fentanyl.

B. Sample Analysis

The HPLC apparatus consisted of a Beckman 110B solvent delivery module, a Beckman 164 variable wavelength UV detector, a Varian 8055 auto sampler with 100

µl sample loop and a Beckman Ultrasphere C-8, 5 µm, 4.6 x 250 mm HPLC column with an inline C-8 guard column. The wavelength was set to 210 nm and 205 nm for morphine and fentanyl, respectively.

Mobile phases for morphine or fentanyl were 0.04 M KH_2PO_4 in 10% or 50% acetonitrile/water, respectively. pH of the mobile phase was adjusted to 3.0 after adding acetonitrile. The flow rate was 1.0 ml/min. Typical chromatograms for morphine and fentanyl are shown in Fig. II-8 and Fig. II-9, respectively.

C. Partition Coefficient Measurement

Equal volumes of octanol and 0.1 mg/ml morphine or fentanyl in buffer solution were mixed and maintained at 37°C overnight in an incubator. Drug concentrations in the aqueous phase were measured using HPLC, and drug concentrations in the organic phase were calculated from the amount of drug found in the aqueous phase. The apparent partition coefficient was simply the ratio of the octanol to aqueous drug concentration.

D. Tissue Collection and Preparation

Male Albino New Zealand rabbits (Bakkom's Rabbitry, Viroqua, WI), weighing 2.5 to 3.0 Kg, were maintained in standard caging facilities with unrestricted diet and a 12 hour light/dark cycle.

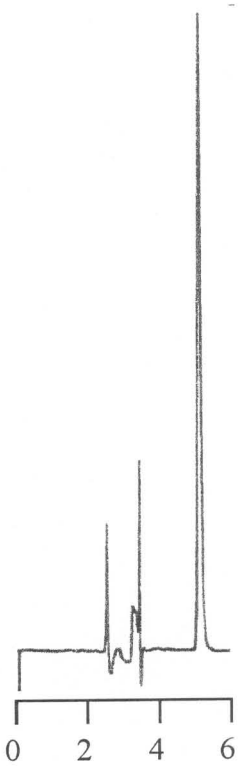


Fig. II-8. HPLC chromatogram of morphine. The retention time is about 5.5 minutes.

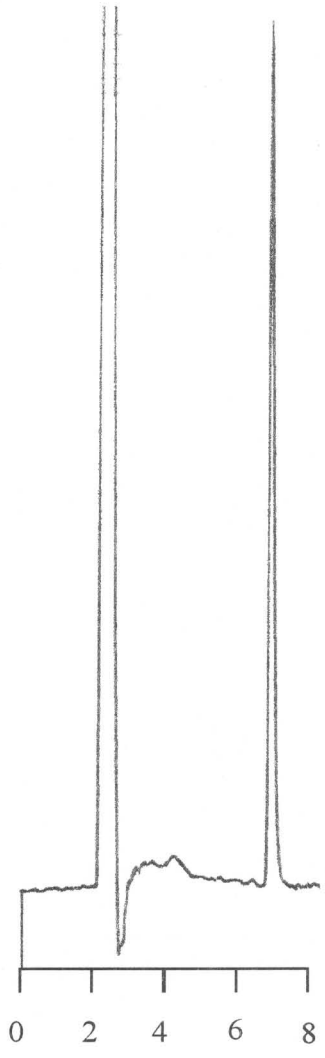


Fig. II-9. HPLC chromatogram of fentanyl. The retention time is about 7 minutes.

Shortly before the experiment, rabbits were sacrificed by an I.V. injection of 10 ml of a 5% sodium pentobarbital solution into a marginal ear vein. The oral cavity was held open with rubber bands on the upper and lower front teeth. Buccal and sublingual mucosa, with some connective tissue, was cut free from the underlying tissue. The tissue was then rinsed and stored in ice-cold buffer solution before further cleanup.

To remove the connective tissue, the buccal and sublingual tissue were pinned epithelial side down onto a dissecting dish. The connective tissue was cut away using fine-pointed forceps and dissection scissors. The cleaned mucosa was stored in ice-cold buffer solution until mounting. The entire tissue collection and cleaning process was completed within one hour. Tissues were then mounted onto diffusion cells and rinsed in oxygen bubbled buffer solution for at least twenty minutes before the experiments.

E. Diffusion Study

Morphine diffusion studies were performed using plastic side-by-side diffusion cells purchased from Precision Instrument Design (Los Altos, CA) (Fig. II-10). Both donor and receiver chambers had the same buffer solution except that 0.1 mg/ml of morphine was present on the donor side only. The donor and receiver solutions were stirred by oxygen gas bubbles, which also provided aeration for the tissues. The diffusion cells were kept in jacketed aluminum blocks to maintain the temperature at 37±1°C. The

area of the tissue to be studied was 0.875 cm^2. The volume of the donor and receiver chambers was 1.5 ml. Samples were taken periodically from the receiver chamber and replaced by fresh buffer solution maintained at 37±1°C. The diffusion study lasted for about four hours. The entire experiment, including tissue collection and the diffusion study, was completed within six hours, which was earlier established as the limit of tissue viability (Dowty, 1992).

Procedures for the fentanyl diffusion studies were identical to that of morphine except that glass diffusion cells were used because of surface adsorption of fentanyl onto the plastic diffusion cells. A schematic diagram of the glass diffusion cell is shown in Fig. II-11. The area of the opening was 0.503 cm^2. The volume of the chambers were 2.0 ml. The diffusion cells were immersed in a 37±1°C water bath to maintain temperature. Oxygen was used to supply aeration and stirring.

pH of the buffer solutions in both donor and receiver chambers were checked after the experiments and the changes were within 0.1 unit.

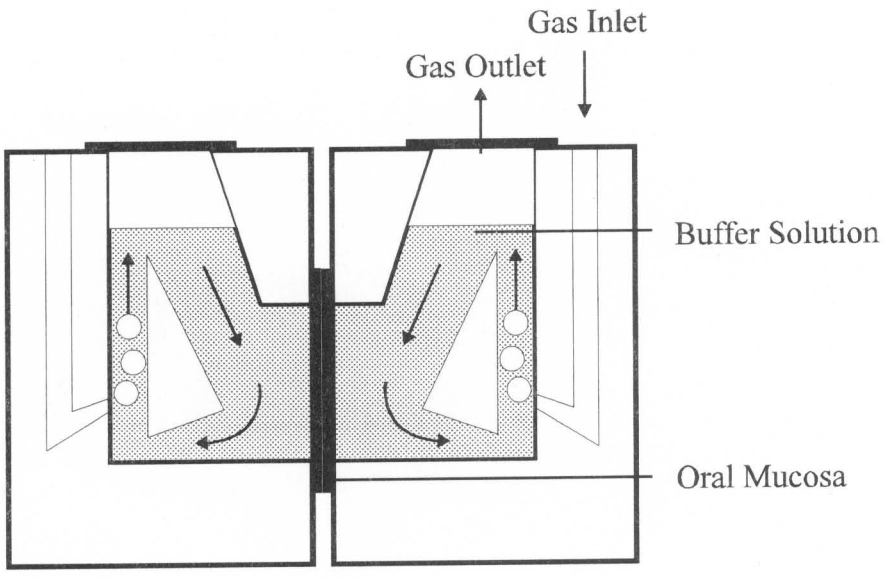


Fig. II-10. Schematic diagram of the plastic diffusion cell used for morphine diffusion studies.

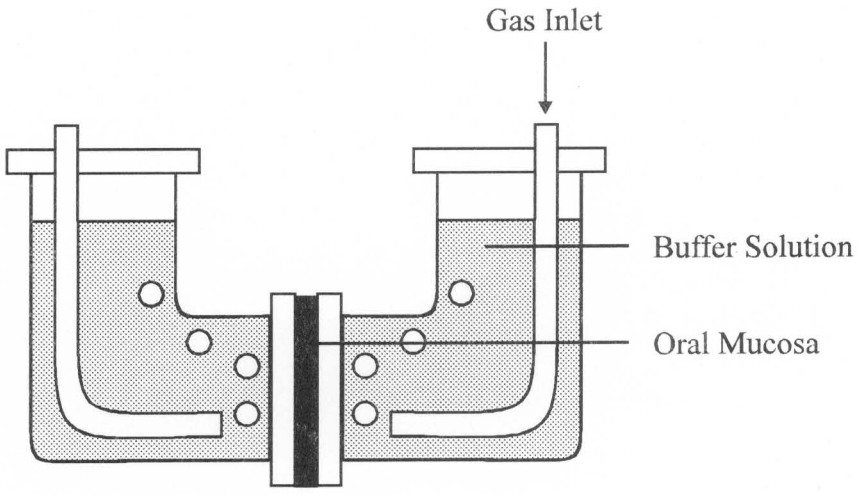


Fig. II-11. Schematic diagram of the glass diffusion cells used for fentanyl diffusion studies.

IV. Results and Discussion

A. Apparent Partition Coefficient of Morphine and Fentanyl

There are two potential charged groups, the alkylamino group and the phenol group, on the morphine molecule (Fig. II-12). At low pH, morphine is positively charged, and it has a low partition coefficient. With a rise in pH, morphine becomes unionized or forms a zwitterion because the ionization constants of the alkylamino and phenol groups are close and overlap each other. At a still higher pH, morphine is negatively charged. Increasing the buffer pH from 6.5 to 9.5, the ionization of morphine is decreased to a minimum, and then starts to increase. The hydrophilicity is changed accordingly. Therefore, the partition coefficient increases with an increase of pH up to a maximum, and decreases after that (Table II-2) (Fig. II-13). The dependency of the partition coefficient on pH does not follow Equation (II-14), i.e., the plot of $\frac{1}{K_P}$ vs. $[H^+]$ is not linear (Fig. II-14). This is because the ionization constant of alkylamino and phenol groups are 7.93 and 9.63 (Schill, 1964; Kaufman, 1975), respectively, and are close to each other. At pH 8.5, there are four forms of morphine existing in the solution, the positively charged morphinium ion, the negatively charged morphinate, the uncharged and the zwitterion forms. Equation (II-14) was developed assuming that there is only one ionized group on the molecule. Nevertheless, the trend is clear that morphine has a low partition coefficient at low pH and a high partition coefficient at high pH.

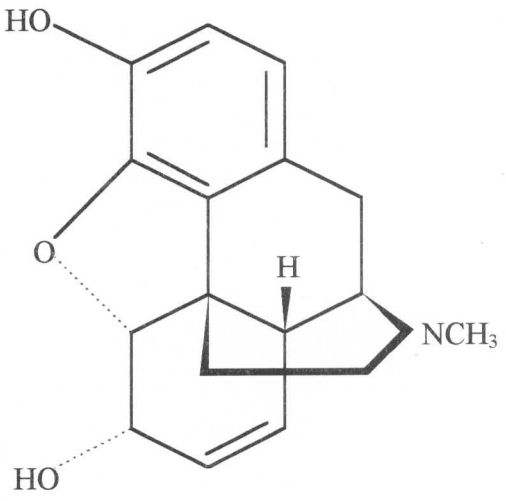


Fig. II-12. Molecular structure of morphine. There are two possible charged groups: the positively charged alkylamino group and the negatively charged phenol group.

Table II-2. Octanol/buffer partition coefficient of morphine at various pH's.

	pH 6.5	pH 7.4	pH 8.5	pH 9.5
K_P	0.065	1.052	4.564	3.749
S.D.	0.012	0.023	0.081	0.052
R.S.D.	18.5%	2.2%	1.8%	1.4%

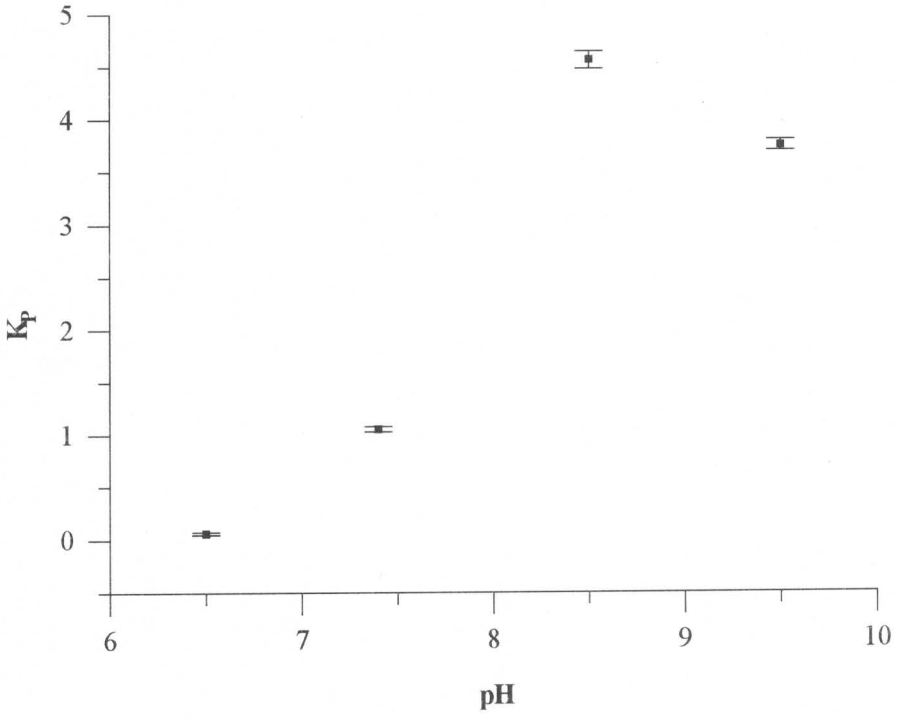


Fig. II-13. Octanol/buffer partition coefficient of morphine at various pH's.

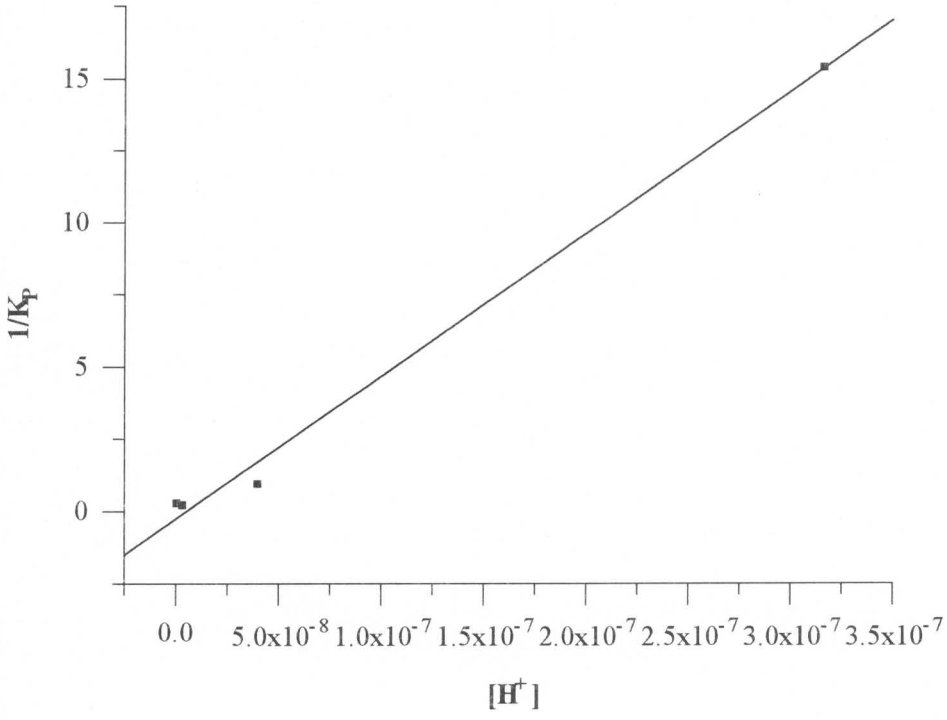


Fig. II-14. $1/K_P$ versus $[H^+]$ plot of morphine at various pH's.

Fentanyl has only one ionizable group, which is positively charged at pH's lower than the pK_a and uncharged at pH's higher than the pK_a (Fig. II-15). The hydrophilicity of fentanyl decreases with an increase in pH. Table II-3 and Fig. II-16 shows the dependency of partition coefficient on pH.

The pK_a of fentanyl is difficult to measure because the solubility of fentanyl is very low, especially at high pH. The value of fentanyl pK_a varies depending on the methods of measurement. It is 8.43 by a potentiometric method at 25°C (Meuldermans, 1982), 8.99 by a solubility method and 8.73 by a graphical method (Roy, 1989). Using partition coefficient method, as shown in previous section, the pK_a of the fentanyl can be obtained by a $\frac{1}{K_P}$ vs. $[H^+]$ plot (Fig. II-17). Using this method at 37°C, the pK_a of fentanyl is 7.55, and the intrinsic partition coefficient is 1031. From $\frac{1}{K_P}$ vs. $[H^+]$ plot (Fig. II-17), the partition coefficient of fentanyl at pH 5.5, 6.5, 7.4 and 8.5 can be calculated (Table II-4).

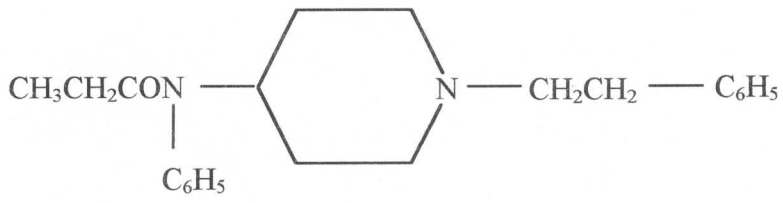


Fig. II-15. Molecular structure of fentanyl shows there is a potential charged group.

Table II-3. Octanol/buffer partition coefficient at various pH's.

	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 7.4	pH 8.0	pH 8.5
K_P	9.13	27.11	87.20	254.26	458.11	812.61	1038.20
S.D.	0.18	0.32	1.54	9.18	55.39	100.24	136.54
R.S.D.	1.9%	1.2%	1.8%	3.6%	12.1%	12.3%	13.2%

Table II-4. Calculated fentanyl octanol/buffer partition coefficient at pH's where diffusion experiments were performed.

	pH 5.5	pH 6.5	pH 7.4	pH 8.5
K_P	9.10	84.51	433.08	955.76

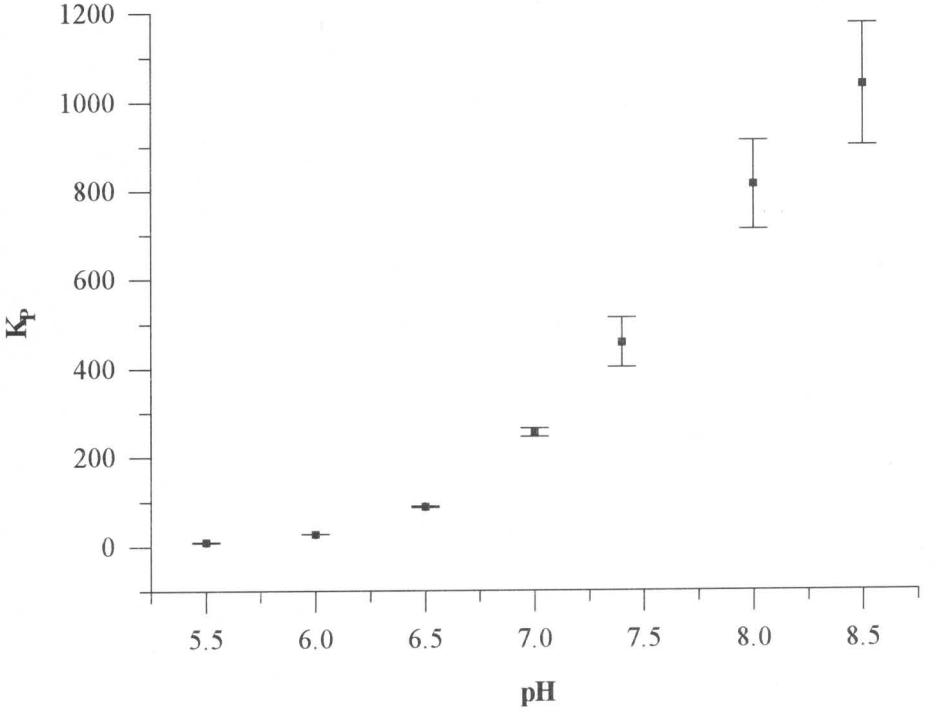


Fig. II-16. Octanol/buffer partition coefficient of fentanyl at various pH's.

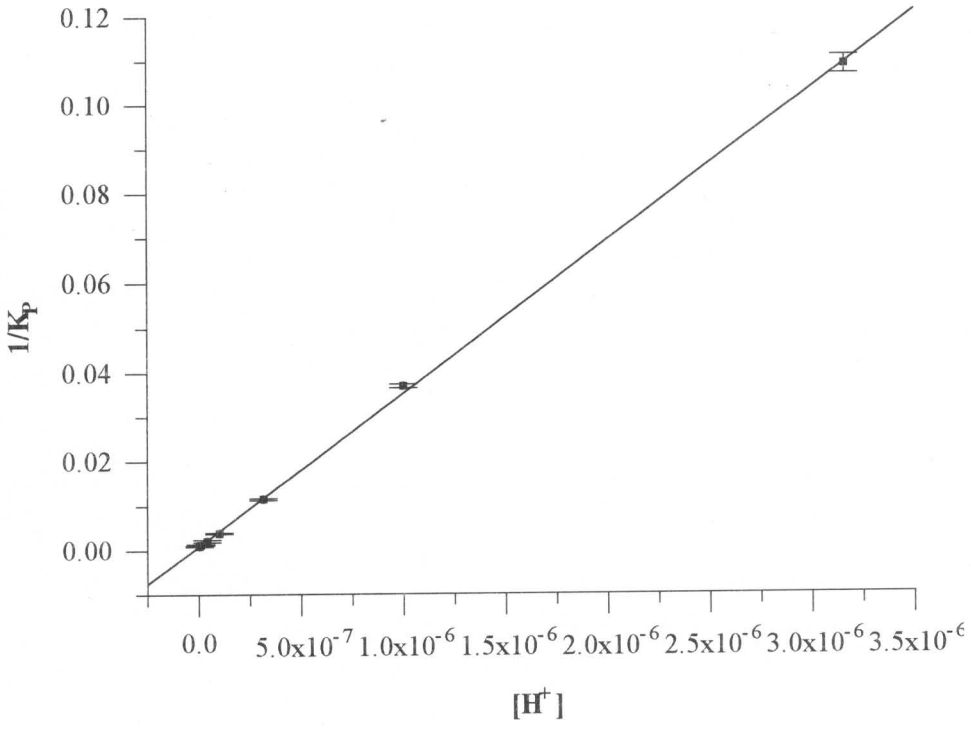


Fig. II-17. $1/K_P$ versus $[H^+]$ plot of fentanyl at various pH's.

B. In Vitro Rabbit Oral Mucosal Morphine and Fentanyl Diffusion Study

In vitro morphine and fentanyl diffusion experiments were performed using rabbit buccal and sublingual mucosa at pH's ranging from 5.5 to 8.5 (Table II-5). This pH range is chosen to accomplish two goals, minimize tissue alteration and maximize diffusant lipophilicity change.

Table II-5. Permeability coefficient ($x10^7$ sec/cm) of morphine and fentanyl at various pH's. The errors are standard deviation based on 4-6 replications.

Diffusant	Region	pH 5.5	pH 6.5	pH 7.5	pH 8.5
Morphine	**Buccal**	—	2.70±0.38	2.58±0.57	3.4±1.5
	Sublingual	—	3.3±1.1	4.0±1.6	4.8±1.7
Fentanyl	**Buccal**	21.2±4.5	88.7±9.1	214±11	328±25
	Sublingual	46.3±18.0	207±32	431±74	650±52

The tissue alteration by the buffer pH should be minimized because the concept of changing buffer pH to change the lipophilicity of the diffusants is based on the assumption that the pH change in the buffer solution would not change permeability of the tissue. Gandhi and Robinson studied the pH effect on buccal tissue by measuring

the diffusion potential and streaming potential (Gandhi, 1991). The diffusion potential is defined as the potential difference produced across a charged membrane by the movement of ions under the influence of a concentration gradient(s). The streaming potential is defined as the potential difference produced by flow of ions across the charged membrane under the influence of either a hydrostatic or an osmotic pressure gradient. Both potential measurements use ions as a probe to determine characteristics of the tissue and are very sensitive to tissue change. Their study found that the rabbit buccal mucosa has an isoelectric point of 2.7±0.25 by diffusion potential measurement and 2.6 by streaming potential measurement. At pH's below the isoelectric point, the buccal mucosa possesses a net positive charge and is anion selective. At pH's above the isoelectric point, from pH 3.0 to 9.0, the buccal mucosa maintains a constant diffusion potential and streaming potential at 8 mV and 3.7 mV, respectively, and the tissue is cation selective. Their study showed that the buccal mucosa is not affected by a bathing buffer solution from pH 3.0 to 9.0. At pH's lower than the isoelectric point, the tissue may undergo dramatic changes. In this study, the pH range was 5.5 to 8.5, which is within the "safe" pH range. Therefore, the pH effect on tissue appears to be small and can be ignored. The pH effect on the sublingual mucosa has not been measured. Since the buccal and sublingual mucosa are very similar in many aspects, it is believed to have a similar response to the surrounding pH.

The lipophilicities of morphine and fentanyl are extremely sensitive to the buffer pH as shown previously. From pH 6.5 to 8.5, the partition coefficient of morphine increased 70 times, although morphine is still relatively hydrophilic at pH 8.5. Further increase of the buffer pH would not increase the partition coefficient of morphine because at higher pH's the phenol group starts to ionize, which will increase the hydrophilicity. Similarly, the partition coefficient of fentanyl increased more than 100 times from pH 5.5 to pH 8.5. Further increase in pH would not increase the partition coefficient because at pH 8.5, the partition coefficient is very close to the intrinsic partition coefficient of fentanyl.

Combining morphine and fentanyl, permeability versus partition coefficient plots for the buccal and sublingual mucosa were constructed to cover the partition coefficient range from 0.065 to 956 (Fig. II-18, Fig. II-19). In the low partition coefficient region, morphine permeability coefficient is independent of the partition coefficient. In the high partition coefficient region, on the other hand, fentanyl permeability coefficient is highly partition coefficient dependent.

For both the buccal and sublingual cases, the permeability coefficient versus partition coefficient plots are very similar to Fig. II-5, which is the hypothetical plot using computer generated permeability coefficient data from Equation (II-8) with some reasonable parameter estimates. Although the lipophilicity of morphine increases 70 times from pH 6.5 to 8.5, the permeability coefficient does not change appreciably. This result implies that morphine is transported primarily through the paracellular

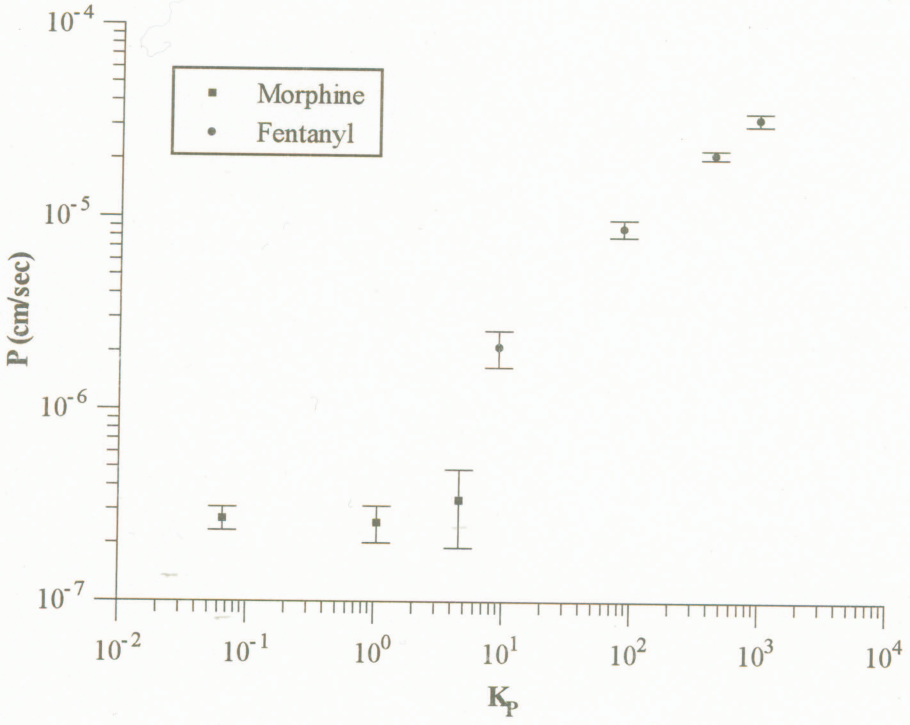


Fig. II-18. The permeability coefficient versus partition coefficient plot of the rabbit buccal mucosa. The error bars represent the standard deviation of 4 to 6 replicas.

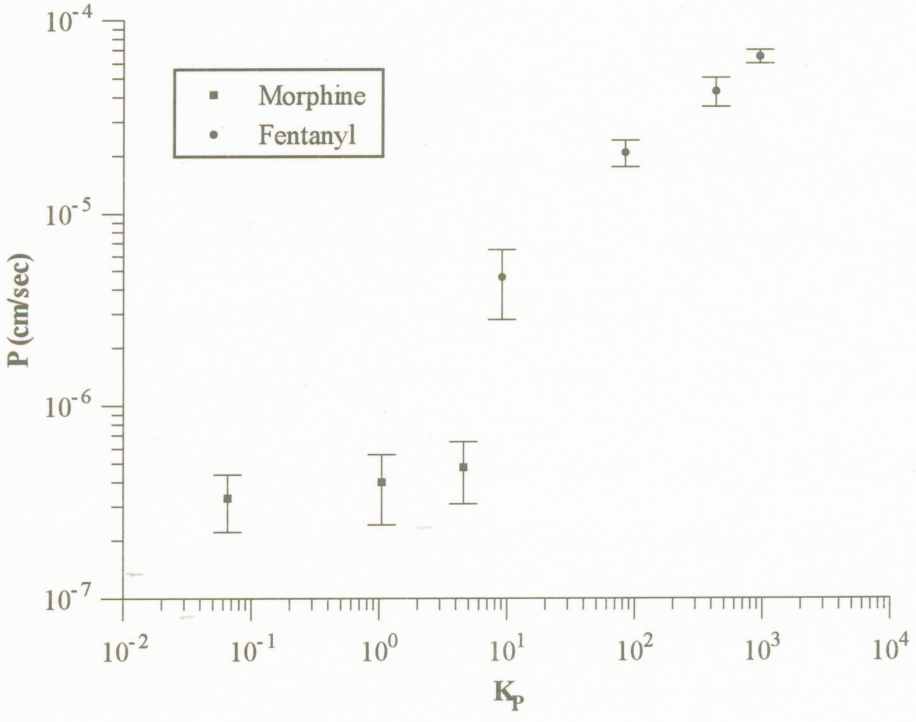


Fig. II-19. The permeability coefficient versus partition coefficient plot of the rabbit sublingual mucosa. The error bars represent the standard deviation of 4 to 6 replicas.

route, where no preferential partitioning is involved. Even at pH 8.5, the morphine molecule is too hydrophilic to use the transcellular pathway. Fentanyl, conversely, is transported through the transcellular pathway because the permeability coefficient of fentanyl is highly sensitive to its lipophilicity.

Note that the morphine and fentanyl data cannot be connected together to form a smooth curve like the curve in Fig. II-5. This is because morphine and fentanyl are different molecules and their physicochemical properties are different. Consequently, the model parameters for morphine and fentanyl are different. As shown earlier in Table II-1, many properties of the diffusant can change the model parameters. This is also the reason why it is difficult to experimentally prove the two-pathway model via indirect kinetic data.

The slope of the log (permeability coefficient) versus log (partition coefficient) plot in the high partition coefficient region is the linear free energy coefficient, which is the dependence of the permeability coefficient on the partition coefficient, and is also the index of the relative lipophilicity of the tissue. The linear free energy coefficients for the rabbit buccal, sublingual mucosa, human skin, and hairless rat skin are listed in Table II-6.

Table II-6. The linear free energy coefficients of rabbit buccal, sublingual mucosa and human and hairless rat skin.

	Rabbit Buccal	Rabbit Sublingual	Human Skin [a]	Hairless Rat Skin [a]
Linear Free Energy Coefficient	0.588	0.563	0.751	0.589

a: From reference Morimoto, 1991.

It is interesting to note that the linear free energy coefficient of the skin is higher than that of the oral mucosa, which means that the lipophilic barrier of the skin is more hydrophobic than that of the oral mucosa. This observation agrees with our general understanding of the skin and oral mucosa lipophilicity characteristics. Therefore, it is possible to use this index to characterize other biological membranes.

Compared to human and hairless rat skin (Morimoto, 1992), it is also interesting to note that the permeability coefficient of the hydrophilic compounds (the permeability of the hydrophilic pathway) is much lower in the skin than in the oral mucosa (Table II-7). The permeability of the hydrophilic pathway is a measure of how much area is devoted to the hydrophilic pathway, how thick is the epithelium, and how tortuous is the pathway. Results from the rabbit oral mucosa and human and hairless rat skin show that the skin has a much lower area available for the hydrophilic pathway and the

route of the hydrophilic pathway is much more tortuous than that in the rabbit oral mucosa.

Table II-7. Permeability coefficient of hydrophilic pathway for drugs with molecular weight around 300 D in the rabbit oral mucosa and the human and hairless rat skin.

	Rabbit Buccal	Rabbit Sublingual	Human Skin [a]	Hairless Rat Skin [a]
P ($x10^{-7}$ cm/sec)	2.89	4.03	0.273	0.833

a: From reference Morimoto, 1991.

V. Conclusion

The permeability of morphine and fentanyl in the rabbit oral mucosa demonstrates that there are two pathways in the oral mucosa, presumably a hydrophilic paracellular pathway and a lipophilic transcellular pathway. A mathematical model of these two pathways was developed based on the general understanding of the cellular structure of the oral mucosa and basic diffusion theory. The relationship between the model parameters and the physicochemical properties of the tissue, diffusants, and the

delivery vehicle were discussed and it is concluded that although the two-pathway model is not difficult to understand, experimentally testing this model is rather difficult. This work also showed that in the rabbit oral mucosa, the lipophilic pathway is less lipophilic, and the hydrophilic pathway is more accessible than that of human skin. All these results agree with our intuitive understanding of the characteristics of the human skin and oral mucosa. The two-pathway model can be used to characterize the diffusion process of other biological membranes. A basic understanding of the diffusion process will eventually lead to better strategies to deliver drug through the oral mucosa efficiently and in a controlled manner.

CHAPTER 3: DRUG TRANSPORT PATHWAYS AND DRUG DEPOSITION INSIDE THE RABBIT ORAL MUCOSA

I. Introduction

As mentioned in Chapter 1, the main problem of an *in vivo* drug absorption experiment is measuring the amount of drug absorbed. The problem is that drug disappearing from the delivery solution is not necessarily equal to drug available to the general circulation. Drug disappearing from the delivery solution may undergo three routes of loss, i.e., be metabolized or degraded by enzymes in the oral epithelium (Dowty, 1992; Garren, 1989b), be deposited (adsorbed or bound) somewhere in the oral mucosa tissue (Davis, 1979; Henry, 1980; Zhang, 1991b), or be delivered into the circulation. Drug metabolism during transmucosal experiments is common for peptides or protein drugs, or other metabolically unstable drugs. Since the oral mucosa is not designed for digestion, the enzymatic activity is relatively low compared to the gastrointestinal tract. Thus, for many drugs, metabolism inside the oral mucosa is not significant. Therefore, drug deposition in oral mucosal tissue is usually the primary reason for the difference between drug disappearance from the delivery solution and drug appearance in the general circulation.

Except for specific binding by a particular molecule in the oral mucosa, drug deposition is generally considered as a distribution issue and therefore subject to equilibrium between regions inside the oral mucosa. Thus, drug deposition is largely determined by the physicochemical nature of the tissue and the drug. These parameters also control the penetration pathway(s) of drug transport across the oral mucosa. There must therefore be a correlation between penetration pathways, lipophilicity of the drug (partition coefficient), and drug deposition. It is the purpose of this chapter to examine these relationships from both a theoretical and experimental point of view.

II. Theory

A. Paracellular pathway

As discussed earlier in Chapter 2, the paracellular pathway is a hydrophilic pathway where drug diffuses through tortuous intercellular spaces. The epithelial cells are only the physical obstacles in the diffusion media, and not directly involved in the diffusion process. Therefore, diffusion in the paracellular pathway can be considered as simple diffusion in a medium with a surface area smaller than the entire tissue and a pathlength longer than the thickness of the tissue (Fig. III-1). The amount of drug deposited inside the tissue is determined by the surface area of the tissue, the thickness of the tissue, drug concentration on the donor side and the fraction of area devoted to

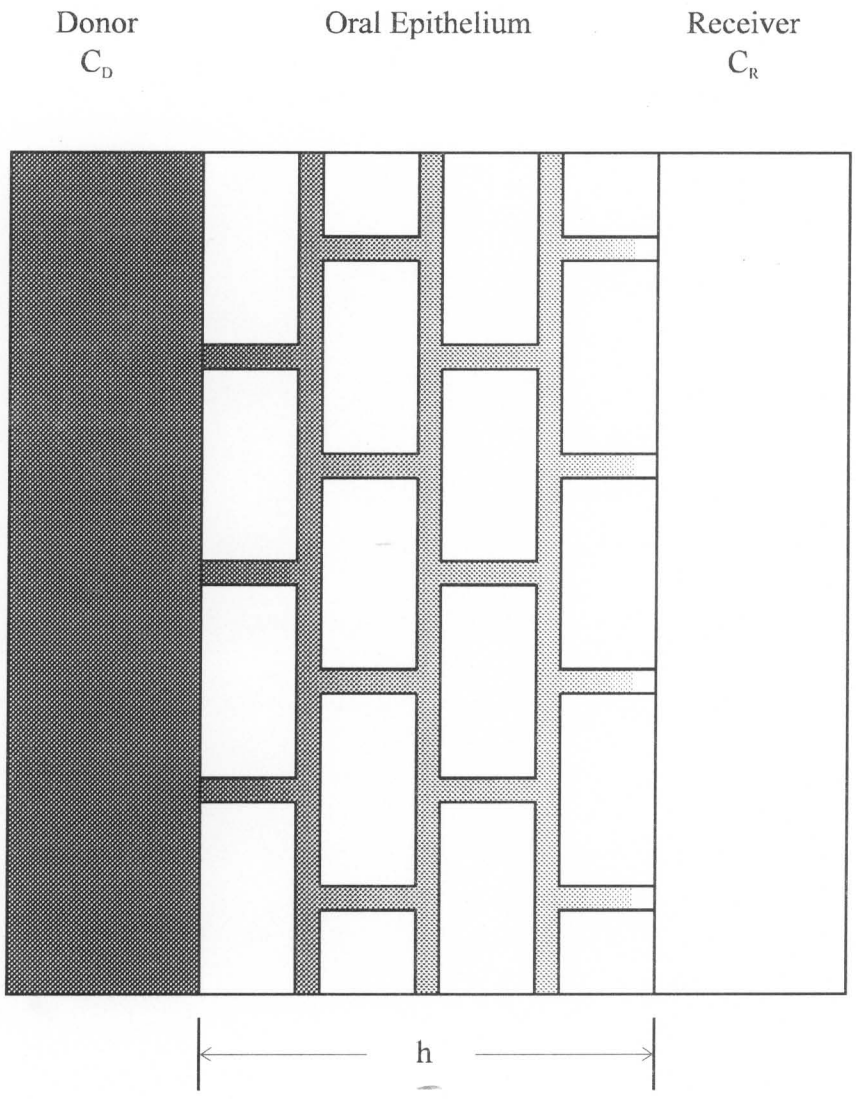


Fig. III-1. Drug transport across the oral mucosa through the paracellular pathway. The density of gray represents the concentration of drug. The surface area of the tissue is fixed at S. The thickness of the tissue is h. Drug concentration on the donor side is C_D. Drug concentration on the receiver side is zero.

the paracellular pathway. Since there is no partitioning involved, the amount of the drug inside the tissue is independent of drug lipophilicity (partition coefficient). In addition, since the drug never enters epithelial cells, the pH difference between the epithelial cytoplasm and the media of the intercellular space should not affect the amount of drug deposited inside the oral mucosa.

B. Transcellular pathway

Drug transport through the transcellular pathway is more complicated than through the paracellular route. In this case, drug diffuses across a cell membrane into the epithelial cell from the epithelial side and diffuses across the cell membrane again into the endothelial side. If the interior of the epithelial cell differs from the medium in the intercellular space, it is possible that the activity of the drug inside the cell differs from activity outside the cell. Therefore, drug concentrations inside and outside the epithelial cell at the same distance from the surface are different, and the drug can accumulate in epithelial cells (Fig. III-2). The amount of drug accumulated is dependent on the drug concentration difference, which reflects the physicochemical property difference, between the epithelial cell cytoplasm and the media of the intercellular space.

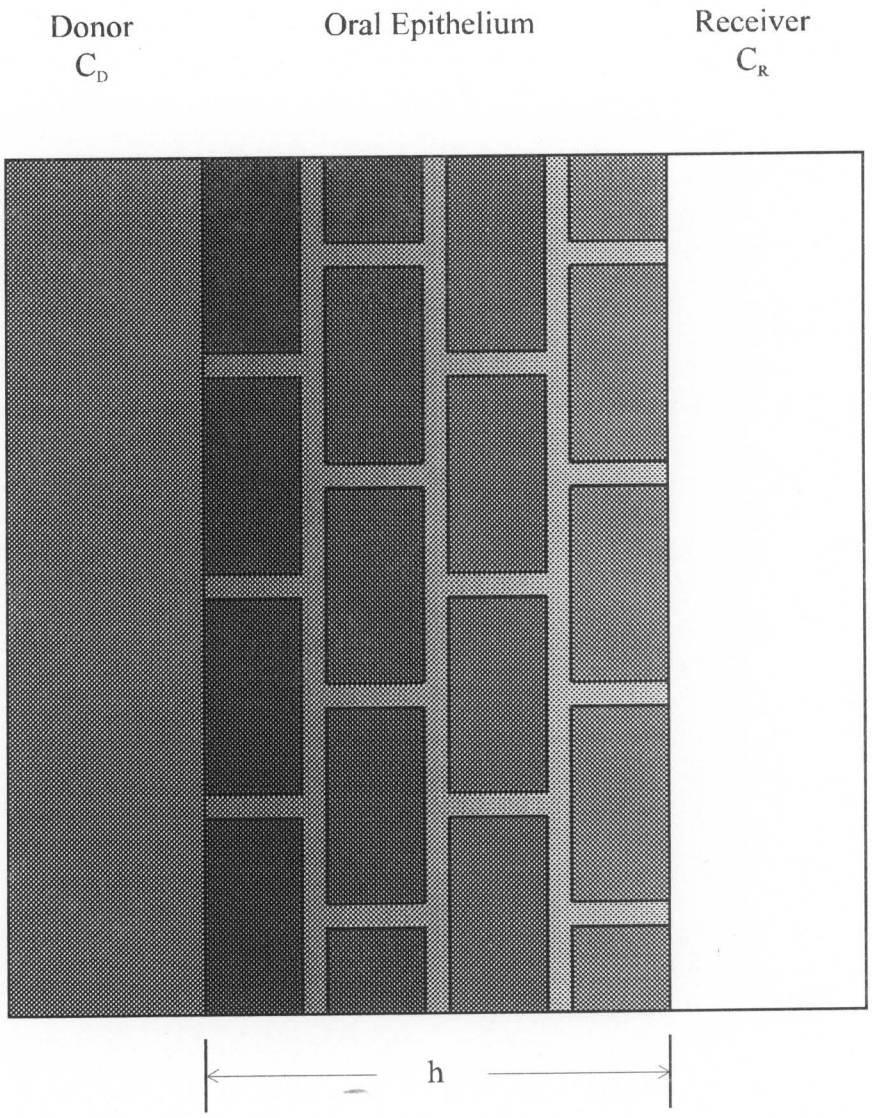
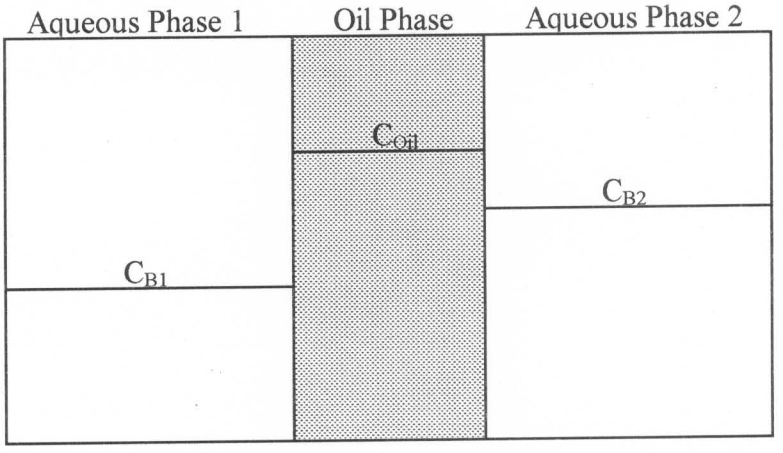


Fig. III-2. Drug transport across the oral mucosa through the transcellular pathway. Since the interior of the epithelial cell and the intercellular space are different, the drug concentration inside the epithelial cell is higher than the drug concentration inthe intercellular space at the same distance from the surface.

If the tissue is placed in a buffer solution that has a pH other than pH 7.4, pH of the intercellular space will quickly adjust to the surrounding pH because hydrogen ion can easily diffuse through the intercellular space and reach equilibrium. The pH of the epithelial cell cytoplasm, on the other hand, is regulated and relatively stable (Dowty, 1991; Frelin, 1988; Moolenaar, 1986). This may be because the cell membrane can significantly reduce the diffusion of hydrogen ion. Other active transport mechanism(s), i.e., Na^+/H^+ exchanger, may also be employed to maintain a constant cytoplasm pH for normal biological functions (Moolenaar, 1986; Aronson, 1985). Therefore, the cytoplasmic pH will never equal pH of the bathing solution.

For an ionizable compound, the partition coefficient is usually highly pH dependent (see Chapter 2). The distribution between two aqueous phases separated by an oil phase is therefore also pH dependent (Fig. III-3). The distribution coefficient between these two aqueous phases equals the inverse ratio of the octanol/buffer partition coefficients of these aqueous phases. Thus, the distribution of an ionizable compound between epithelial cytoplasm and intercellular space is dependent on the bathing solution pH. Conversely, if drug accumulation inside the oral mucosa is bathing solution pH dependent, the drug must be transported via the transcellular pathway, otherwise, there will not be distribution between two aqueous phases and no pH dependence.



$$K_{P1} = \frac{C_{Oil}}{C_{B1}}$$

$$K_{P2} = \frac{C_{Oil}}{C_{B2}}$$

$$K_{P1/P2} = \frac{K_{P1}}{K_{P2}} = \frac{C_{B2}}{C_{B1}}$$

Fig. III-3. Schematic diagram shows two aqueous phases separated by an oil phase. The distribution coefficient between these separated aqueous phases equals the concentration ratio of these two phases.

Using data on drug deposition inside the oral mucosa, the pathway used for drug transport can be easily detected. In the meantime, the difference in pH dependency of drug deposition can be used as evidence to show that there are two drug transport pathways in the oral mucosa.

III. Experimental

A. Material

Materials, solution preparation, tissue collection, and sample assay procedures are the same as stated in Chapter 2.

B. Oral mucosal absorption of morphine

Oral mucosal tissues were prepared as if they would be used for a diffusion study. The tissue was then cut into small pieces (0.050-0.150 gram) and placed in 0.5 ml 0.1 mg/ml morphine solution at 37°C for one hour. The incubation time was set because previous diffusion studies showed that the lag time of diffusion was 30 to 60 minutes for rabbit buccal and sublingual tissues (Zhang, 1992b). Morphine absorption should reach equilibrium after one hour. The concentration difference before and after tissue incubation was measured and the amount of morphine absorbed per gram of tissue in various pH's was calculated.

C. Oral mucosa absorption of fentanyl

Fentanyl absorption across oral mucosal tissue was measured after the diffusion studies described in Chapter 2. The total amount of fentanyl at the end of the experiment, including the receiver and donor chambers, was measured, and the difference was calculated as the amount of fentanyl absorbed.

IV. Results and Discussion

A. Rabbit oral mucosal absorption of morphine

The oral mucosa absorption of morphine was measured in rabbit buccal and sublingual tissues at pH 6.5, 7.4, 8.5 and 9.5 (Table III-1, Fig. III-4, Fig. III-5). In both the buccal and sublingual experiments, there are no significant differences between the pH groups (99% confidence level). It appears that morphine molecules did not penetrate into or across any hydrophobic region because morphine absorption is independent of buffer solution pH as is the partition coefficient. As shown in Fig. III-1, this type of independency means that morphine molecules did not transport into the cytoplasm of the epithelial cells. Thus, this result indirectly demonstrated that morphine transport across the oral mucosa occurs via the paracellular pathway.

Table III-1. Morphine absorbed (mg/g tissue) in the rabbit buccal and sublingual mucosa at various pH's. Error represents the standard deviation based on 4 to 7 replications. Statistic analysis (99% confidence level) shows that there is no significant difference between pH groups.

Region	pH 6.5	pH 7.4	pH 8.5	pH 9.5
Buccal	0.093±0.017	0.086±0.011	0.104±0.014	0.105±0.019
Sublingual	0.130±0.018	0.142±0.029	0.153±0.013	0.115±0.044

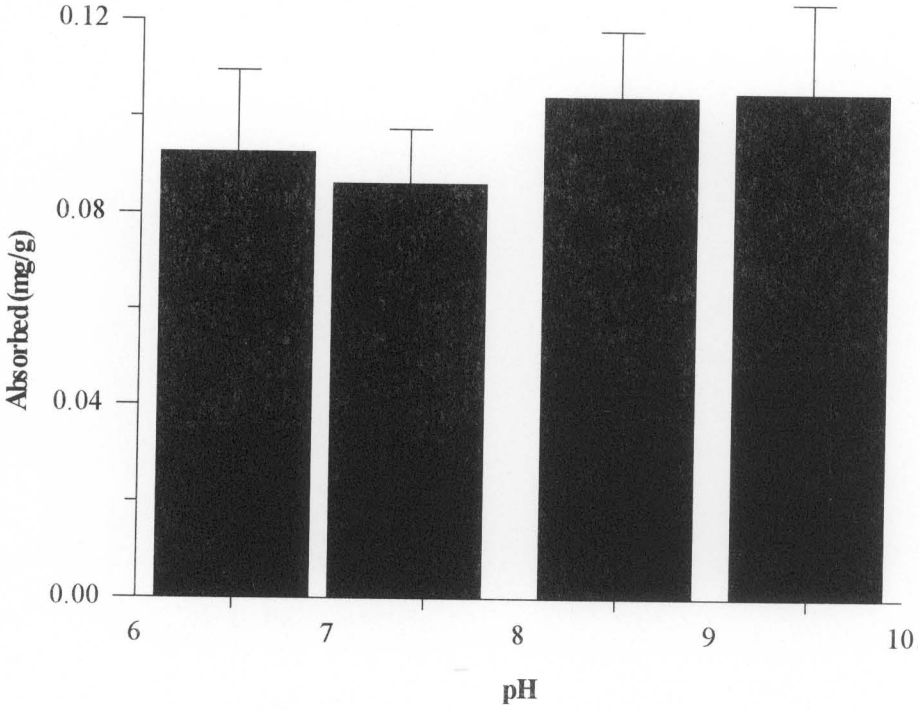


Fig. III-4. Morphine absorption in rabbit buccal tissue at various pH's. Error bars represent the standard deviation of 4 to 6 replications.

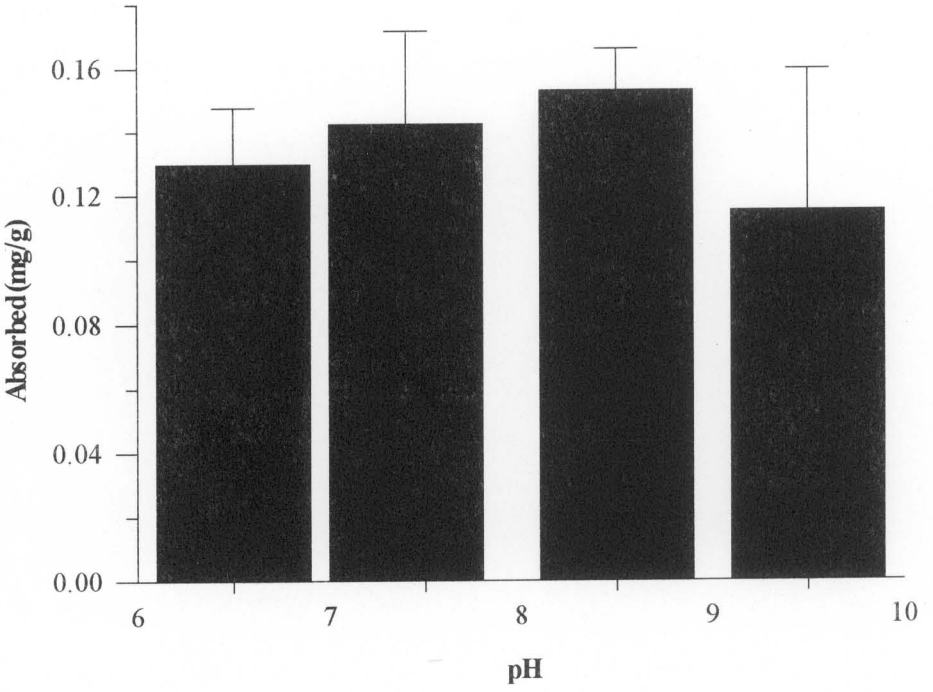


Fig. III-5. Morphine absorption in rabbit sublingual tissue at various pH's. Error bars represent the standard deviation of 4 to 6 replications.

B. Accumulation of fentanyl in rabbit oral mucosa

The concentration of fentanyl in the donor and receiver chambers was measured after each diffusion experiment. The amount of fentanyl absorbed was calculated from the difference before and after the experiment. Since the surface areas of the tissue exposured to fentanyl are constant for all diffusion studies, the weights of the tissue are also constant, assuming that the thicknesses of the oral mucosa are identical. Therefore, the amount of fentanyl absorbed during the diffusion study can be compared directly.

The amount of fentanyl absorbed was measured after each experiment with the buffer solution pH at 5.5, 6.5, 7.4 and 8.5 (Table III-2, Fig. III-6, Fig. III-7). The amount of fentanyl absorbed increases significantly with an increase in solution pH. It is clear that fentanyl molecules are penetrating into a hydrophobic region and accumulating, and the amount of accumulation depends on the aqueous phase pH. This result agrees with the hypothesis that a hydrophobic compound can diffuse through the epithelial cell membrane and accumulate inside the cytoplasm as shown Fig. III-2 and Fig. III-3.

Table III-5. Fentanyl accumulated ($x10^3$ mg) in rabbit buccal and sublingual mucosa. Error represents the standard deviation of 4 to 6 replicas.

Region	pH 5.5*	pH 6.5*	pH 7.4*	pH 8.5*
Buccal	8.4 ± 1.2^a	10.6 ± 2.1^a	28 ± 11^b	54 ± 27^b
Sublingual	8.4 ± 2.0^c	10.10 ± 0.52^c	27 ± 10^d	39 ± 12^d

*: No significant difference (99% conficence level) within group a, or b, or c, or d. There is significant difference between group a and b, c and d.

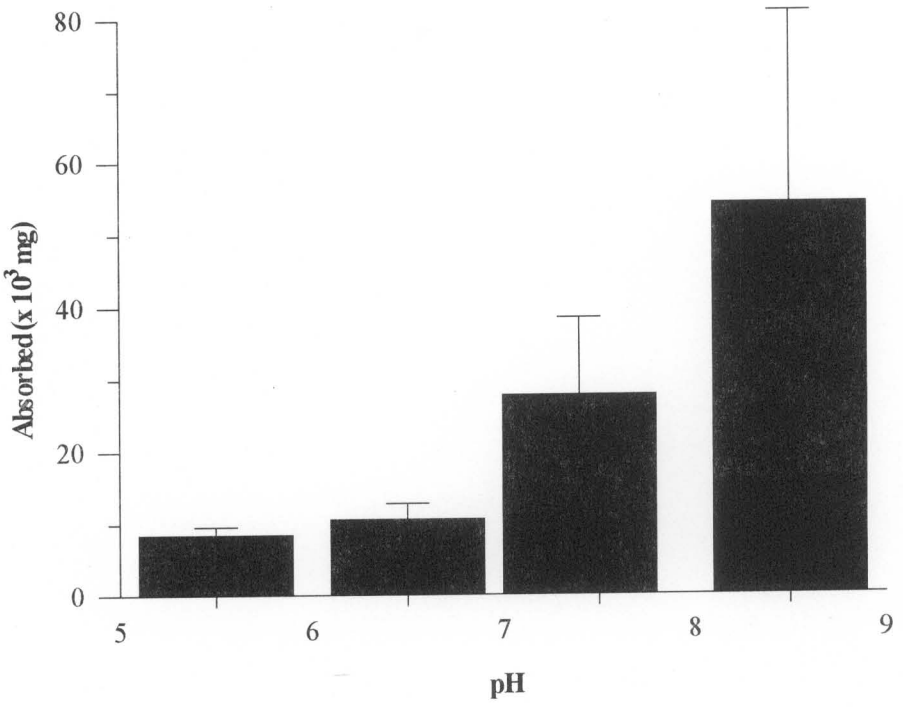


Fig. III-6. Fentanyl accumulated in rabbit buccal mucosa at various pH's. The error bars represent the standard deviation of 4 to 6 replications.

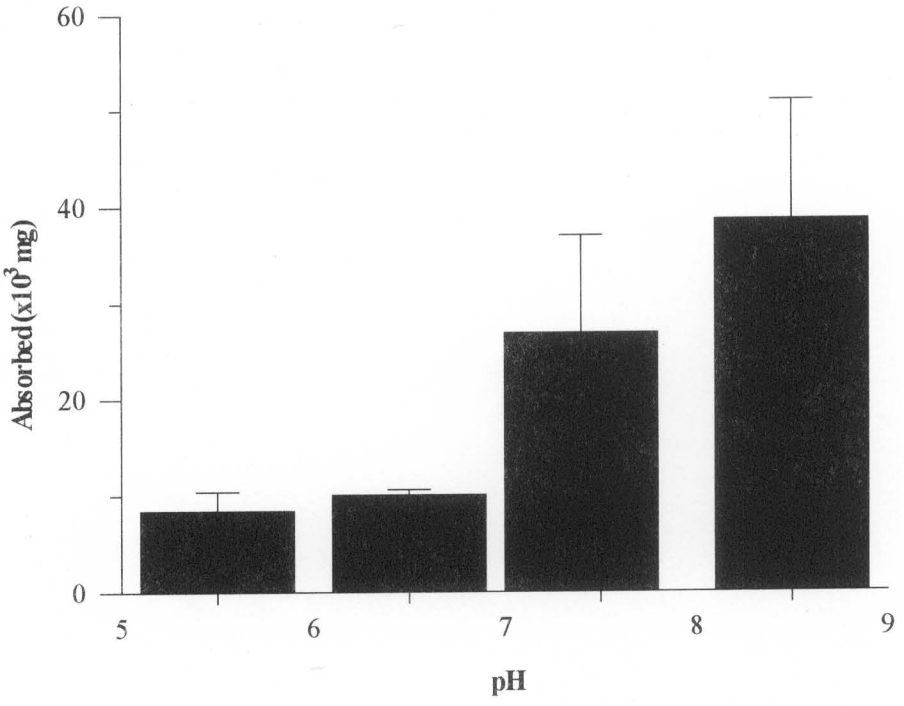


Fig. III-7. Fentanyl accumulated in rabbit sublingual mucosa at various pH's. The error bars represent the standard deviation of 4 to 6 replications.

V. Conclusion

Drug absorption into/onto rabbit oral mucosa reveals that the amount of drug accumulated, the lipophilicity of the drug, the pH of the solution, and the transport pathways are related to each other. Among these, lipophilicity is the key parameter which determines the transport pathway, the amount of drug accumulation inside the epithelial cell, and the overall permeability coefficient. The main contribution of the bathing solution pH is to change the partition coefficient between the cell membrane and the bathing solution. The effect of pH on tissue integrity is assumed small because the range of the study is close to normal biological pH.

CHAPTER 4: EFFECT OF pH GRADIENT ON DRUG TRANSPORT

I. Introduction

pH is an important parameter influencing permeability of ionizable drugs across biological membranes. It is also a complicated issue because a simple pH difference in a delivery solution can change certain aspects of the delivery system that may be crucial to understanding the mechanism of drug absorption.

In Chapters 2 and 3, the impact of the buffer solution pH on the transport pathway and drug accumulation inside the oral mucosa have been discussed. However, all these experiments were done with no pH gradient, which means that the pH of the donor chamber is identical to the pH of the receiver chamber. This setting is unable to mimic the *in vivo* drug delivery situation because the receiver side, i.e., blood circulation, is always at a biological pH of 7.4 for an *in vivo* system while the delivery vehicle is often adjusted to pH other than 7.4 for stability, solubility, or other purposes. Therefore, there is a pH gradient across the oral mucosa.

For an ionizable compound, the unionized form has high lipophilicity and will preferentially use the transcellular pathway, while both the ionized and unionized form will use the paracellular route. For many drugs, to deliver a sufficient amount of drug through the oral mucosa, the transcellular pathway is the primary pathway because it

has a larger surface area and shorter pathlength than the paracellular pathway. To improve absorption, pH of the donor solution is often adjusted so that a greater fraction of drug is in an unionized form (Beckett, 1967; Beckett, 1968; Beckett, 1969a). In these cases, however, the effect of donor pH on the pH gradient across the tissue was ignored.

The receiver side pH effect on delivery of ionizable drug through biological membranes has been recognized recently by Kou, et al (Kou, 1993) for transdermal systems. A model was developed based on the structure of the skin. The barrier function of the skin is performed by both the superficial layer of the stratum corneum and the underlying epidermis/dermis. Therefore, the Kou model consists of two barriers in series, the lipophilic barrier (stratum corneum) that is permeable to the unionized species only, and the hydrophilic barrier (epidermis/dermis) that is permeable to both the ionized and unionized species and has the same pH as the receiver side. The receiver side pH will influence the flux of the hydrophilic barrier and consequently the overall flux.

This model, however, is not suitable for other biological membranes, such as buccal and sublingual tissues, because these membranes are not covered by a stratum corneum. The diffusion barrier of these membranes consists of parallel paracellular and transcellular pathways. The purpose of the present study is to examine the effect of a

pH gradient across the oral mucosa on the permeation of ionizable drugs, and to establish an appropriate mathematical model.

II. Theory

A. Paracellular Pathway

For drugs diffusing through the paracellular pathway, the diffusion medium, or the intercellular space, is hydrophilic, as are the donor and receiver side media. Therefore, there is no partitioning involved in the diffusion process. The flux of drug absorption can be expressed as:

$$J = \frac{D_H}{h_H} \cdot C_D \qquad \text{(IV-1)}$$

This equation is valid only under the assumptions that the drug concentration gradient inside the paracellular route is linear, the diffusion coefficient is constant throughout the paracellular pathway, and drug concentration on the receiver side is zero (sink condition). For an ionizable drug, if there were no pH gradient across the tissue, all these assumptions would be fulfilled. However, if there is a pH gradient, the drug

concentration gradient is no longer linear. The flux, therefore, must be determined by the concentration gradient $\frac{dC}{dx}$ at the membrane/receiver solution interface:

$$J \propto -\left(\frac{dC}{dx}\right)_{x \to h_H} \qquad \text{(IV-2)}$$

where x is the distance from the tissue/donor interface.

The equation for drug concentration inside the paracellular pathway can be derived from the pH on donor and receiver sides, the pK_a of the diffusant and the paracellular pathlength assuming that hydrogen ion concentration and unionized drug concentration gradients inside the paracellular pathway are linear and ionization of the drug reaches equilibrium throughout the diffusion route.

If hydrogen ion on the donor and receiver side are $[H^+]_D$ and $[H^+]_R$, respectively. The hydrogen ion concentration inside the paracellular pathway is:

$$[H^+]_x = [H^+]_D + \frac{[H^+]_R - [H^+]_D}{h_H} \cdot x \qquad \text{(IV-3)}$$

For a weak base that is ionized at low pH and unionized at high pH, the ionization equilibrium can be expressed as:

$$K_a = \frac{[H^+]\cdot[B]}{[HB^+]} \qquad \text{(IV-4)}$$

where K_a is the ionization equilibrium constant.

Since the total drug concentration on the donor side is the sum of the ionized and unionized drug concentration:

$$C_D = [HB^+]_D + [B]_D \qquad \text{(IV-5)}$$

therefore, the unionized drug concentration on the donor side is:

$$[B]_D = \frac{K_a}{K_a + [H^+]_D}\cdot C_D \qquad \text{(IV-6)}$$

and the unionized drug concentration inside the paracellular pathway is:

$$[B]_x = [B]_D - \frac{[B]_D}{h_H}\cdot x \qquad \text{(IV-7)}$$

The ionized drug concentration inside the paracellular pathway is:

$$[HB^+]_x = \frac{[H^+]_x \cdot [B]_x}{K_a} \qquad \text{(IV-8)}$$

Substituting Equations (IV-3), (IV-6) and (IV-7) into Equation (IV-8), the ionized drug concentration inside the paracellular route is:

$$[HB^+]_x = \left(1 - \frac{x}{h_H}\right) \cdot \frac{[H^+]_D \cdot (h_H - x) + [H^+]_R \cdot x}{(K_a + [H^+]_D) \cdot h_H} \cdot C_D \qquad \text{(IV-9)}$$

Equation (IV-9) shows that the pH gradient across the tissue will affect the ionized drug concentration inside the paracellular pathway (Fig. IV-1, Fig. IV-2). It will also affect total drug concentration because total drug concentration inside the paracellular pathway is:

$$C_x = [HB^+]_x + [B]_x \qquad \text{(IV-10)}$$

Substituting Equations (IV-3), (IV-6), (IV-7), and (IV-8) into Equation (IV-10), the total drug concentration is (Fig. IV-3, Fig. IV-4):

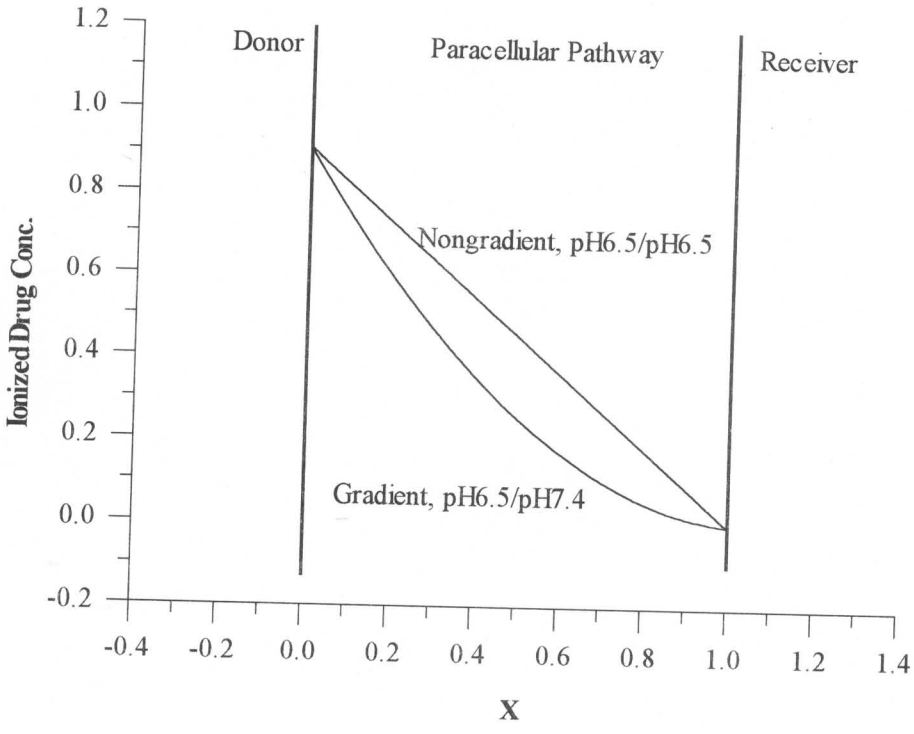


Fig. IV-1. Ionized drug concentration of gradient and nongradient pH inside the intercellular space. The donor side pH is 6.5. The receiver side pH is 7.4 for gradient pH and 6.5 for nongradient pH. The model drug has pK_a of 7.5.

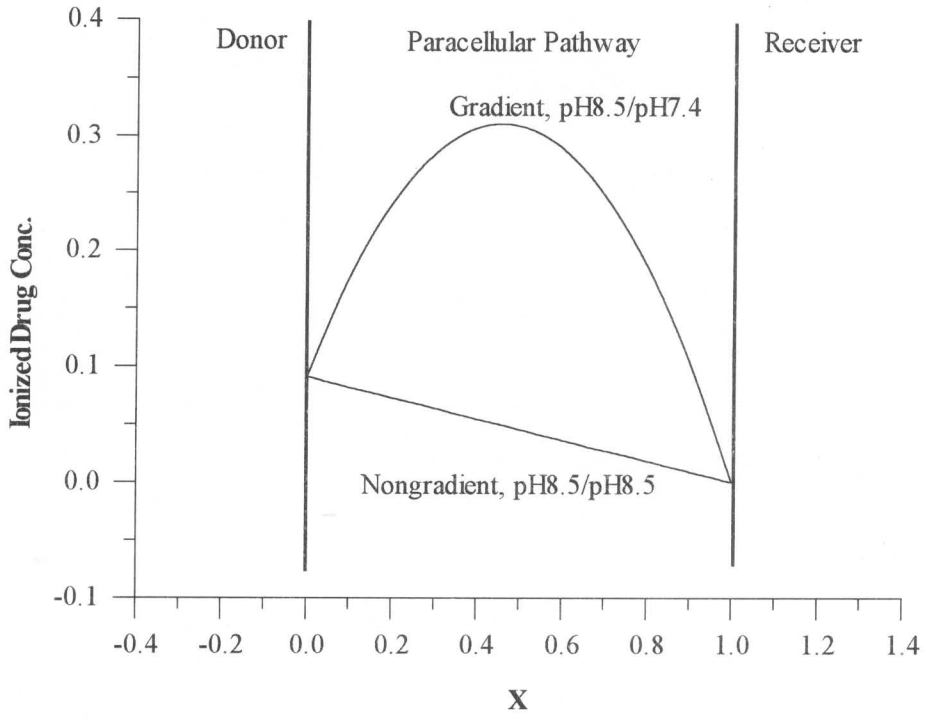


Fig. IV-2. Ionized drug concentration of gradient and nongradient pH inside the intercellular space. The donor side pH is 8.5. The receiver side pH is 7.4 for gradient pH and 8.5 for nongradient pH. The model drug has pK_a of 7.5.

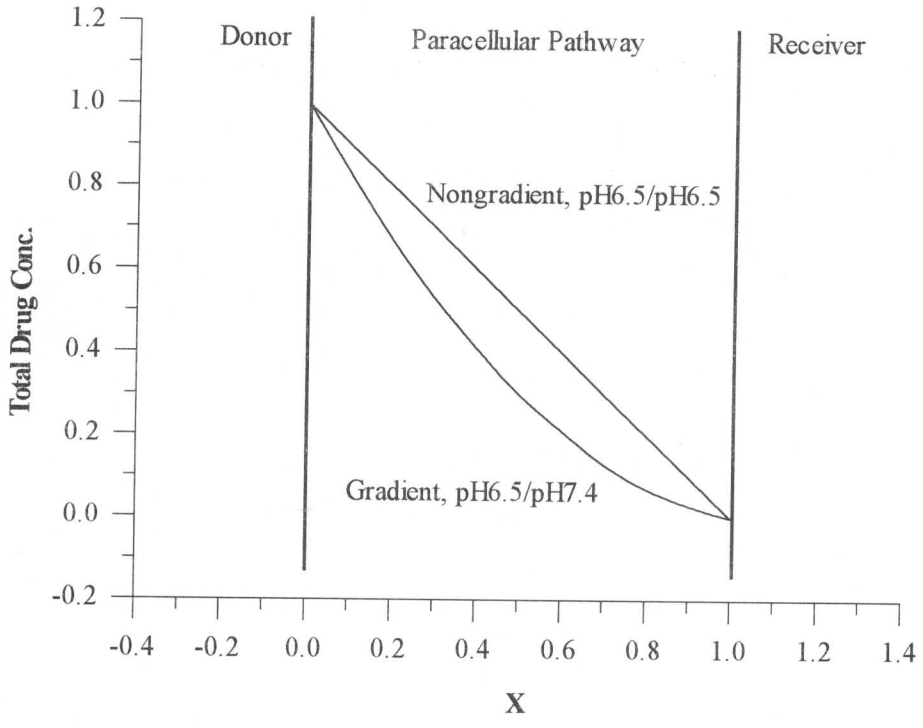


Fig. IV-3. Total drug concentration of gradient and nongradient pH inside the intercellular space. The receiver side pH is 7.4. The donor side pH is 6.5 for gradient pH and 7.4 for nongradient pH. The model drug has pK_a of 7.5.

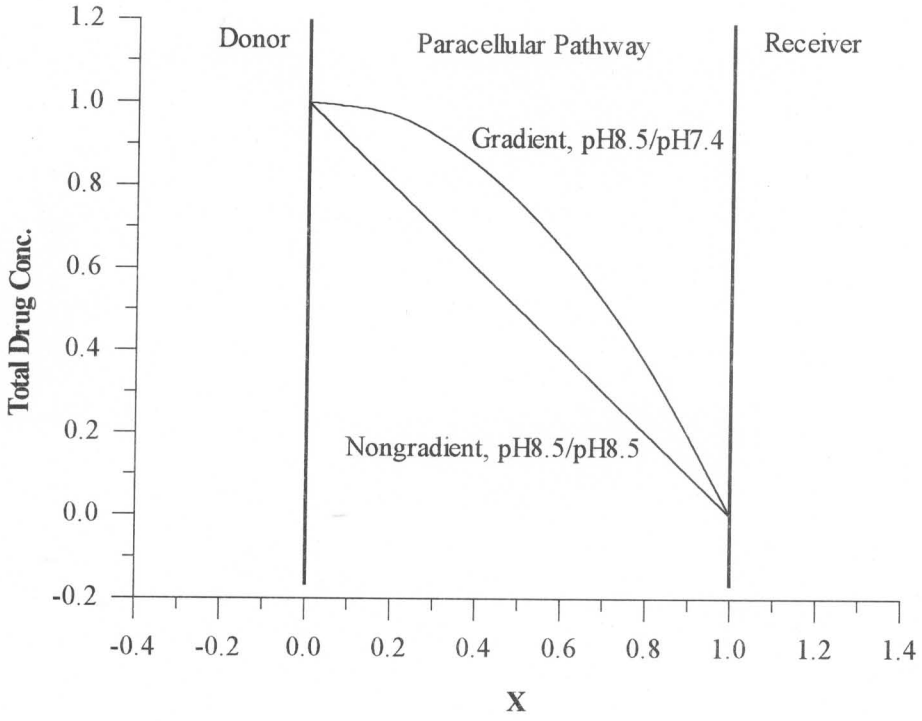


Fig. IV-4. Total drug concentration of gradient and nongradient pH inside the intercellular space. The receiver side pH is 7.4. The donor side pH is 8.5 for gradient pH and 7.4 for nongradient pH. The model drug has pK_a of 7.5.

$$C_x = \left(1 + \frac{[H^+]_R - [H^+]_D}{K_a + [H^+]_D} \cdot \frac{x}{h_H}\right) \cdot \left(1 - \frac{x}{h_H}\right) \cdot C_D \qquad \text{(IV-11)}$$

The total drug concentration gradient inside the paracellular pathway is:

$$\frac{dC_x}{dx} = \left(-\frac{x}{h_H^{\,2}} + \frac{[H^+]_R - [H^+]_D}{K_a - [H^+]_D} \cdot \frac{h_H - 2x}{h_H^{\,2}}\right) \cdot C_D \qquad \text{(IV-12)}$$

The flux of drug movement across the tissue is determined by how fast the drug can leave the tissue, which means that it is determined by:

$$J = -D_H \cdot \left(\frac{dC_x}{dx}\right)_{x \to h_H} \qquad \text{(IV-13)}$$

thus the flux is:

$$J = \frac{D_H}{h_H} \cdot \left(1 + \frac{[H^+]_R - [H^+]_D}{K_a + [H^+]_D}\right) \cdot C_D \qquad \text{(IV-14)}$$

Note the difference between Equation (IV-14) and Equation (IV-1) is the pH gradient parameter F:

$$F = \left(1 + \frac{[H^+]_R - [H^+]_D}{K_a + [H^+]_D}\right) \qquad \text{(IV-15)}$$

which is determined by pH's on both sides of the tissue and the pK_a of the drug. If both donor and receiver sides have the same pH, F becomes zero. If K_a is much larger than $[H^+]_D$ and $[H^+]_R$, F is not sensitive to pH on the donor and receiver sides, and the pH gradient has less effect.

The same procedure can be used to derive an equation for drugs that are unionized at pH lower that their pK_a and ionized at pH's higher than their pK_a. In this case, the effect of the pH gradient is opposite, that is the lower receiver side pH will decrease the permeation and higher receiver side pH will increase the permeation. The parameter of pH gradient effect in this case is:

$$F = \left(1 + \frac{[H^+]_R - [H^+]_D}{K_a + [H^+]_D}\right) \cdot \frac{[H^+]_D}{[H^+]_R} \qquad \text{(IV-16)}$$

B. Transcellular Pathway

The primary barrier of the transcellular pathway is the cell membrane. To move across a cell layer, drug molecules have to cross two cell membranes, one on the donor side and the other on the receiver side (Fig. IV-5). If the partition coefficients, membrane/donor ($K_{M/D}$), membrane/cytoplasm ($K_{M/C}$), and membrane/receiver ($K_{M/R}$), are different, the flux of drug transport is:

$$J_1 = \frac{D_M}{h_M}\left(C_D \cdot K_{M/D} - C_{Cyto} \cdot K_{M/C}\right) \qquad \text{(IV-17)}$$

$$J_2 = \frac{D_M}{h_M}\left(C_{Cyto} \cdot K_{M/C} - C_R \cdot K_{M/R}\right) \qquad \text{(IV-18)}$$

Since at steady state, the overall flux equals to the flux of each step, that is:

$$J = J_1 = J_2 \qquad \text{(IV-19)}$$

Substituting Equation (IV-17) and (IV-18) into Equation (IV-19), the term $C_{Cyto} \cdot K_{M/C}$ can be solved:

$$C_{Cyto} \cdot K_{M/C} = \frac{1}{2}\left(C_D \cdot K_{M/D} - C_R \cdot K_{M/R}\right) \qquad \text{(IV-20)}$$

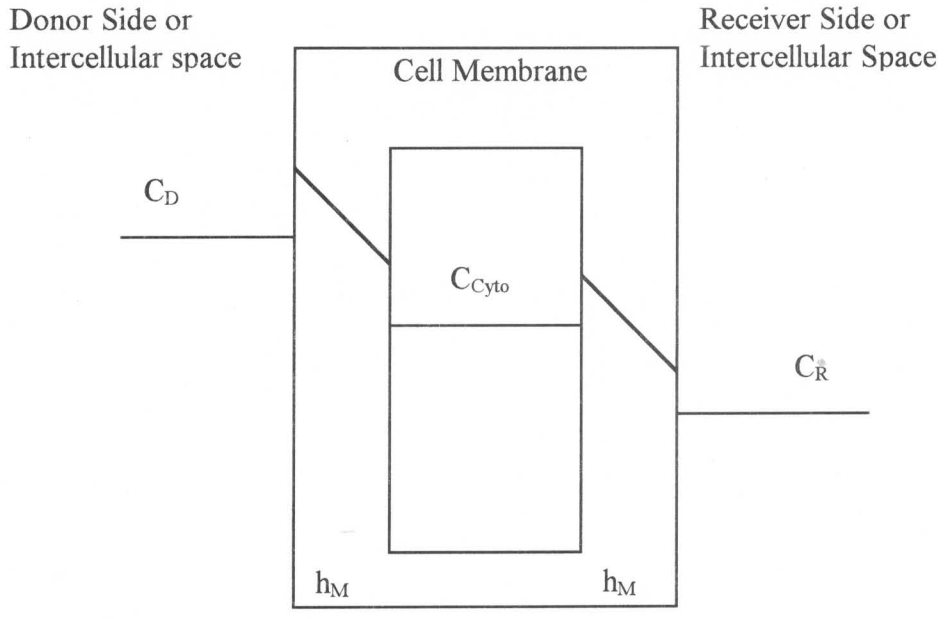


Fig. IV-5. Drug transport across a epithelial cell layer (two cell membrane) at steady state. The diffusion inside the cytoplasm is faster than inside the cell membrane, and is assumed instantaneously.

and the overall drug flux is:

$$J = \frac{D_M}{2 \cdot h_M}\left(C_D \cdot K_{M/D} - C_R \cdot K_{M/R}\right) \qquad \text{(IV-21)}$$

Equation (IV-21) shows that the flux of drug transport across a cell at steady state is a function of drug concentration on the donor and receiver sides and the membrane/buffer partition coefficients on the donor and receiver sides. The drug flux is independent of the cytoplasm.

If the aqueous solution on the donor, receiver, and intercellular space are identical in pH, the membrane/aqueous partition coefficients are the same as K_P, and the total flux can be written as:

$$J = \frac{D_M \cdot K_P}{2 \cdot n \cdot h_M}\left(C_D - C_R\right) \qquad \text{(IV-22)}$$

where n is the number of cell layers the drug molecules has to cross in order to move across the entire oral mucosa. Equation (IV-22) can be transformed into Equation (IV-23) assuming the receiver side drug concentration is zero, the diffusion coefficient inside the cell membrane is D_L and the sum of the cell membrane thickness is h_L.

$$J_L = \frac{D_L \cdot K_P}{h_L} C_D \qquad \text{(IV-23)}$$

The gradient pH will not affect the transcellular pathway because in Equation (IV-23), flux is a function of donor side partition coefficient. The drug flux has nothing to do with the receiver side partition coefficient, and by inference the intercellular space. However, recall, from Fig. II-3, II-4 and IV-5, that the transcellular pathway involves both the cell membrane and the intercellular space. Drug molecules in the transcellular pathway have to cross the lipophilic barrier (cell membrane), as well as the hydrophilic barrier (intercellular space). If there is a pH gradient existing in the intercellular space, the diffusion process becomes complicated and is beyond our ability to describe this process mathematically. Qualitatively, however, the effect of pH gradient on the transcellular pathway is similar to the effect on the paracellular pathway, that is for ionizable compounds which are ionized at a pH lower than their pK_a, lower receiver side pH will increase the permeability across the oral mucosa, and *vice versa*.

III. Materials and Methods

A. Material

Materials, solution preparation, tissue collection, and sample assay procedures are the same as described in Chapter 2.

B. Gradient pH Diffusion Study

The procedures for gradient pH diffusion studies were similar to the nongradient pH diffusion studies described in Chapter 2 except that the pH of the receiver chamber is fixed at 7.4 to mimic the biological pH in the blood circulation. Appropriate buffer solution was placed in the diffusion cells 20 minutes prior to the experiment to stabilize the pH gradient across the oral mucosa. Morphine diffusion studies were performed using plastic side-by-side diffusion cells purchased from Precision Instrument Design (Los Altos, CA), and fentanyl diffusion studies were performed using glass diffusion cells.

IV. Results and Discussion

A. Morphine Gradient pH Diffusion Study

Effect of receiver side pH on morphine transport across rabbit buccal and sublingual tissues were performed *in vitro* (Table IV-1, Table IV-2, Fig. IV-6, and Fig. IV-7).

Statistical analysis (99% confidence level) revealed that the difference between gradient pH, pH6.5/pH7.4 and pH8.5/pH7.4 (donor/receiver), was significant. There was no significant difference between nongradient pH studies, pH6.5/pH6.5 and pH8.5/pH8.5. It is obvious that the permeation of morphine increases with a lower receiver side pH and decreases with a higher receiver side pH.

As discussed earlier in Chapters 2 and 3, morphine molecules were transported through the oral mucosa via the paracellular route, and the change of pH or partition coefficient would not affect the permeability. However, the gradient pH study shows that the donor side pH can significantly change the permeability coefficient of drugs transported paracellularly if the receiver side pH is fixed at 7.4. This result may explain why many *in vivo* studies show a pH dependence (Al-Sayed-Omar, 1987; Barsuhn, 1988; Beckett, 1967; Hicks, 1973; Randhawa, 1986; Yamahara, 1990) while *in vitro* studies did not (Dowty, 1992 #57, Zhang, 1989). It is important to understand that a pH gradient always exists in an *in vivo* experiment. Therefore, the effect of donor side pH is always a mixture of the pH effect on both the transcellular and paracellular pathways. It is easy to mistake the pH effect on the paracellular pathway for the pH effect on transcellular pathway and conclude that the drug is transported via the transcellular pathway.

Table IV-1. Morphine permeability in rabbit buccal mucosa in gradient and nongradient pH. The receiver side pH is fixed at 7.4 for gradient pH. Error represent the standard deviation of 4 to 6 replications.

	Nongradient pH			Gradient pH	
	6.5	**7.4**	**8.5**	**6.5**	**8.5**
P (x 10^7 cm/sec)*	2.70 $^{a\,b\,c}$	2.58 $^{a\,b\,c}$	3.4 $^{a\,b\,c}$	2.09 ^{b}	4.20 ^{c}
S.D.	0.38	0.57	1.5	0.69	0.81

*: No statistical difference within groups. Significant difference between b and c (99% confidence level).

Table IV-2. Morphine permeability in rabbit sublingual mucosa in gradient and nongradient pH. The receiver side pH is fixed at 7.4 for gradient pH. Error represent the standard deviation of 4 to 6 replications.

	Nongradient pH			Gradient pH	
	6.5	**7.4**	**8.5**	**6.5**	**8.5**
P (x 10^7 cm/sec)	3.3 $^{a\,b\,c}$	4.0 $^{a\,b\,c}$	4.8 $^{a\,b\,c}$	1.91 ^{b}	4.9 ^{c}
S.D.	1.1	1.6	1.7	0.74	1.2

*: No statistical difference within groups. Significant difference between b and c (99% confidence level).

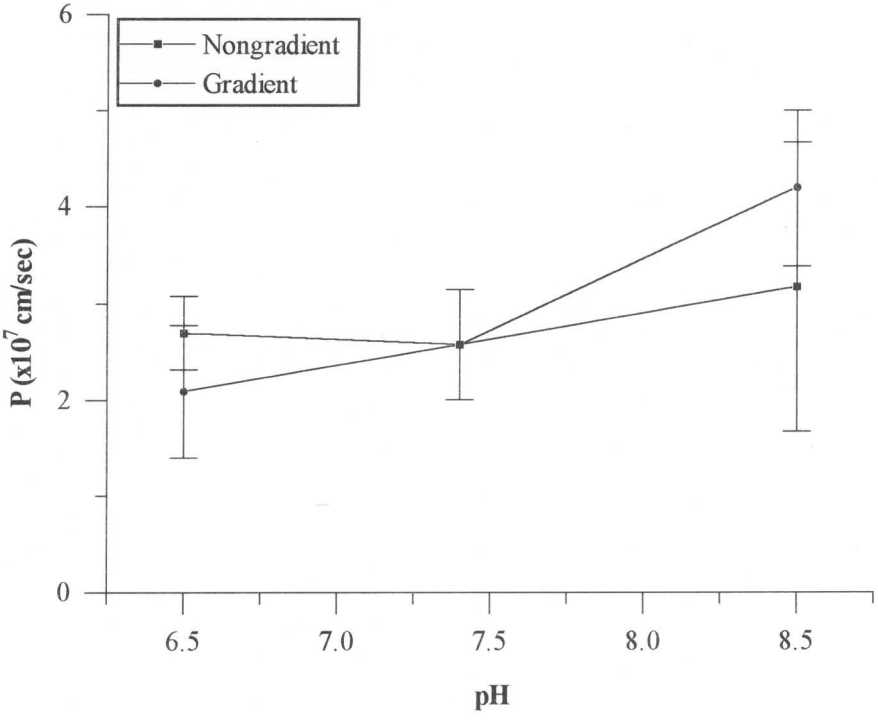


Fig. IV-6. Permeability coefficient of morphine in gradient and nongradient pH in rabbit buccal mucosa. The receiver side pH is 7.4 for gradient pH. The error bars represent standard deviation of 4 to 6 replications.

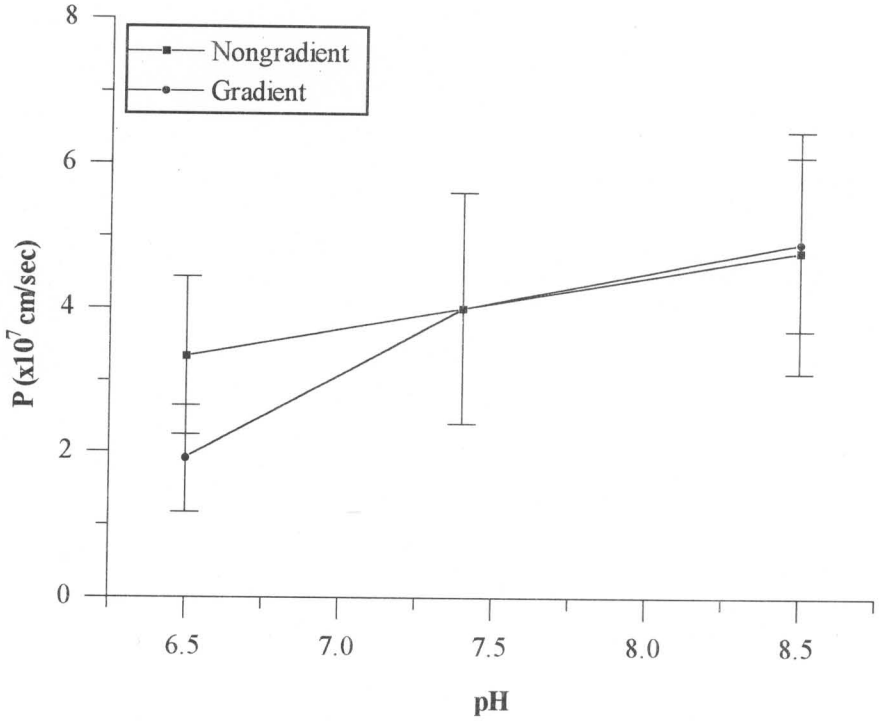


Fig. IV-7. Permeability coefficient of morphine in gradient and nongradient pH in rabbit sublingual mucosa. The receiver side pH is 7.4 for the gradient pH. The error bars represent standard deviation of 4 to 6 replications.

The real pH gradient effect on morphine permeability is smaller than the predicted pH gradient effect for both the buccal and sublingual tissues (Fig. IV-8). There are several possible reasons. The prediction is made assuming that there is only one ionized group in morphine and the pK_a is 7.93. As mentioned earlier, morphine molecules possess a second ionizable group, the phenol group, which has a pK_a of 9.63 (Schill, 1964; Kaufman, 1975). Ionization of the phenol group will affect the overall morphine concentration inside the oral mucosa. The effect of the second ionizable group is not included in the mathematical model due to complexity of the model. The pK_a of 7.93 is measured in different aqueous phases, thus, the pK_a of morphine in this study may differ from 7.93, in accord with the predicted pH gradient effect. In addition, the mathematical model is based on the assumption that hydrogen ion has a linear gradient inside the oral tissue. This assumption may not be true because the concentration of hydrogen ion is very low compared to the morphine concentration, which is about 3.5×10^{-4} M. The movement of morphine will also affect the hydrogen ion concentration inside the tissue. Therefore, the actual concentration gradients inside the oral mucosa of morphine and hydrogen ion are nonlinear. In general, this pH gradient model is only a qualitative assessment to show that the pH gradient across the oral mucosa will affect the permeability of ionizable compounds.

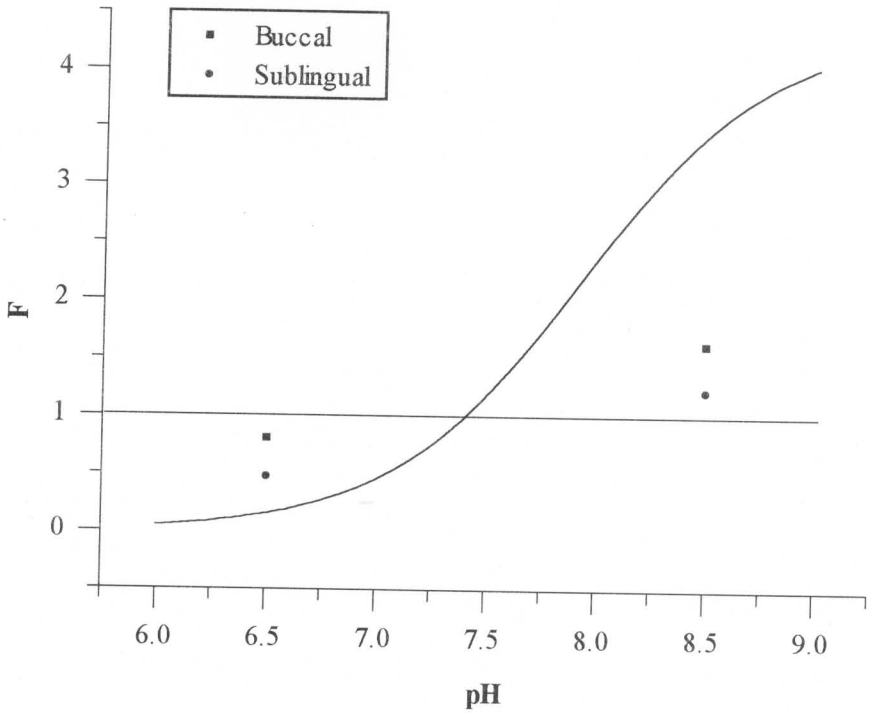


Fig. IV-8. Effect of pH gradient on morphine permeability across rabbit oral mucosa. The solid line is the theoretical prediction using Equation (IV-15) with morphine pK_a of 7.93 (Schill, 1964; Kaufman, 1975). Experimental F values were the ratio of gradient pH study over nongradient pH study at pH 7.4.

Morphine diffusion studies had a relatively high standard deviation because permeation was low and was close to the limit of assay sensitivity. Tissue variation could also have a large influence if the permeability coefficient is small.

B. Fentanyl pH Gradient Diffusion Study

Fentanyl diffusion studies (Table IV-3, Table IV-4, Fig. IV-9, and Fig. IV-10) showed a similar effect of receiver side pH on the permeability of fentanyl across the rabbit oral mucosa. Fentanyl, a lipophilic drug, will penetrate buccal and sublingual tissue via the transcellular and paracellular pathways simultaneously (see Chapter 2 and 3). Although the effect of donor side pH dominates over the effect of receiver side pH, the receiver side pH had a significant impact (99% confidence level) on fentanyl permeation across the rabbit oral mucosa.

As discussed earlier in the **Theory** section, the pH gradient effect on the transcellular pathway is more complicated than that on the paracellular pathway. The pH gradient across the oral mucosa will increase more than just the flux in the paracellular pathway. To prove this point, we can assume that the diffusion of fentanyl at pH 5.5 is entirely through the paracellular pathway. Thus, the calculated pH gradient effect on the permeability coefficient can be obtained by the change of the pH gradient parameter times the permeability coefficient at pH 5.5 [$(1\text{-}F)xP_{5.5}$] (Table 5). Comparing the calculated and the experimental permeability coefficient change due to

Table IV-3. Fentanyl permeability in rabbit buccal mucosa in gradient and nongradient pH. The receiver side pH is fixed at 7.4 for gradient pH. Error represent the standard deviation of 4 to 6 replications.

	Nongradient pH				Gradient pH		
	5.5	6.5	7.4	8.5	5.5	6.5	8.5
P *	2.12×10^{-6}	8.87×10^{-6}	2.14×10^{-5}	3.28×10^{-5}	4.53×10^{-7}	3.78×10^{-6}	6.32×10^{-5}
S.D.	4.5×10^{-7}	9.1×10^{-7}	1.1×10^{-6}	2.5×10^{-6}	1.1×10^{-7}	5.6×10^{-7}	8.9×10^{-6}

*: Statistical difference between any groups (99% confidence level).

Table IV-4. Fentanyl permeability in rabbit sublingual mucosa in gradient and nongradient pH. The receiver side pH is fixed at 7.4 for gradient pH. Error represent the standard deviation of 4 to 6 replications.

	Nongradient pH				Gradient pH		
	5.5	6.5	7.4	8.5	5.5	6.5	8.5
P *	4.63×10^{-6}	2.07×10^{-5}	4.31×10^{-5}	6.50×10^{-5}	9.69×10^{-7}	7.37×10^{-6}	1.10×10^{-4}
S.D.	1.8×10^{-6}	3.2×10^{-6}	7.4×10^{-6}	5.2×10^{-6}	2.7×10^{-7}	9.4×10^{-7}	1.7×10^{-5}

*: Statistical difference between any groups (99% confidence level).

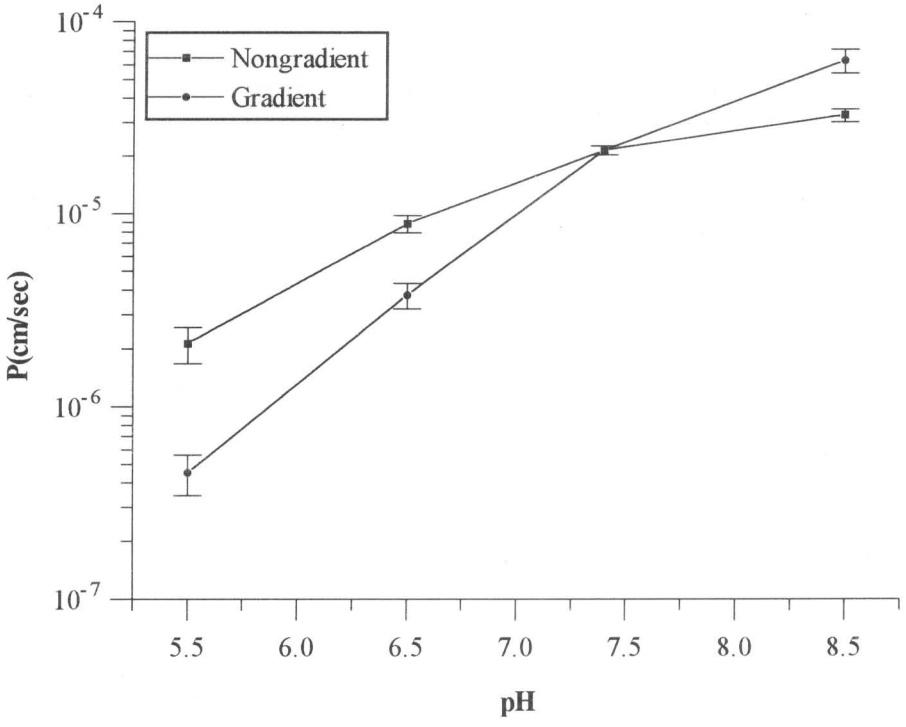


Fig. IV-9. Fentanyl permeability coefficient across rabbit buccal mucosa in gradient and nongradient pH. The receiver side pH is fixed at 7.4 for gradient pH. Error bars represent the standard deviation of 4 to 6 replications.

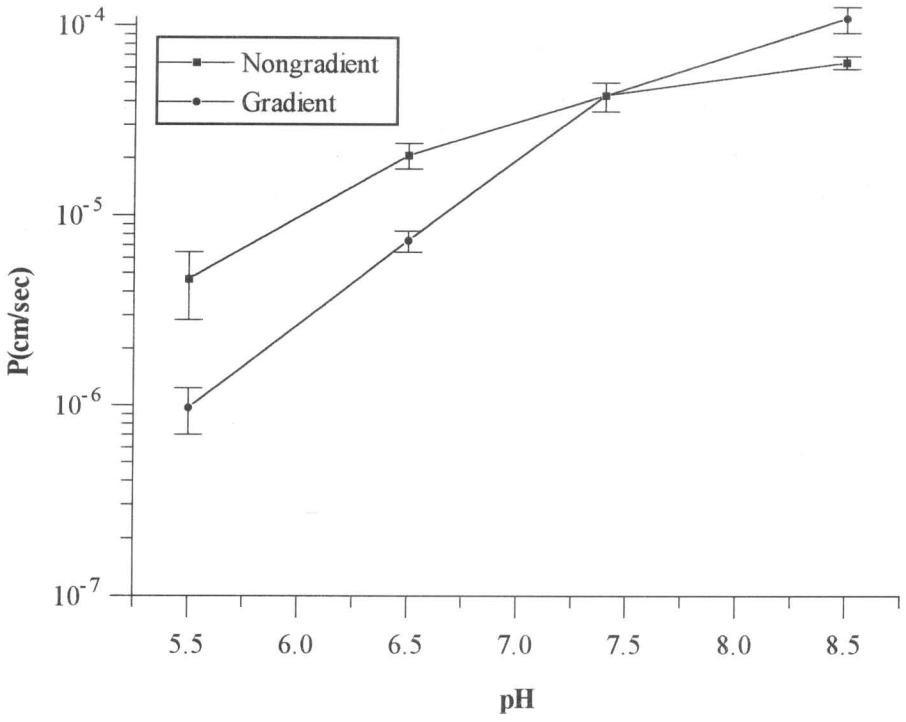


Fig. IV-10. Fentanyl permeability coefficient across rabbit sublingual mucosa in gradient and nongradient pH. The receiver side pH is fixed at 7.4 for gradient pH. Error bars represent the standard deviation of 4 to 6 replications.

Table IV-5. Comparison of the experimental and calculated pH gradient effect on fentanyl permeability across the rabbit oral mucosa. The calculated permeability change is obtained by (1-F) times the permeability coefficient at pH 5.5 [(1-F)x$P_{5.5}$].

		pH 5.5	pH 6.5	pH 8.5
	F	0.02131	0.1974	2.169
Buccal	ΔP_{Exp}	-1.667×10^{-6}	-5.09×10^{-6}	3.04×10^{-5}
	ΔP_{Cal}	-2.075×10^{-6}	-1.702×10^{-6}	2.478×10^{-6}
Sublingual	ΔP_{Exp}	-3.661×10^{-6}	-1.333×10^{-5}	4.500×10^{-5}
	ΔP_{Cal}	-4.531×10^{-6}	-3.716×10^{-6}	5.412×10^{-6}

the pH gradient (Fig. IV-11, Fig. IV-12), it is clear that if drug molecules diffuse entirely through the paracellular pathway, the model reasonably predicts the pH gradient effect. However, if drug molecules move across the oral mucosa through both the transcellular and paracellular pathway, the model cannot predict the pH gradient effect. The more the transcellular pathway is involved, the worse the prediction is.

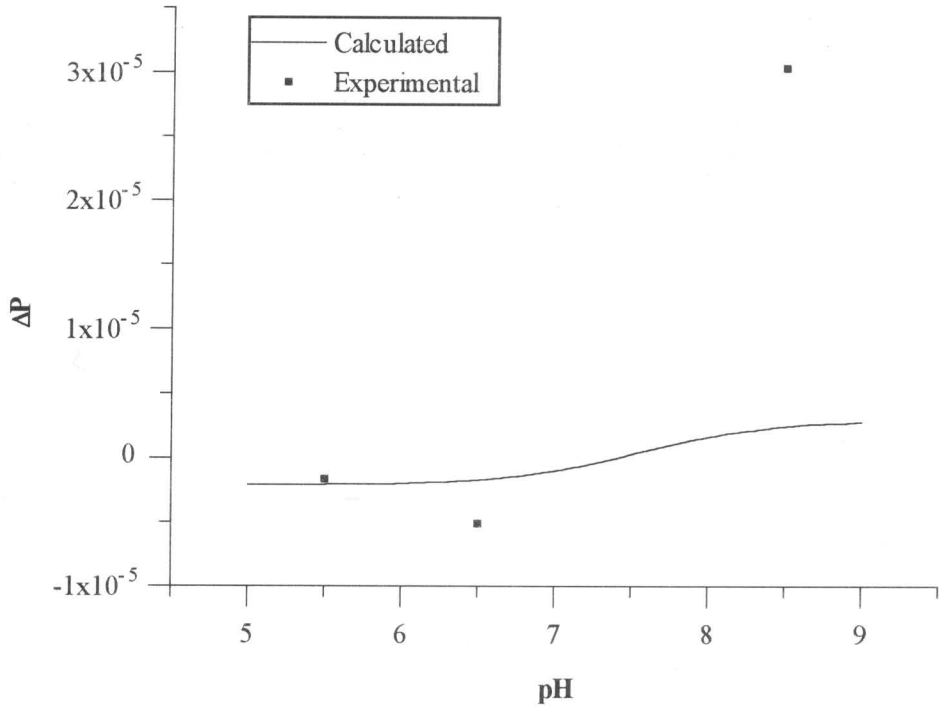


Fig. IV-11. Model prediction and experimental fentanyl permeability change due to the pH gradient across rabbit buccal mucosa. Calculated pH gradient effect is based on the assumption that the permeability of fentanyl at pH 5.5 is entirely through the paracellular pathway and the pH gradient can only affect permeability in the paracellular pathway.

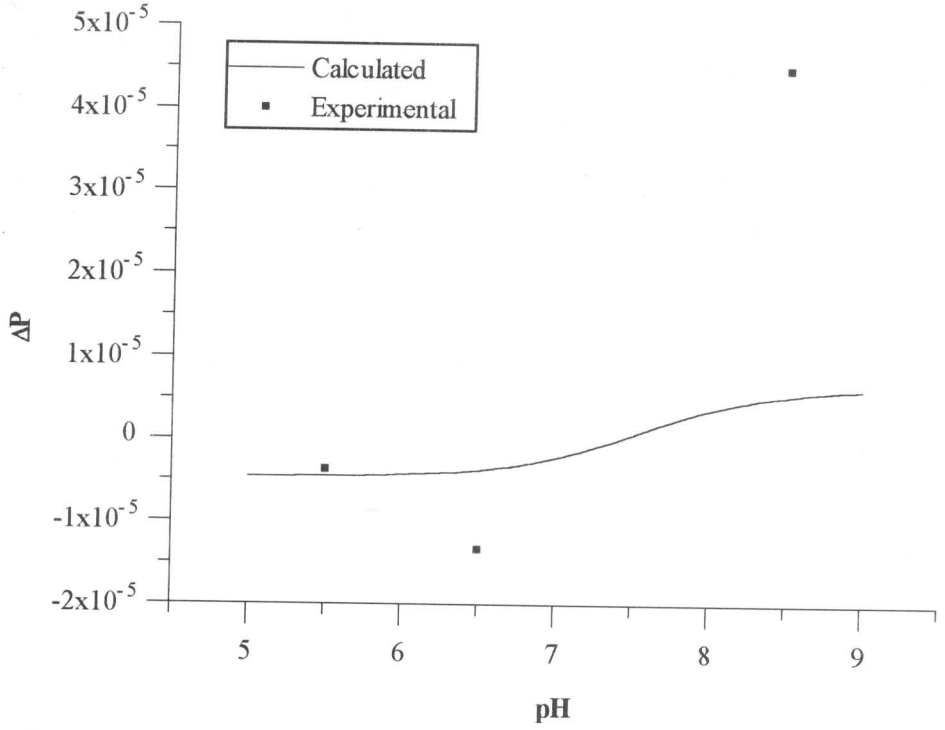


Fig. IV-12. Model prediction and experimental fentanyl permeability change due to the pH gradient across rabbit sublingual mucosa. Calculated pH gradient effect is based on the assumption that the permeability of fentanyl at pH 5.5 is entirely through the paracellular pathway and the pH gradient can only affect permeability in the paracellular pathway.

The pH gradient effect on the transcellular pathway is too complicated to be described by a simple mathematical model. Nevertheless, it appears that the effect is on the hydrogen ion gradient in the intercellular space. Qualitatively, the mechanism of the effect is similar to that of the paracellular pathway.

V. Conclusion

Both morphine and fentanyl studies showed that the receiver side pH will affect the overall permeability of ionizable compounds across the oral mucosa. It is important to recognize this issue when interpreting the results of *in vivo* or *in vitro* pH dependent studies. *In vivo* studies will always have a pH gradient if the donor side is not at pH 7.4. The pH gradient effect may overwhelm the effect of lipophilicity if the compound is small, water soluble and hydrophilic in nature. With *in vitro* studies, on the other hand, it is common to use the same pH buffer solution on both sides of the tissue and hence eliminate the pH gradient issue. Doing both gradient and nongradient studies *in vitro* is the only way to demonstrate the effect of pH gradient.

The effect of the pH gradient across the oral mucosa appears based on the intercellular hydrogen ion gradient, which changes the drug concentration gradient inside the paracellular pathway. The transcellular pathway is also affected due to the fact that

diffusion of drugs through the transcellular pathway has to cross the intercellular space, and, thus, are affected by the pH gradient.

The mathematical model developed here is primitive and is difficult to predict the magnitude of the pH gradient effect using this model directly because the real diffusion process is considerably more complicated than the model suggests. Nevertheless, this model provides a starting point in understanding the pH gradient effect.

CONCLUDING REMARKS

The main obstacle in using the oral mucosa as a systemic drug delivery site is the relatively low permeability of the oral mucosa. To increase, as well as control, oral mucosal permeability, a basic understanding of drug transport mechanism(s) is essential. The drug transport pathway, as a key step of understanding the drug absorption process, has been the focus for many studies. However, to successfully address this issue requires an appropriate theoretical model (hypothesis) and a carefully designed experiment.

An appropriate hypothesis must be supported by the basic understanding of the cellular structure of the oral mucosa. This dissertation developed a two-pathway model primarily based on these postulates: 1) the interior of the cell membrane consists of hydrophobic fatty acid chains and is hydrophobic, 2) the intercellular space is hydrophilic in general although there are lipophilic compounds or regions, 3) there are no tight intercellular junctions in the oral epithelial, thus, the intercellular space is large enough for diffusion of small water-soluble compounds. This two-pathway model, i.e., the hydrophilic paracellular pathway conjugates with a lipophilic transcellular pathway, addresses the relationship between the oral mucosal permeability and the physicochemical properties of the oral epithelial, the diffusant and the bathing solution.

Theoretically, to understand this model may not be difficult. Experimentally, however, to show the correlation between the permeability coefficient and the partition coefficient is a difficult task because a change of physicochemical property can change several model parameters simultaneously, e.g., change of molecular size may change the diffusion coefficient, the area devoted for paracellular pathway, and the paracellular pathlength. To overcome this problem, ionizable compounds were employed because 1) the partition coefficient of the compounds can be controlled by the bathing solution pH, 2) the size, shape and other physicochemical properties are identical for ionized and unionized molecules, and, thus, the other model parameters are identical. Using morphine and fentanyl as probes, the drug transport pathways were explored and the two-pathway model was tested. Both diffusion and accumulation studies revealed that the hypothesis was correct, and that there are two general transport pathways in the oral mucosa.

This dissertation also addressed an important issue, i.e., the pH gradient across the oral mucosa. If the pH of donor side is not a biological pH of 7.4, a pH gradient across the oral mucosa exists. From theoretical point of view, how and why the pH gradient can affect the permeability of ionizable compounds was the focus of this study. Although the mathematical model developed here is primitive, this model creates the need for further investigation of this issue.

The main contribution of this dissertation is building a bridge to connect oral mucosal permeability and the physicochemical properties of the diffusant and the delivery vehicle.

APPENDIX A: NON-STEADY STATE DIFFUSION THEORY

Equations for non-steady state diffusion of a substance across a membrane can be derived from Fick's second law in one dimension assuming that the concentration at the donor side surface is constant, initial drug concentration inside the membrane is zero, and the concentration of the substance on the receiver side is zero.

$$C = C_D, \qquad x = 0, \qquad t \geq 0$$

$$C = 0, \qquad x = h, \qquad t \geq 0$$

$$C = 0, \qquad 0 < x < h, \qquad t = 0$$

If the diffusion coefficient (D) is constant throughout the media, the concentration inside the membrane can be obtained by solving Fick's second law in one dimension (Crank, 1975) as:

$$C_x = C_D - C_D \cdot \frac{x}{h} + \frac{2}{\pi} \sum_{n=1}^{\infty} -\frac{C_D}{n} \cdot \mathrm{Sin}\left(\frac{n\pi x}{h}\right) \cdot e^{-\frac{D \cdot n^2 \cdot \pi^2 \cdot t}{h^2}} \qquad \text{(A-1)}$$

Then the concentration gradient inside the membrane is:

$$\frac{dC_x}{dx} = -\frac{C_D}{h} - \frac{2 \cdot C_D}{h} \sum_{n=1}^{\infty} Cos\left(\frac{n\pi x}{h}\right) \cdot e^{-\frac{D \cdot n^2 \cdot \pi^2 \cdot t}{h^2}} \qquad \text{(A-2)}$$

The rate of diffusant movement across the membrane at time t, i.e., the rate at which the diffusant emerges from unit area of the membrane surface at x = h, is given by:

$$J' = -D \cdot \left(\frac{dC_x}{dx}\right)_{x=h} \qquad \text{(A-3)}$$

or

$$J' = \frac{D \cdot C_D}{h} + \frac{2 \cdot C_D}{h} \sum_{n=1}^{\infty} (-1)^n \cdot e^{-\frac{D \cdot n^2 \cdot \pi^2 \cdot t}{h^2}} \qquad \text{(A-4)}$$

Integrating Equation (A-4) with respect to t, the amount of diffusant passing through the unit area of the membrane at time t is:

$$Q = \int_0^t \left(\frac{D \cdot C_D}{h} + \frac{2 \cdot C_D}{h} \sum_{n=1}^{\infty} (-1)^n \cdot e^{-\frac{D \cdot n^2 \cdot \pi^2 \cdot t}{h^2}} \right) \cdot dt \qquad \text{(A-5)}$$

and

$$Q = \frac{D \cdot C_D \cdot t}{h} - \frac{C_D \cdot h}{6} - \frac{2 \cdot C_D \cdot h}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot e^{-\frac{D \cdot n^2 \cdot \pi^2 \cdot t}{h^2}} \qquad \text{(A-6)}$$

Then, the overall flux, i.e., the amount of diffusant across the membrane at the unit area and the unit time $\left(\frac{Q}{t}\right)$, equals:

$$J = \left(\frac{D}{h} - \frac{h}{6 \cdot t} - \frac{2 \cdot h}{\pi^2 \cdot t} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot e^{-\frac{D \cdot n^2 \cdot \pi^2 \cdot t}{h^2}} \right) \cdot C_D \qquad \text{(A-7)}$$

At time approaches infinity, the series term in Equation (A-4) equals zero, the flux is:

$$J_{S.S.} = \frac{D}{h} \cdot \left(1 - \frac{h^2}{6 \cdot D \cdot t} \right) \cdot C_D \qquad \text{(A-8)}$$

Notice the difference between the flux at steady state assuming steady state as an initial condition (Equation (II-1)) and the flux at steady state derived from a non-steady state (Equation (A-8)) is the intercept on the time axis, which is called the lag time:

$$L = \frac{h^2}{6 \cdot D} \qquad \text{(A-9)}$$

Equation (A-9) shows that the lag time of a membrane is a function of the thickness of the diffusion media and the diffusion coefficient of the diffusant inside the diffusion media.

If there is preferential partitioning between solution and membrane, a partition coefficient (K_P) can be introduced into Equation (A-9):

$$J_{S.S.} = \frac{D \cdot K_P}{h} \cdot \left(1 - \frac{h^2}{6 \cdot D \cdot t}\right) \cdot C_D \qquad \text{(A10)}$$

This one dimensional diffusion across a membrane model is the most commonly used model in the drug delivery field. Although many modifications have been made, e.g., adding stagnant layers, separate epithelial and connective tissue, etc., the fundamentals remain the same.

APPENDIX B: IN VITRO MEASUREMENT OF DRUG PERMEATION ACROSS A BIOLOGICAL MEMBRANE

There are many mathematical models to describe the process of drug transport across the oral mucosa. For most drugs, however, drug absorption via the oral mucosa is a passive diffusion process and can be described by Fick's first law:

$$J = \frac{D \cdot K_P}{h} \cdot C_D \qquad \text{(B-1)}$$

J is the flux of drug defined as the amount of drug moving across the oral mucosa per unit time and surface area, D is the diffusion coefficient of the drug inside the oral mucosa, K_P is the partition coefficient between the buffer solution and the oral mucosa, h is the pathlength of drug diffusion across the oral mucosa, and C_D is drug concentration in the donor chamber.

It is impractical, if not impossible, to measure the diffusion coefficient, partition coefficient and pathlength separately. However, the permeability coefficient, which is defined as:

$$P = \frac{D \cdot K_P}{h} \tag{B-2}$$

is easily obtained. In a typical diffusion experiment, the amount of drug (A) moving across the tissue at time t is determined by measuring drug concentration on the receiver side. The surface area of the tissue (S) (the opening of the diffusion cell) and the initial drug concentration in the donor chamber are known and remain constant during the course of the experiment. Thus, the flux is:

$$J = P \cdot C_D = \frac{A}{S \cdot t} \tag{B-3}$$

and the amount of drug transported at time t is:

$$A = P \cdot S \cdot C_D \cdot t \tag{B-4}$$

Since the total amount of drug (A_T) on the donor side is:

$$A_T = V_D \cdot C_D \tag{B-5}$$

where V_D is the volume of the donor chamber, the percent of drug transported (T%) at time t is:

$$T\% = \frac{A}{A_T} = \frac{P \cdot S}{V_D} \cdot t \qquad \text{(B-6)}$$

A plot of T% versus t will give a slope of $\frac{P \cdot S}{V_D}$, and from this slope the permeability coefficient can be obtained.

Equation (B-6) shows how to determine the permeability coefficient at steady state. Practically, however, almost all diffusion studies do not begin at steady state, and, as a matter of fact, steady state is theoretically never reached because the donor and receiver drug concentrations are continuously changing. If the concentration changes are small, we can assume that the donor drug concentration is constant and the receiver drug concentration is zero. Under this assumption, the diffusion process can be described, in one dimension, by Fick's second law, as described in Appendix A. The drug flux across the oral mucosa at steady state is:

$$J_{S.S.} = \frac{D \cdot K_P}{h} \cdot \left(1 - \frac{h^2}{6 \cdot D \cdot t}\right) \cdot C_D \qquad \text{(B-7)}$$

and the amount of drug across the oral mucosa at time t is:

$$A = \frac{S \cdot D \cdot K_P \cdot C_D}{h} \cdot \left[t - \frac{h^2}{6 \cdot D} \right] \qquad \text{(B-8)}$$

Then, the percent of drug transported at time t is:

$$T\% = \frac{A}{A_T} = \frac{P \cdot S}{V_D} \cdot \left[t - \frac{h^2}{6 \cdot D} \right] \qquad \text{(B-9)}$$

Equation (B-9) shows that a plot of percent transported versus time has a slope of $\frac{P \cdot S}{V_D}$ and an x-axis intercept of $\frac{h^2}{6 \cdot D}$. Table B-1. shows data for a typical fentanyl diffusion study, in which six data points were taken during the experiment. It is important to keep in mind that the amount of drug crossing the oral mucosa is cumulative, which means that the amount taken out as a sample must be added back to calculate the amount transported. The T% versus time plot of this data set is shown in Fig. B-1. The linear portion of the curve is used to calculate the permeability coefficient and lag time.

Table B-1. A typical fentanyl diffusion study. HPLC peak heights were used to determine fentanyl concentration. The peak height to concentration conversion factor is 1.27×10^{-5} mg/ml/mm. Donor side fentanyl concentration is 0.1051 mg/ml. Area is 0.503 cm^2. Volume of donor and receiver chambers are 2.0 ml. Sample size is 1.0 ml.

Time (min)	Peak Height (mm)	Concentration (mg/ml)	Sample (mg)	Total Flux (mg)	T%
15	7.40	9.39×10^{-5}	9.39×10^{-5}	1.88×10^{-4}	0.0894
45	87.90	1.12×10^{-3}	1.21×10^{-3}	2.32×10^{-3}	1.1061
75	165.00	2.09×10^{-3}	3.30×10^{-3}	5.40×10^{-3}	2.5678
105	209.00	2.65×10^{-3}	5.96×10^{-3}	8.61×10^{-3}	4.0953
135	232.00	2.94×10^{-3}	8.90×10^{-3}	1.18×10^{-2}	5.6349
165	237.00	3.01×10^{-3}	1.19×10^{-2}	1.49×10^{-2}	7.0960

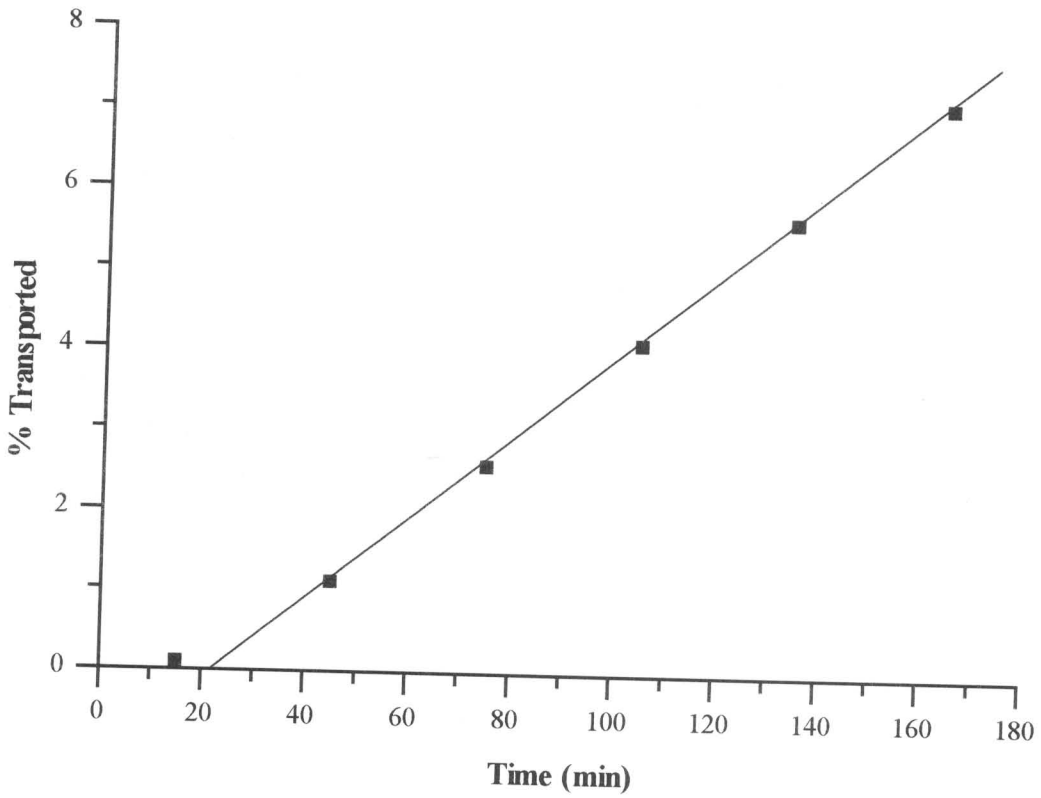


Fig. B-1. A typical %Transported versus time plot of a fentanyl diffusion experiment. The linear portion of the curve is fitted by linear regression to obtain the slope and x-axis intercept. These are then used to calculate the permeability coefficient and lag time.

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