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Transport in the Mammary Glands

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by

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This dissertation is dedicated to my family.

ABSTRACT OF THE DISSERTATION

Transport in the Mammary Glands

by

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Doctor in Philosophy, Graduate Program in Bioengineering
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The transport of toxins moving from the blood stream into the ducts of the mammary glands is analyzed in this work. The model predictions are compared with experimental data from the literature. The utility of the model lies in its potential to improve our understanding of toxin transport as a pre-disposing factor to breast cancer. The model presented in Chapter I is based on a multi-layer transport model to analyze the toxins present in the breast milk. The breast milk in comparison with other sampling strategies allows us to understand the mass transport of toxins once inside the bloodstream of breastfeeding women. The multi-layer model presented describes the transport of caffeine, DDT and Cimetidine. The analysis done takes into account the unique transport mechanisms for each of the toxins. The model presented in Chapter II is based on a multi-layer transport model to analyze the concentration of toxins present in the breast ducts. The multi-layer model presented describes the transport of caffeine, cimetidine, aspirin and nicotine during the resting mammary gland period. Additionally, the dermal transport of drugs such as nicotine and aspirin into the resting mammary gland is analyzed. In a unique approach we also present the impact of introducing an external heat flux at the boundaries to increase the diffusion of these particles into the breast ducts. Our model predicts the movement of toxins and/or drugs within the resting mammary glands.

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1. CHAPTER I. INTRODUCTION

In comparison with other organs in the human body, the mammary glands are continuously evolving after birth. The most important role the mammary glands need to fulfill is the transport of milk through the ducts after giving birth. The process of breast feeding mostly depends on the milk ducts which are composed of the ductal lumen as well as myoepithelial and luminal cells. Unfortunately, the changes in diet and the environmental factors are affecting the reliability of the milk transported through the milk ducts. Considering that in Mexico 9 out of 10 women breast feed for more than the first semester after giving birth it is important to understand the genotoxicity as well as the protection it provides to women to prevent breast cancer. The relationship between breast cancer and breast milk is important, because breast milk or fluid is easier to sample to detect abnormalities in the epithelial cells of the mammary glands. The sampling is non-invasive and gives reliable information of the DNA damage present in the sample. The toxins found in the breast milk were transported from the blood supply or from the adipose tissue, which provides a clue of the toxins stored in our body. During the non-lactating period this toxins accumulate in the adipose tissue and in the mammary glands increasing the probability of abnormal cell growth. Basically, during the lactation period the toxins stored in the mammary glands are mostly removed.

Breast cancer research has established a correlation among life style, obesity and environmental factors with the increasing number of cancer patients in industrialized countries. Even though, cancer has a strong correlation with genetic factors, it has been found that there is

a strong relationship between the country of origin and women habits [1]. Solely in the United States of America it is expected that 1 out of 8 women will be diagnose with breast cancer [2].

The aim of this research is to develop and analyze a sophisticated multi-layer mass transfer model of the mammary glands mainly the lobular system in which most of the breast tumors initiate. The aim of the research is divided in two scenarios a lactating and a non-lactating mammary gland.

Background

The mammary gland is the only organ in our body that is continuously changing throughout the different life stages. This organ has a unique structure that is capable of providing both nutrition and immune protection to the infant during the lactating period [3]. In order for the new mother to fulfill the needs of the newborn, the mammary gland undergoes several changes which include alveologenesis and a complete remodel of the ductal system. All these changes occur after the pregnancy hormones sent a signal to initiate the growth of the ductal system. An important consideration during this process is the differentiation of two main cell types: Myoepithelial and luminal epithelial cells. These two cell types are in control of the contraction and secretion of breast milk during the lactation period [4].

As mentioned in the previous paragraph, the mammary gland undergoes several changes along a life cycle. The mammary gland of a newborn is limited to a primitive ductal system with fewer endbuds that will differentiate into epithelial cells until the infant reaches puberty. During the pubertal stage additional branching occurs and the fatty tissue content decreases to be replaced with a more complex ductal system. After this growth, the mammary

glands wait until a signal is sent by the pregnancy hormones in order to complete the branching development as shown in figure 1.1. After receiving the signal the alveolar systems prepares for milk production [5]. In order to prepare for the lactating period the ductal system needs to develop completely. The ductal system of the mammary gland is surrounded by both a Myoepithelial and luminal epithelial cell layer.

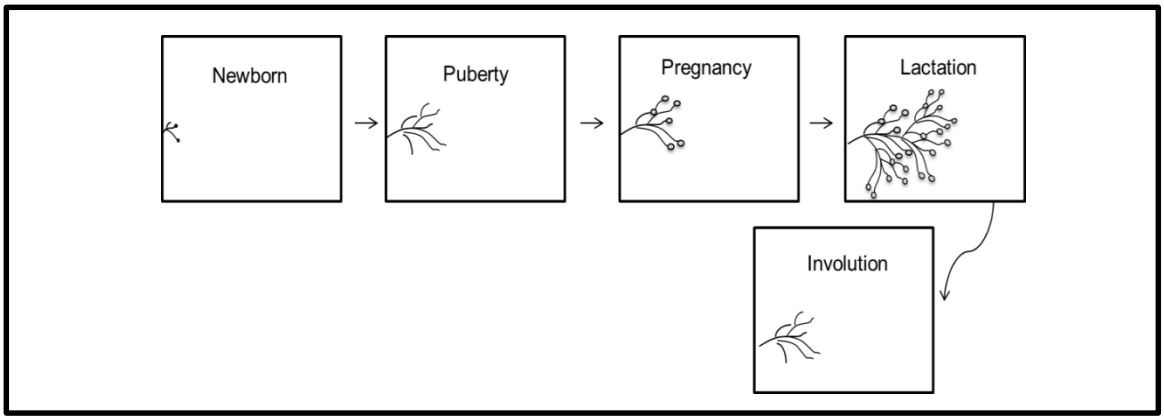

FIGURE 1.1 ALVEOLOGENESIS

Alveologenesis occurs along with the ductal system formation, this process account for the formation of alveoli which are microstructural cavities responsible for the breast milk production. The alveoli is also surrounded by a layer of Myoepithelial and luminal epithelial cells as well as an arterial system responsible of transferring immune factors, nutrients and some toxins into the breast milk [3]. During the pregnancy and lactation period, the mammary glands contain proteins responsible for providing protection against breast cancer. These proteins are called BCRP breast cancer resistant proteins these proteins are accounted for being responsible of detoxifying the mother and helping boost the immune system of the newborn by releasing the toxins into the breast milk [6].

The transport mechanism of breast milk initiates in the alveoli. A cluster of alveoli is denominated a lobule and a group of lobules is named lobe. The lobe is connected to the nipple through a system of lactiferous ducts. These ducts are responsible for the collection and transport of the breast milk out of the mammary gland through the nipple. During breastfeeding the alveoli is stimulated and the production of milk continues for future feeding.

Toxicity in the breast fluids

Breast milk samples have been identified as a precise indicator of the mammary gland actual condition. Sampling the milk fluid provides a simple and a non-invasive procedure to test the existence of DNA damage as well as the presence of toxins inside the lactiferous ducts. All these testing are important to fully understand the substances stored in the lactiferous ducts. Prior to pregnancy, the mammary glands are not fully developed and most of the space is occupied by 80-90% of adipose tissue [7]. Since the calorie expenditure for our body to store toxins in the fat is considerably lower than their complete removal, in occasions the toxins are stored in the adipose tissue. As the adipose tissue is remodeled to initiate the transition to the lactation period most of these toxins are transported into the blood stream.

Toxins are transported from the blood stream into the breast milk or from the adipose tissue into the blood stream or other organs for complete removal by active or passive diffusion. The method for the particle to be diffused depends entirely on the properties of the particles and their affinity with the tissue. Some of the smaller particles can freely diffuse into the breast milk such as caffeine. The malignant effect of caffeine in the newborn is still not completely understood, but its presence has been verified in women during pregnancy and the lactation period [8]. Organochloride pesticides are other particles that diffuse into the breast milk without difficulty. More than a dozen organochloride pesticides have been identified in breast milk sample in women living in the tropical areas in Mexico [9].

In the mid 1900's DDT was a popular pesticide used to eradicate the mosquito plagues in order to prevent West Nile and other diseases [10]. Even though it was banned in many countries decades after, the bioaccumulation of these pesticides in the tissue and the

environmental has been difficult to eradicate completely. As a control method for example Japan has been constantly monitoring breast milk samples over the last 4 decades to determine the levels and the complications infants are presenting depending on the levels of organochloride present in the breast milk [11]. Even in the United States and Canada, where high restrictions exist on the utilization of these pesticides, DDT is still present in the breast milk samples [10].

In contrast with passive diffusion, active diffusion requires sets of proteins serving as transporters. These transporters are responsible for moving the toxins/elements against the concentration gradient. Some studies have indicated the presence of an ATP binding cassette transporters responsible for the expulsion of compounds through the breast milk [12]. The BCRP breast cancer resistant protein, an ATP binding cassette transporter, has been linked to the transport of several drugs. Since BCRP is responsible of the transport of many substances, the concentration of drugs is greater in the breast milk in comparison with the maternal plasma [12]. This is completely different from molecules transported through passive diffusion in which the concentration in the maternal plasma is larger than the breast milk.

Considering the malignancy of the bioaccumulation of toxins inside the mammary glands several studies have been developed to understand its relationship with breast cancer. If we consider that during the lactation period most of these toxins are removed, it is not complicated to be aware that in a non-lactating period all these substances are just stored in the breast without being eliminated. In alignment with this hypothesis, a research group analyzed women in different countries to determine a correlation among environmental, dietary and country of residence in the incidence of breast cancer. Women from UK, India, Singapore and

Hong Kong volunteered to provide blood and milk samples. Both of the samples were tested for genotoxicity. After the analysis it was concluded a higher presence of toxins in the UK samples. As a result, the research group was able to establish a relationship between the UK high risk of developing breast cancer and the higher presence of toxins inside the samples in comparison with the other countries with lower rates of breast cancer which showed a reduced amount of toxins [7].

2. CHAPTER II. MODELING AND ANALYSIS OF TRANSPORT IN THE MAMMARY GLANDS

INTRODUCTION

The female breast initiates development after the sixth week of gestation and reaches its complete development during pregnancy and childbirth [13]. Inside the female breast there is a unique structure called the mammary glands. The uniqueness of the mammary glands is their capability to provide both nutrition and immune protection to the infant during “the lactation period” [3]. Mammals are the only ones that possess the capability of milk production and excretion after giving birth [3].

In comparison with other organs in the body, the mammary glands are constantly evolving throughout different life stages of female mammals. The mammary gland of a newborn is limited to a primitive ductal system with limited end buds that ultimately differentiate into epithelial cells once the infant reaches puberty. During the pubertal stage additional branching occurs and the fatty tissue content decreases to be replaced with a more complex ductal system. After this growth, the mammary glands go through a pause period until a signal is sent by the pregnancy hormones in order to complete the development of the branches. Once the signal is received, the ductal system development will be completed in order to prepare for the lactating period [14].

In order for the new mother to fulfill the nutritional needs of the newborn, the mammary gland undergoes several changes, which include alveologenesis (Figure 2.1A) and a complete remodel of the ductal system. All these changes occur after the pregnancy hormones sent a

signal to initiate the growth of the ductal system. An important consideration during this process is the differentiation of two main cell types: myoepithelial and luminal epithelial cells (Figure 2.1B). These two cell types are in control of the contraction and secretion of breast milk during the lactation period [15].

Alveologenesis occurs along with the ductal system formation. This process accounts for the formation of alveoli which are microstructural cavities responsible for the breast milk production. The alveolus (singular for alveoli) is also surrounded by a layer of myoepithelial and luminal epithelial cells (Figure 2.1C) as well as an arterial system responsible for transferring immune factors, nutrients and some toxins into the breast milk [3]. During the pregnancy and lactation period, the mammary glands contain proteins responsible for providing protection against breast cancer. These proteins are referred to as Breast Cancer Resistant Proteins (BCRP). These proteins are credited for being responsible for detoxifying the mother and helping boost the immune system of the newborn by releasing the toxins into the breast milk [6].

The transport mechanism of breast milk initiates in the alveoli. A cluster of alveoli is denominated a lobule and a group of lobules is named lobe (Figure 2.1D). The lobe is connected to the nipple through a system of lactiferous ducts. These ducts are responsible for the collection and transport of the breast milk out of the mammary gland through the nipple. During breastfeeding the alveoli is stimulated and the production of milk continues for future feeding. The changes in diet and the environmental factors affect the reliability of the milk transported through the milk ducts [7].

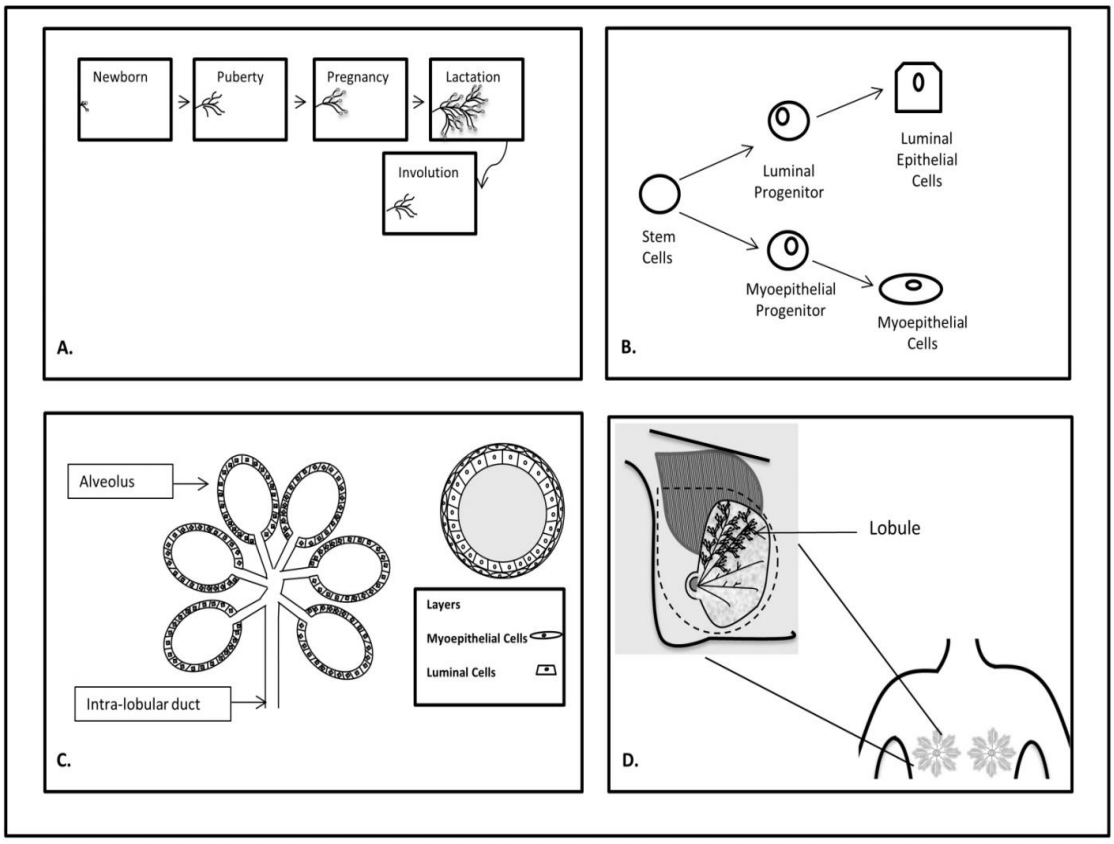

FIGURE 2.1 THE MAMMARY GLAND DEVELOPMENT. A) ALVEOLOGENESIS B) STEM CELL DIFFERENTIATION C) LOBULE D) BREAST STRUCTURE

It is believed that the increased number of toxins present in the mammary tissue are responsible for the onset of the breast cancer [16]. Most of the toxins accumulated in the breast milk are from contaminants that are lipophilic and have a low molecular weight. Mostly these components have a non-pharmaceutical origin and could eventually affect the health of both the mother and the child [17]. For this reason it is recommended that breast milk samples should be monitored in order to check and control the persistent organic pollutants (POP) as well as other particles in different regions around the world [18]. It is critical to understand the

effects of banned and regulated products on the breastfeeding population in comparison with the previous studies [17].

Breast milk sampling has become one of the preferred testing methods. Many countries such as Mexico, Japan, Germany, and China among others are continuously developing field studies at different regions of their countries in order to establish correlations between breast milk samples, lifestyle and incidence of breast cancer [11, 19, 20].Breast milk samples have been identified as a precise indicator of the mammary gland actual condition. Sampling the milk fluid provides a simple and a non-invasive procedure to test the existence of DNA damage as well as the presence of toxins inside the lactiferous ducts. Continuous testing is important to fully understand the substances stored in the lactiferous ducts. Prior to pregnancy, the mammary glands are not fully developed and most of the space is occupied by 80-90% of adipose tissue [7].

Different lifestyles, environmental factors as well as a delay in starting a family are increasing the probabilities of being diagnosed with breast cancer [21]. In the United States it is expected that 1 out of 8 women will be diagnosed with breast cancer [2]. Research groups have established a relationship between breast milk samples with higher presence of toxins and countries with higher rates of breast cancer [7, 22, 23].

Considering these factors, breast cancer research has established a correlation among life style, obesity and environmental factors with the increasing number of cancer patients in industrialized countries [7, 22, 24, 25]. Even though, cancer has a strong correlation with genetic factors, it has been found that there is a strong relationship between the country of origin, environment and dietary factors [1].The aim of this research is the development and analysis of

comprehensive multi-layer mass transfer models of the mammary glands mainly the lobular system in which most of the breast tumors initiate. Research groups have shown the importance of using mathematical models to address biological questions [26]. The quantitative analysis of the transport of toxins provides additional insight for the investigators regarding the condition of the mammary gland. The utilization of multi-layer mathematical models to predict and analyze the transport of molecules has been proven previously by our research group [27-30].

FORMULATION

MULTI-LAYER MODEL

Extensive experimental procedures done in the human mammary glands can be challenging and sometimes almost impossible [31]. Even with these limitations, we are able to provide an alternative to understanding how the toxins get into the breast milk. A pertinent computational model offers a non-invasive alternative to simulate the transport mechanism. Our approach is that it will provide an alternative to estimate the milk to plasma ratio for breastfeeding women. Research publications have shown that toxins are able to penetrate the epithelial layers and get into the breast milk in higher concentrations compared to the resting mammary gland [7, 11, 17, 18, 20, 31-36]. The present work takes into consideration the particle size and properties of the layers to estimate the percentage of toxins that are introduced into the breast milk. The properties of the layers and the particles are shown in Tables 2.1-2.5. Our work demonstrates how the toxins enter various layers over a determined period of time, which makes it closer to physiological conditions.

TABLE 2.1 SOLUTE PROPERTIES

Caffeine Particle	
Particle Radius	$3.7x10^{-10}$[m]
Molecular Weight	194.1[g]
DDT Particle	
Particle Radius	$6.3x10^{-10}$[m]
Molecular Weight	354.9[g]
Cimetidine Particle	
Particle Radius	$5.5x10^{-10}$[m]
Molecular Weight	252.3[g]

TABLE 2.2 PHYSIOLOGICAL PARAMETERS USED IN THE SIMULATION OF ALVEOLAR SYSTEM

Layers	**Parameters**	**Value**	**Units**	**References**
Plasma Layer	Thickness	$5x10^{-6}$	$[m]$	[37]
	Density	1139	$[kg/m^3]$	[38]
	Dynamic Viscosity	$1.5x10^{-3}$	$[Pa\ s]$	[39]
Myoepithelial Layer	Thickness	$8x10^{-6}$	$[m]$	[40]
	Density	1060	$[kg/m^3]$	[41]
	Dynamic Viscosity	$1.1x10^{-3}$	$[Pa\ s]$	[42]
	Porosity	$5x10^{-4}$	-	[28]
Luminal Epithelial Layer	Thickness	$17x10^{-6}$	$[m]$	[40]
	Density	1160	$[kg/m^3]$	[43]
	Dynamic Viscosity	$1.1x10^{-3}$	$[Pa\ s]$	[42]
	Porosity	$5x10^{-4}$	-	[28]
Breast Milk Layer	Diameter	$100x10^{-6}$	$[m]$	[40]
	Density	1139	$[kg/m^3]$	[38, 44]
	Dynamic Viscosity	$14.7x10^{-3}$	$[Pa\ s]$	[45, 46]

TABLE 2.3. DIFFUSION COEFFICIENTS FOR THE TRANSPORT OF CAFFEINE INTO THE BREAST MILK

Layers	Parameters	Value	Units
Plasma Layer	Diffusion Coefficient	$4.0x10^{-10}$	$[m/s^2]$
Myoepithelial Layer	Diffusion Coefficient	$5.5x10^{-10}$	$[m/s^2]$
Luminal Epithelial Layer	Diffusion Coefficient	$5.5x10^{-10}$	$[m/s^2]$
Breast Milk Layer	Diffusion Coefficient	$4.1x10^{-11}$	$[m/s^2]$

NOTE: The diffusion coefficients were estimated using the Stokes-Einstein Relationship (Equation 1.1)

TABLE 2.4. DIFFUSION COEFFICIENTS FOR THE TRANSPORT OF DDT INTO THE BREAST MILK

Layers	Parameters	Value	Units
Plasma Layer	Diffusion Coefficient	$2.3x10^{-10}$	$[m/s^2]$
Myoepithelial Layer	Diffusion Coefficient	$3.2x10^{-10}$	$[m/s^2]$
Luminal Epithelial Layer	Diffusion Coefficient	$3.2x10^{-10}$	$[m/s^2]$
Breast Milk Layer	Diffusion Coefficient	$2.5x10^{-11}$	$[m/s^2]$

NOTE: The diffusion coefficients were estimated using the Stokes-Einstein Relationship (Equation 1.1)

TABLE 2.5. PHYSIOLOGICAL PARAMETERS USED IN THE SIMULATION OF CIMETIDINE IN THE ALVEOLAR SYSTEM

Layers	Parameters	Value	Units	References
Plasma Layer	Diffusion Coefficient	$2.7x10^{-10}$	$[m/s^2]$	
	Relative Permittivity	0.2	[-]	[47]
	Electrical Conductivity	$1x10^{7}$	[S/m]	[47]
Myoepithelial Layer	Diffusion Coefficient	$3.6x10^{-10}$	$[m/s^2]$	
	Relative Permittivity	$2.5x10^{7}$	[-]	[47]
	Electrical Conductivity	0.2	[S/m]	[47]
Luminal Epithelial Layer	Diffusion Coefficient	$3.6x10^{-10}$	$[m/s^2]$	
	Relative Permittivity	80	[-]	[48]
	Electrical Conductivity	0.3	[S/m]	[49]
Breast Milk Layer	Diffusion Coefficient	$2.5x10^{-11}$	$[m/s^2]$	
	Relative Permittivity	96	[-]	[50]
	Electrical Conductivity	740	[S/m]	[50]

NOTE: The diffusion coefficients were estimated using the Stokes-Einstein Relationship (Equation 1.1)

The transport mechanism in the mammary gland is shown schematically in figure 2.2. The arterial blood supplies the toxins, nutrients and immune factors through the luminal cells until it reaches the breast milk cavity [36]. As seen in figure 2.2, milk globules represent the breast milk produced. The transport occurs by diffusion, it can be active or passive diffusion. The

active diffusion utilizes membrane potential or proteins to reach the alveolar cavity while the passive or free diffusion takes advantage of the high to low concentration gradient. The diffusion considered in our models is unidirectional. The alveolus shown on the left side of figure 2.2 can have a diameter fluctuating from 100 to 300 µm. The myoepithelial and luminal epithelial layers are considered as a diffusion barrier responsible for the blockage of certain toxins. These layers have a thickness of 8 and 17 µm respectively.

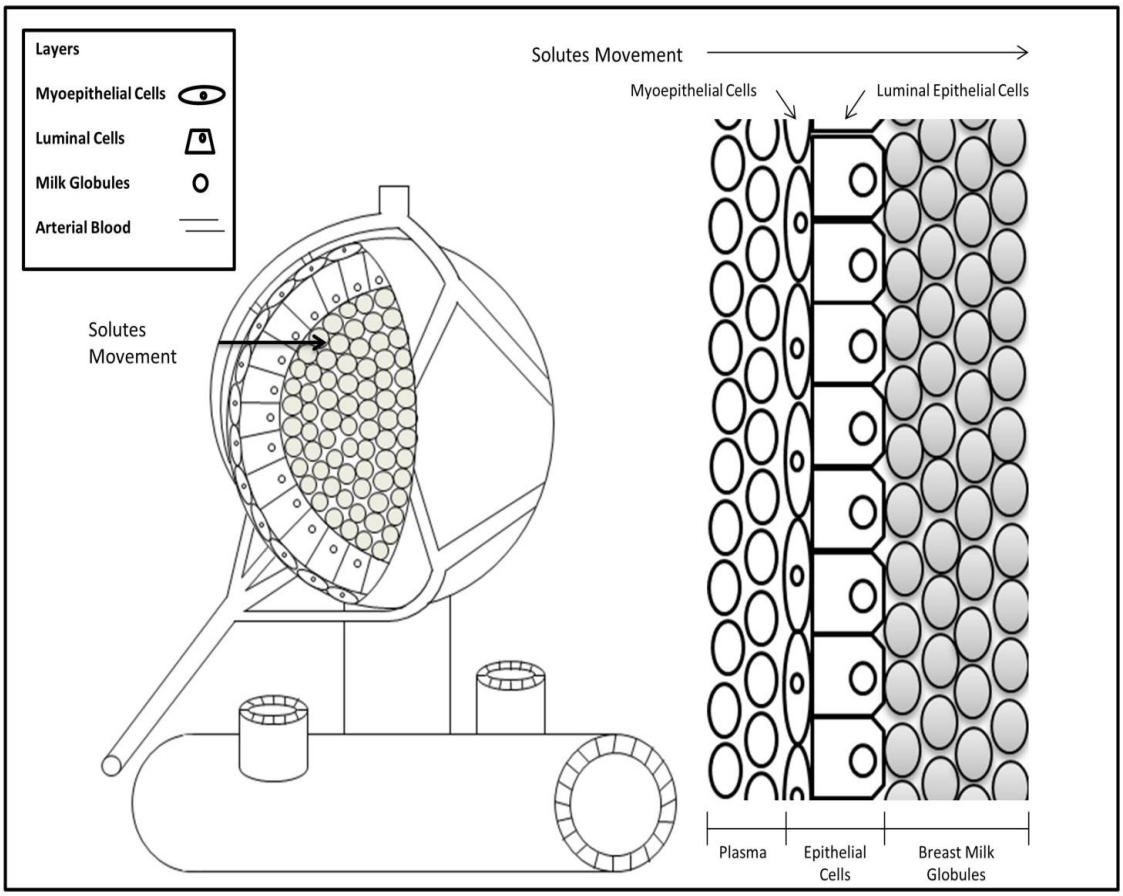

FIGURE 2.2 TRANSPORT OF SOLUTES INTO THE BREAST MILK LAYER

Some limitations do exist for our parameter estimation due to the invasiveness of developing experimental work while women are breastfeeding in order to estimate diffusion coefficients. For this reason, we will consider Stokes-Einstein equation to estimate the diffusion coefficients for the in vivo parameters. The Stokes-Einstein relationship has proven to be useful as a starting point to estimate the diffusion coefficients of proteins, sugar and other small molecules in, prior works [51-56].

In the epithelial cells the fluid phase viscosity of the medium is not much higher from the one estimated for water. In this case the viscosity used in our work is 1.1 cP for the epithelial layers [42]. To estimate the diffusion coefficient of the solute in a "cell layer" we approximated it by utilizing the Stokes-Einstein equation (equation 2.1) as a starting point. Using this equation we are able to consider the effect of the particle size and the viscosity of the cells.

$$D = \frac{k_B T}{6\pi\eta r}$$

$$\begin{aligned} &D = Diffusion\ Coefficient \\ &k_B = Boltzmann's\ Constant = 1.38x10^{-23}\ m^2 kg/s^2 K \\ &T = Temperature = 310\ K \\ &\eta = Viscosity\ of\ cells = 1.1\ cP \\ &r = Radius\ of\ the\ molecule\ (cimetidine, caffeine\ or\ DDT) \end{aligned} \tag{2.1}$$

The diffusion coefficients obtained with the utilization of Stokes-Einstein equation gave us a starting point to estimate the diffusion coefficients for in vivo situations. The mammary gland is unique and during lactation the diffusion barriers are more permeable to molecules that will not be able to penetrate in other circumstances, making the mechanism unique [57]. Most of the experimental work done while women are breastfeeding is minimally invasive consisting

only of blood and breast milk samples to determine concentration levels. Given these conditions, for our computational model we are considering the diffusion coefficient of the solute in solvent as discussed in other publications [58].

A consideration we made for the epithelial layers is to include the transepithelial transport of molecules across a porous membrane and include this as the effective diffusion coefficient [27-29]. The effective diffusion coefficient of the epithelial cells layers can be estimated using equation 2.2.

$$D_{eff} = D\varepsilon$$

$$D = Diffusion\ Coefficient \tag{2.2}$$
$$D_{eff} = Effective\ Mass\ Diffusion\ Coefficient$$
$$\varepsilon = Porosity\ of\ the\ layer$$

The diffusion coefficients in the ductal epithelium of the mammary gland are not available. After extensive literature search we were able to only find the diffusion coefficient of cimetidine and caffeine absorbed by CACO-2 epithelial cells. These epithelial cells are located in the intestine layer and are our closest approximation to the cell type [55]. The solute movement in CACO cells is from the lumen into the bloodstream. Some of the parameters are shown in Table 2.6. Given these parameters we are able to make the appropriate assumptions for our model.

TABLE 2.6. COMPARISON OF THE ESTIMATED DIFFUSION COEFFICIENT OF THE EPITHELIAL CELLS WITH THE AVAILABLE LITERATURE VALUES

Molecule	Porosity [28, 29]	Particle Size [m]	Estimated Diffusion Coefficient [m^2/s]	Literature Diffusion Coefficient [m^2/s]	Estimated Effective Diffusion Coefficient [m^2/s] *	Literature Effective Diffusion Coefficient [m^2/s] *	References
Caffeine	$5x10^{-4}$	$3.7x10^{-10}$	$5.5x10^{-10}$	-	$2.75x10^{-13}$	$3.75x10^{-13}$	[59, 60]
DDT	$5x10^{-4}$	$6.3x10^{-10}$	$3.2x10^{-10}$	-	$1.6x10^{-13}$	-	[55, 59, 60]
Cimetidine	$5x10^{-4}$	$5.5x10^{-10}$	$3.7x10^{-10}$	$7.7x10^{-10}$	$1.8x10^{-13}$	$0.72x10^{-13}$	[55, 56, 59-61]

* Literature references available for epithelial cells surrounding the intestine layer [CACO Cells]

In order to assess the utilization of Stokes-Einstein we ran the model utilizing the diffusion coefficient of CACO epithelial cells vs our model of the mammary gland epithelial cells. As shown in Figure 2.3 and 2.4 our model, utilizing the Stokes-Einstein Equation, is in agreement with the results obtained based on the available literature values for the diffusion coefficients. As such we have utilized the calculated diffusion coefficient values for caffeine, cimetidine and DDT. Additionally, given that the mammary glands properties are not fully understood our work is unique in demonstrating a new methodology to analyze the transport of drugs from the bloodstream into the breast milk layer. The order of magnitude of our approximation is close to the experimental work done in the intestine layers, where drugs move from the lumen into the plasma. Also other factors are important in our model such as porosity which takes into consideration the transport across a porous membrane.

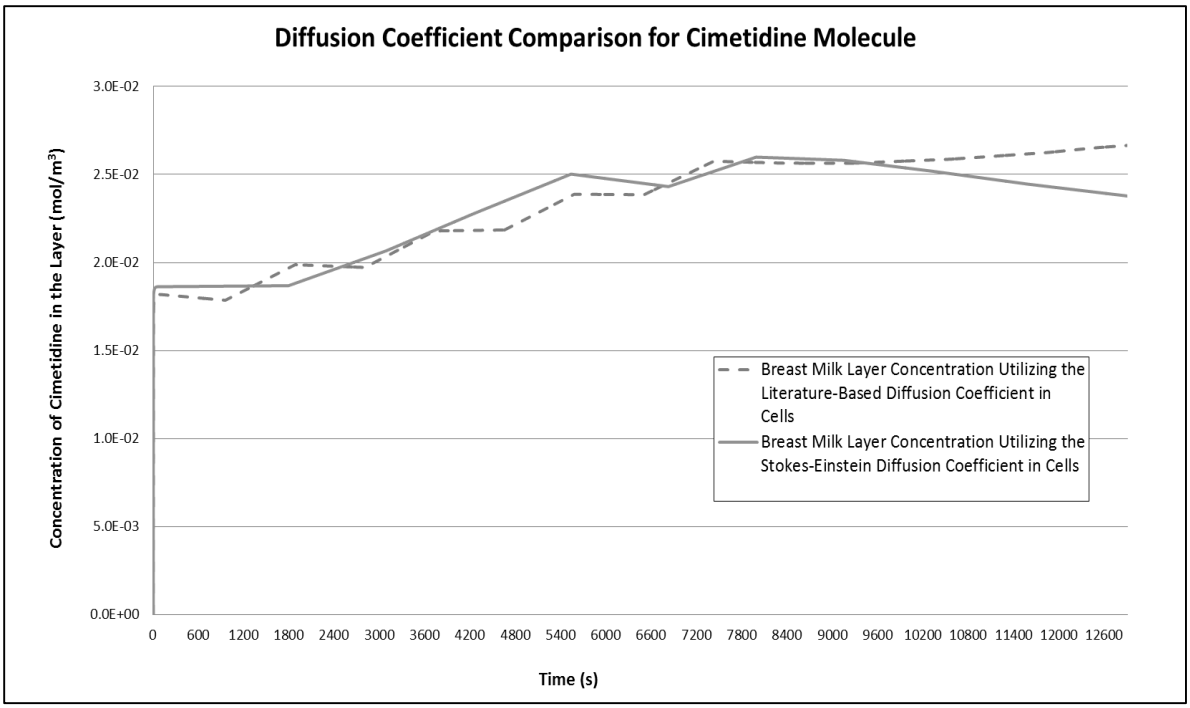

FIGURE 2.3 DIFFUSION COEFFICIENT COMPARISON FOR CIMETIDINE MOLECULE

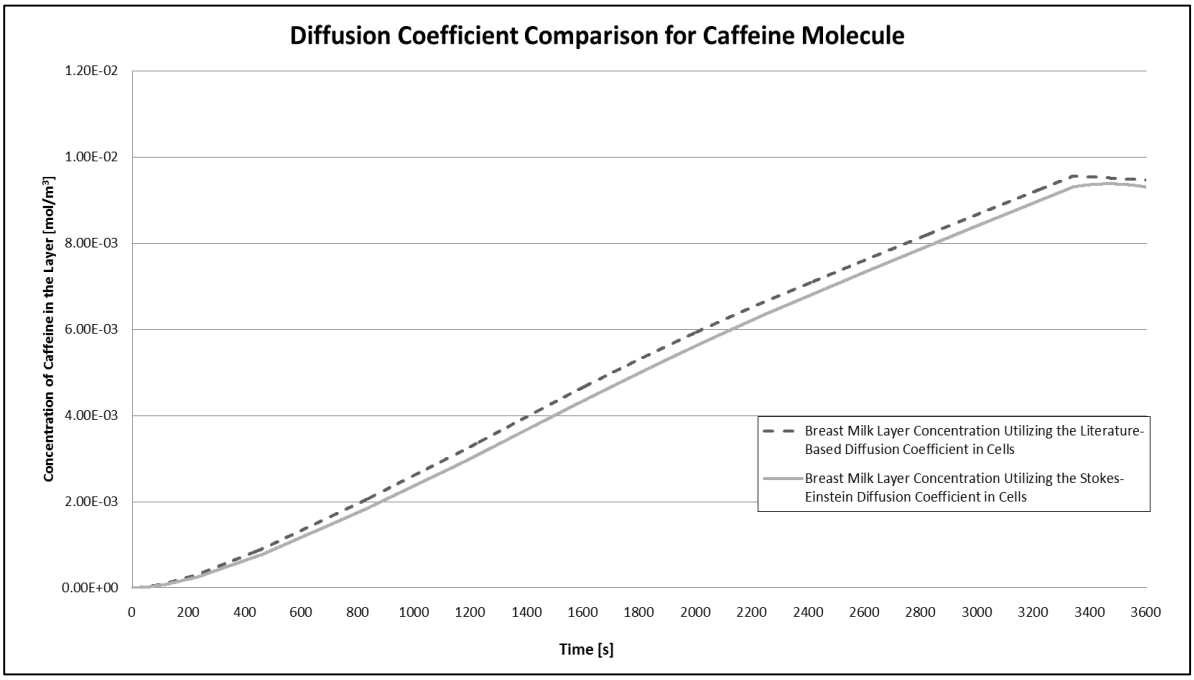

FIGURE 2.4 DIFFUSION COEFFICIENT COMPARISON FOR CAFFEINE MOLECULE

PASSIVE DIFFUSION

THE TRANSPORT MECHANISM OF CAFFEINE INTO BREAST MILK

Caffeine freely diffuses into the breast milk layer directly from the bloodstream [8, 62, 63]. The following two layers, the myoepithelial and luminal epithelial, serve as a thin diffusion barrier capable of blocking at least 30 percent of the caffeine concentration being transported into the breast milk [63, 64]. The caffeine transport mechanism initiates with the intake by the women breastfeeding of this substance in beverages or food. The caffeine enters the bloodstream gradually during the first hour after ingestion [63]. During time zero to the first hour, the concentration of caffeine will increase its presence in the bloodstream. Given this, the expected concentration can be seen increasing over this time period.

After the caffeine increases its presence in the blood stream, it continues to freely diffuse into the contiguous cellular layers. The first diffusion barrier of the breast milk multi-layer system is the myoepithelial layer. In this layer at time zero there will be no concentration present, but due to the caffeine particle size it will easily diffuse into this first barrier. As the species enter the myoepithelial layer a percentage starts leaving this layer to start the diffusion onto the second diffusion barrier called “Luminal Epithelial Layer” [8]. In both of these layers the caffeine solute establishes its presence to finally attempt to diffuse into the breast milk layer [64].

The passive transport mechanism of caffeine into the breast milk layers can be represented by:

$$\varepsilon \frac{\partial c}{\partial t} + \nabla \bullet (-D\nabla \vec{c}) = S \qquad (2.3)$$

where c is the concentration of Caffeine or DDT, D the diffusion coefficient, ε the porosity and S is the source term. Each diffusion barrier has a characteristic porosity, diffusion coefficient and an initial concentration to initiate the transport mechanism. A source term is also incorporated in the governing equation to accommodate the physiological mechanism of the caffeine entering the bloodstream after ingestion.

THE TRANSPORT MECHANISM OF DDT INTO BREAST MILK

In several countries the fields were sprayed with organochloride pesticides for a long period of time to control pests. The damage caused in the air and soil has been monitored and it is believed that the effect is worst in regions with tropical temperatures [65]. If DDT particles are sprayed by workers manually or are located close to a field recently sprayed the regulations stipulate a maximum concentration of $2.8\ \mu mol/m^3$ or a flux of $1.3x10^{-21}\ mol/m^2 s$ in the air as shown in Fig. 2.5 [66].

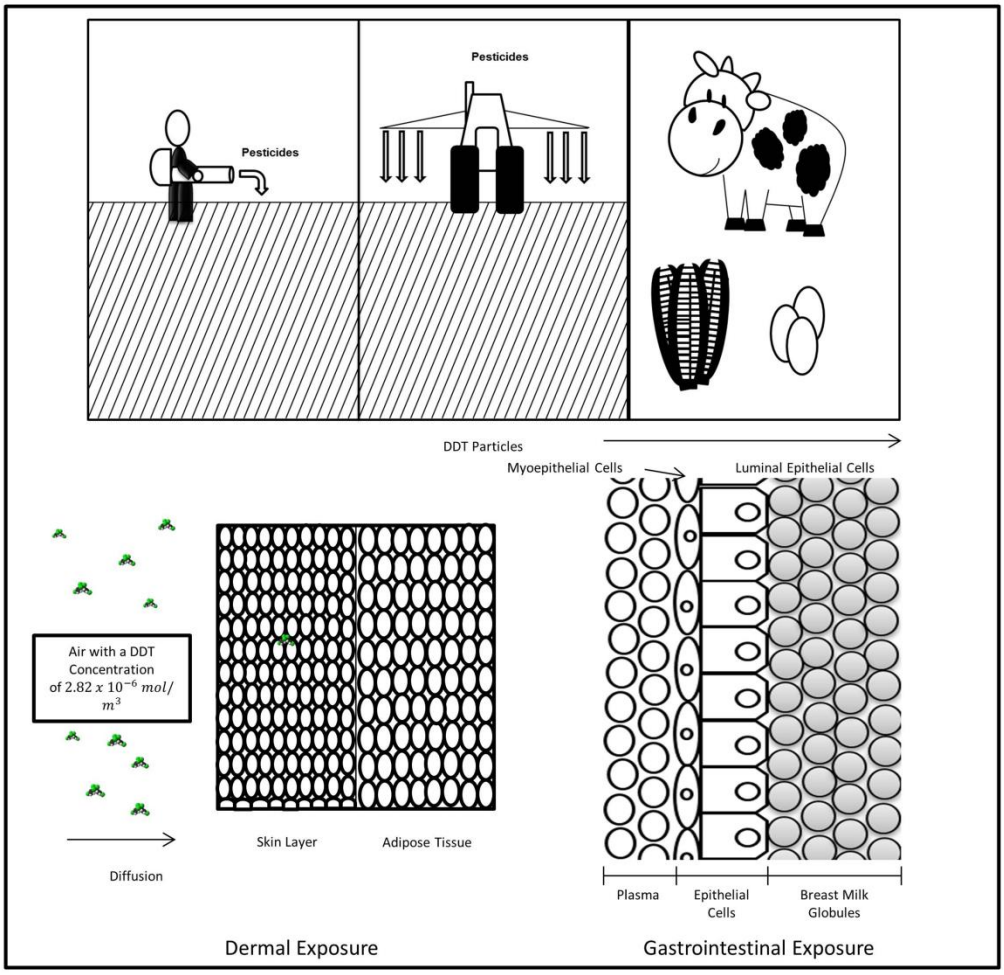

FIGURE 2.5 DDT DERMAL AND GASTROINTESTINAL EXPOSURE

A field worker within an eight hour shift can be exposed to a continuous DDT concentration. The skin blocks the entrance of DDT particles into the adipose tissue. The dermal adsorption fraction is 0.03 compared with the gastrointestinal which is 0.7 [67]. The contrast of the adsorption is due to the affinity of DDT to be stored in the adipose tissue after ingesting products contaminated with DDT particles such as fruits and vegetables. Unfortunately, DDT contamination is not limited to the air as it has been found that it is also accumulated in soils and water. This complicates its complete elimination. For example, in China DDT pesticides were

banned or controlled a couple of decades ago and the particles contamination is still present in the studies done in the breast milk from women of the most affected areas [20].

We consider the scenario where the concentration of DDT is inside the bloodstream feeding the alveoli. Schecter *et al.* [68] presented a study of the concentration of DDT in the blood samples from women in Northern Vietnam. Based on their experimental method it was estimated that they possess an average concentration of $1.3 \pm 0.2 \ ng/ml$ and $4.6 \pm 1.0 \ ng/ml$ at rural and urban zones respectively [68]. In a separate study in Mexico the blood serum DDT concentration was found to be around $1.8 \pm 3.8 \ ng/ml$ [69].

The passive diffusion equation 2.1 was utilized in the analysis of all the multiple layers in the alveoli. The concentrations from the work of Schecter *et al* [68] were taken as the initial values in the plasma layer. As such the initial concentration was taken as $15.9 \ \mu mol/m^3$ based on the rural Northern Vietnam experimental data [68].

ACTIVE DIFFUSION MODEL

In comparison with most of the molecules transported into the breast milk through passive diffusion, cimetidine is one of the few molecules transported through an active diffusion mechanism. Cimetidine is a specific histamine H2-Receptor antagonist that is commonly used for the cure of gastrointestinal ulcers [70]. The main difference for these particles is that they follow an active transport mechanism allowing the molecules to have a higher presence in the breast milk in comparison with the bloodstream. The verification of the active transport

mechanism has been demonstrated through studies of breast milk samples [34]. The milk plasma ratios (M/P) range from 4 – 12 in most of the predicted results [71].

The proposed model includes an electric current in the epithelial layers. Having an electric potential in the epithelial boundary layers simulates the physiological conditions for an actively diffusing cimetidine into the breast milk. The mammary glands have shown an electric potential ranging from -35 to -49 mV [72]. Researchers have demonstrated that human normal breast epithelium has a transepithelial potential difference of approximately +30 mV which is distributed across the cellular wall [73] .

The governing equations for an active diffusion model can be represented by:

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D\nabla c - zU_m Fc\nabla V) = S$$

$$E_m = -\nabla V$$

$$V = V_0 \qquad (2.4)$$

where c is the concentration of cimetidine, D the diffusion coefficient, z the charge number, U_m the mobility and F, V and S are the Faraday constant, voltage and the source term respectively. E is equal to the membrane's voltage potential.

The M/P ratio for the models was based on Oo *et al.* [34] experimental work where they observed an average M/P ratio of approximately 5.5 after single oral doses of 100, 600 and 1200 milligrams. Given these values, the membrane potential of the epithelial cells is estimated utilizing the Goldman–Hodgkin–Katz voltage equation.

$$Milk\ to\ Plasma\ Ratio = 5.5$$

$$E_m = 61.5mV \log\left(\frac{Milk\ Concentration}{Plasma\ Concentration}\right) = 61.5mV \log\left(M/P\right) = 61.5\log\left(5.5\right) = -45.5mV$$

(2.5)

The transepithelial potential difference across the boundary layers as shown in Fig. 2.6 considered for the model is +30 mV based on the suggested breast duct transepithelial potential by McCaig *et al.* [73]. For this reason the voltage of the boundary layer of the epithelial layers was estimated to be -45 mV as estimated in equation [73].

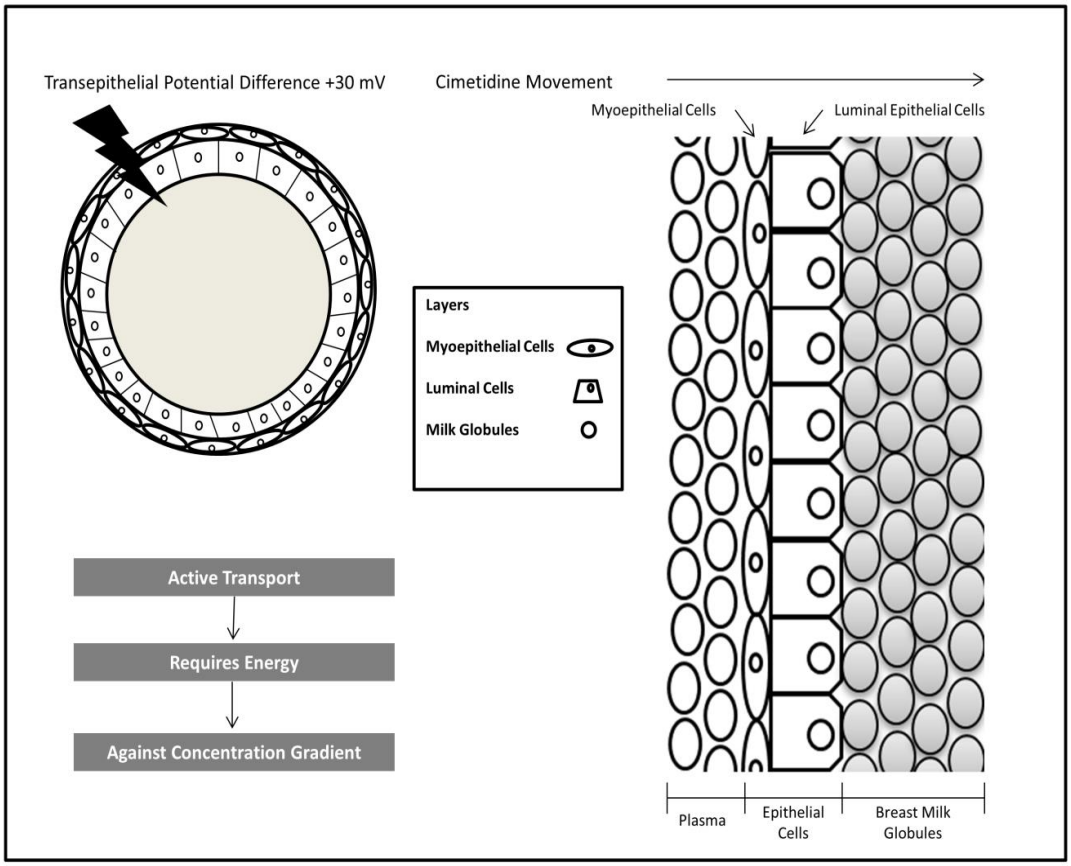

FIGURE 2.6 ACTIVE TRANSPORT OF CIMETIDINE IN THE MAMMARY GLAND

RESULTS AND DISCUSSION

COMPARISONS

The expected milk to plasma ratio of the particles is dependent on different factors such as dosage, delivery of the blood to the breast, the pharmacokinetic characteristics of the particle, period of lactation, frequency of breastfeeding and others [74]. In most of the cases the milk to plasma ratio is considered only on single dosages and in a steady state environment and for simplicity an average ratio is calculated [75]. The literature expected milk to plasma ratio of Cimetidine, caffeine and DDT are presented in Table 2.7.

TABLE 2.7. LITERATURE MILK TO PLASMA RATIOS

Particle	Literature Expected M/P Range	Model M/P Range	References
Caffeine	0.7 – 0.9	0.8	[63, 64, 76]
DDT	0.5 – 2.6	1	[68]
Cimetidine	4.6 – 11.7	2.9-5.4	[55, 56]

For the passive diffusion of caffeine in the breast milk our mathematical model forecasted an M/P (Milk to Plasma ratio) equal to 0.8 which is in agreement with the average experimental results as illustrated in Table 1.8 [63, 64, 76]. In contrast with caffeine DDT has a higher half-life in which the levels of these toxins in the plasma and the breast milk reach an equilibrium pattern [69]. To illustrate the DDT scenario Table 2.8 presents the results of the diffusion of DDT concentration in which our model is able to achieve a steady state that

compares very well with the presented experimental results in which the resulting milk to plasma ratio reaches one [77].

TABLE 2.8. COMPARISON OF THE CALCULATED MILK TO PLASMA RATIOS OF PASSIVE DIFFUSION PARTICLES WITH THE AVAILABLE LITERATURE VALUES

Time [sec]	Model	Plasma		Breast Milk		Experimental M/P	Model M/P	References
		Experimental Concentration [mol/m³]	Model Concentration [mol/m³]	Experimental Concentration [mol/m³]	Model Concentration [mol/m³]			
3600	Passive Diffusion of Caffeine into the Breast Milk	$12.3x10^{-3}$	$11.5x10^{-3}$	$7.4x10^{-3}$	$9.3x10^{-3}$	0.7 - 0.9	0.8	[33, 62]
	Passive Diffusion of DDT into the Breast Milk	-	$6.3x10^{-6}$	-	$6.3x10^{-6}$	1	1	[52, 53, 58]

Furthermore, we also estimated the dermal adsorption of DDT molecules after exposure to these chemicals. The results of our mathematical models shown in Table 2.9 demonstrate that the percentage is lower than one percent compared with the average percentage presented by the health associations [78] .

TABLE 2.9. COMPARISON OF THE CALCULATED DERMAL ADSORPTION OF DDT PESTICIDES WITH THE AVAILABLE LITERATURE VALUES

Time [sec]	Model	Experimental Dermal Adsorption	Model Dermal Adsorption	References
3600	Dermal Exposure to a concentration of DDT	<3%	<1%	[78]

In contrast with DDT and caffeine transport mechanism some particles such as cimetidine follow an active diffusion mechanism. Considering the unique properties of an active transport mechanism Table 2.10 illustrates a comparison between our model and the available pertinent experimental results in the literature. In this Table we present three distinctive scenarios. The passive diffusion of cimetidine was calculated in order to emphasize that the milk to plasma ratio is lower than unity. This mechanism demonstrates that in order to have a higher concentration of cimetidine in the breast milk than in plasma as shown in literature [34, 71] there is a need to include additional parameters as discussed earlier. The second scenario shown in Table 2.10 considers an average concentration and milk to plasma ratio (M/P). Our mathematical model is in agreement with the average experimental M/P. In the third case shown in Table 2.10 we demonstrate that our model has the capability of estimating the resulting M/P when there is a variant concentration of cimetidine entering the bloodstream [34, 71].

TABLE 2.10. COMPARISON OF THE CALCULATED MILK TO PLASMA RATIOS OF ACTIVE DIFFUSION PARTICLES WITH THE AVAILABLE LITERATURE VALUES

Time [sec]	Model	Plasma		Breast Milk		Experimental M/P	Model M/P	References
		Experimental Concentration [mol/m^3]	Model Concentration [mol/m^3]	Experimental Concentration [mol/m^3]	Model Concentration [mol/m^3]			
12600	Passive Diffusion of Cimetidine into the Breast Milk	-	14.3×10^{-3}	-	10.1×10^{-3}	-	0.7	-
	Active Diffusion of Cimetidine into the Breast Milk [Mean Average]	19.8×10^{-3}	19.8×10^{-3}	108.9×10^{-3}	107.2×10^{-3}	5.5	5.4	[55, 56]
	Active Diffusion of Cimetidine into the Breast Milk Subjected to a Variant Concentration	7.9×10^{-3}	8.5×10^{-3}	17.8×10^{-3}	25.6×10^{-3}	2.2	2.9	[55, 56]

EFFECT OF VARIATIONS IN THE DIFFUSION COEFFICIENT ON THE MILK TO PLASMA RATIO (M/P) AND CONCENTRATION DISTRIBUTION

The parameter selection is important for accurate model predictions. Some parameters are known in literature as shown in Tables 2.1-2.5 and others need to be properly calculated. The diffusion coefficient for the epithelial layers was estimated utilizing the Stokes-Einstein equation. In order to ensure the correct parameter selection we compared it with literature-based diffusion coefficients available for intestinal epithelial cells as shown in Figs. 2.3 and 2.4 [55, 56, 59-61]. As such we had established that the use of diffusion coefficient based on the Stokes-Einstein relationship produces results which match closely with those utilizing the literature-based diffusion coefficients. In here, we perform an additional investigation regarding

the variation of the diffusion coefficient by studying the impact of modifying the epithelial layers diffusion coefficient by an order of magnitude from the value predicted by the Stokes-Einstein relationship as shown in Fig 2.7. The alveolus is a microstructure making the transport sensitive to order of magnitude changes. The 150 mg caffeine oral dose remains constant in comparison, but the concentration levels of caffeine in the plasma and the breast milk experiences a mild variation as a result of the increased epithelial layer diffusion coefficient. The variation is expected because as we increase the diffusion coefficient more caffeine molecules are transported into the breast milk resulting in a lower concentration of caffeine in the plasma layer. Our model demonstrates the impact the epithelial layers have on the blockage or passage of molecules into breast milk.

The milk to plasma ratio (M/P) is the amount of drug found in breast milk compared to the concentration available in the plasma. The M/P value is a widely utilized reference number indicative of the percentage of drug that can be transported into breast milk. The M/P value increases as the diffusion coefficient increases substantially as shown in Fig. 2.7. The impact of modifying the diffusion coefficients by an order of magnitude is significant, but relatively moderate considering such a drastic change in the diffusion coefficient. As shown in Fig. 2.8 altering the diffusion coefficient increases the percentage of particles transported into the breast milk. The M/P value increases slightly from 0.8 to 0.9 with the same oral dosage of 150 mg. As mentioned before, the concentration levels in the plasma and the breast milk are different because increasing the diffusion coefficient allows the passage of a larger amount of caffeine particles.

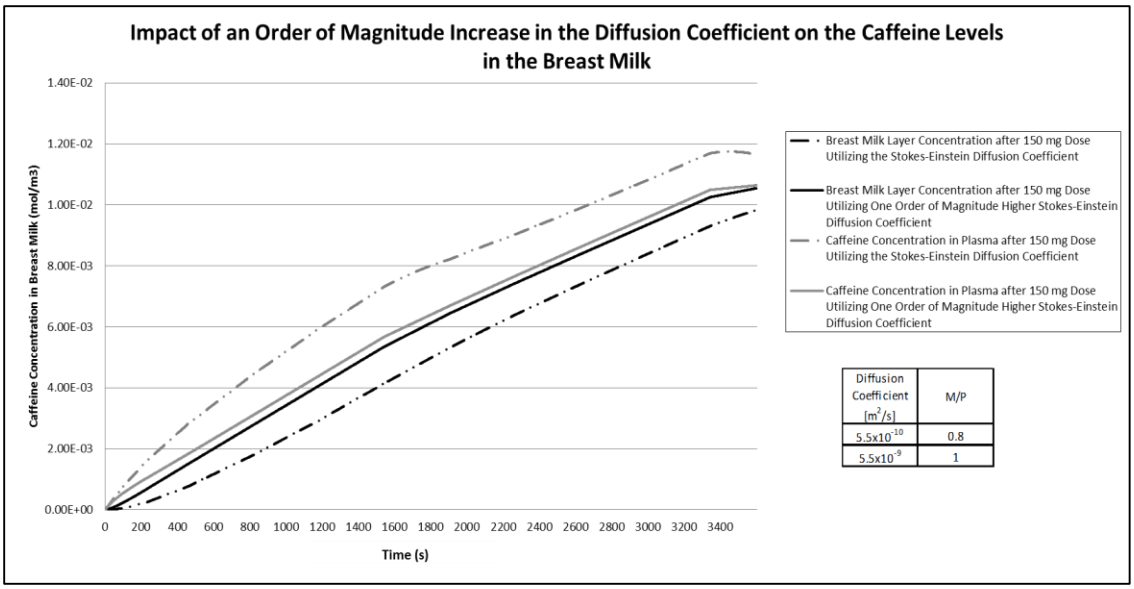

FIGURE 2.7 IMPACT OF AN ORDER OF MAGNITUDE INCREASE IN THE DIFFUSION COEFFICIENT ON THE CAFFEINE LEVELS IN THE BREAST MILK

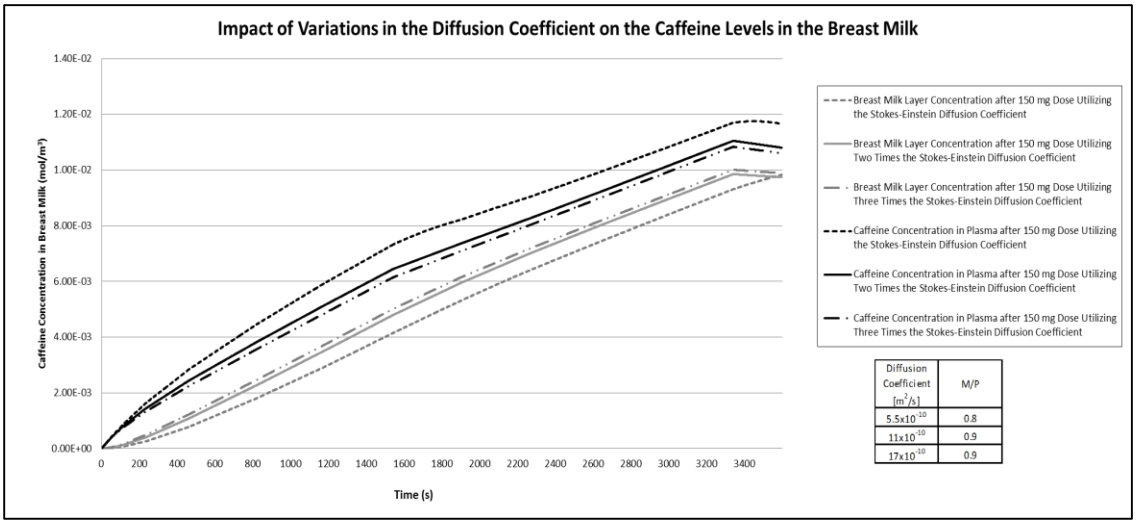

FIGURE 2.8 IMPACT OF VARIATIONS IN THE DIFFUSION COEFFICIENT ON THE CAFFEINE LEVELS IN THE BREAST MILK

Our model is able to predict the M/P value variation for the different molecules. The setup of our model permits the computational simulation of different molecules with multiple concentrations. One important goal for our simulation is to predict the drug concentration entering the breast milk utilizing a non-invasive approach. Caffeine ingestion is not uncommon and does not have a serious impact as other drugs such as cimetidine and DDT have while women are breastfeeding, making it easier to obtain experimental comparisons. The relationship between the caffeine oral dose and the bioavailability in breast milk have been demonstrated experimentally in prior work [8, 31, 62, 63, 76, 79]. For this reason the dosage has been studied and it has been experimentally demonstrated that when the oral dosage increases the milk to plasma ratio remains constant even if the blood and breast milk caffeine concentration increases proportional to the drug dose. The implication of this in the transport mechanism is that if we increase the caffeine consumption the epithelial layers will still be able to block an average of 20 percent of the drug trying to enter the breast milk layer (M/P = 0.8) [63, 64, 76].

In Figure 2.9 we have demonstrated that our model also confirms the experimental results and that as the concentration of caffeine increases the milk to plasma ratio remains constant validating the effect the epithelial layers have on the blockage of molecules before reaching the breast milk. Our model demonstrates an agreement with research publications where it is stated that the concentration will double if the ingestion goes from 150 mg to 300 mg in a single dose [63]. Our model demonstrates how the concentration levels are affected by the barriers which the molecules face prior to entering the breast milk.

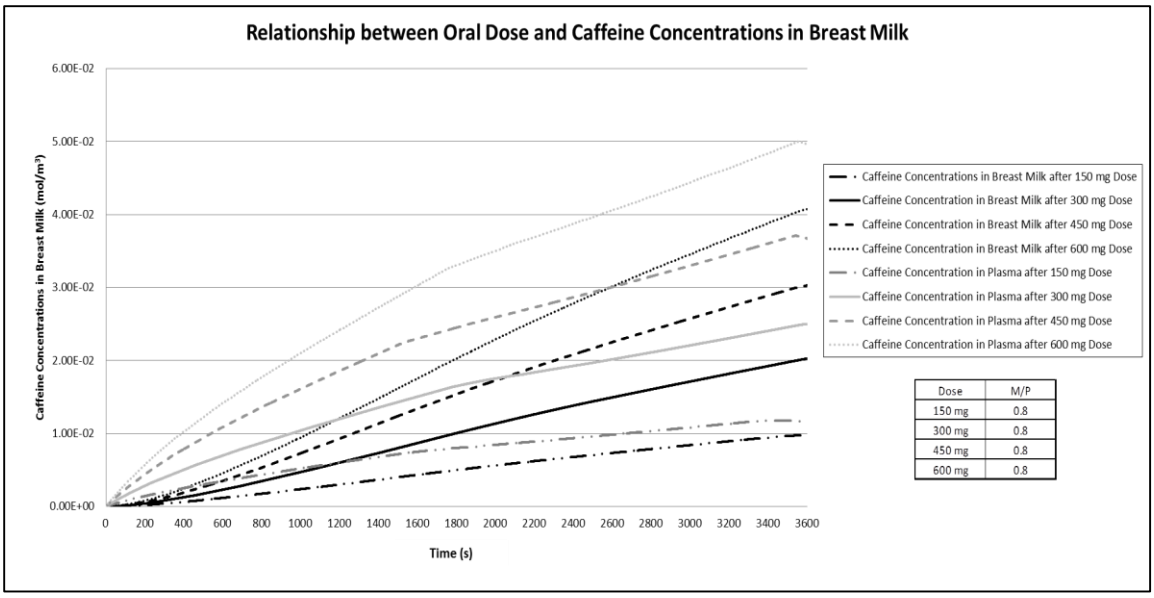

FIGURE 2.9 RELATIONSHIP BETWEEN ORAL DOSE AND CAFFEINE CONCENTRATIONS IN BREAST MILK

STUDY OF THE TRANSPORT OF CAFFEINE INTO THE BREAST MILK

The diffusion barriers block the entrance of caffeine into the breast milk layer. During the first half hour the concentration of caffeine in the bloodstream based on the experimental work of Tyrala *et al.* [63] reaches $8.2\ mmol/m^3$ and after the first hour the peak concentration of caffeine in the breast milk reaches $12.3\ mmol/m^3$ [63] . As can be seen the milk to plasma ratio (M/P) for the present study in Fig. 2.10 is approximately 0.7 which is consistent with the experimental data, which shows that the average M/P ratio is between 0.7-0.8 [63, 64].

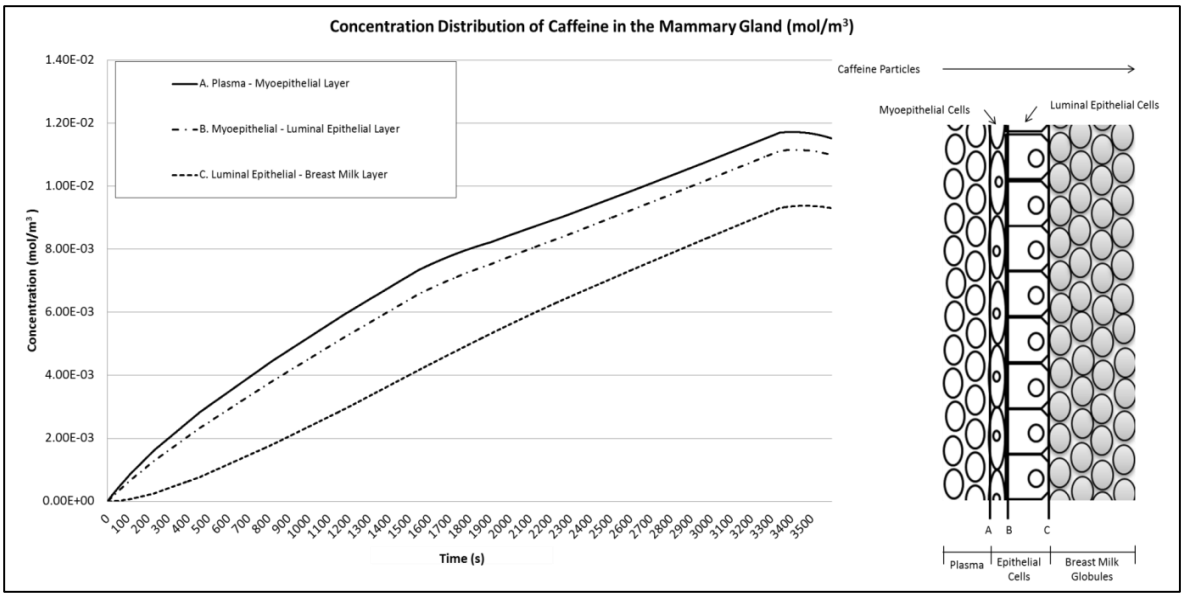

FIGURE 2.10 PASSIVE DIFFUSION OF CAFFEINE INTO THE BREAST MILK

STUDY OF THE TRANSPORT OF DDT INTO BREAST MILK

Organochloride Pesticides are accumulated in food, soils, air and animals [65]. Their persistance in being present in our food chain and in lipid-rich tissues of organisms makes it inevitable to have some levels of DDT present in the bloostream. People depend on their food chain and the air so they are constantly exposed to these persistant pesticides. For this reason, in the present work we have simulated the DDT concentration present in the plasma and breast milk layer of women while breastfeeding. DDT pesticides have demonstrated a high affinity with the fat present in the mammary glands resulting in a high presence in the breast milk fat as well as in the breast adipose tissue [77].

Our results for the passive diffusion of DDT within the mammary gland are shown in Fig. 2.11. The milk to plasma ratio of DDT has an average of 0.5 to 2.6, but if the comparison is done

in the fat levels the ratio reaches almost one [77]. Given these considerations, it is expected that at least the fat contained in the breast milk will contain a high percentage of DDT. Based on the experimental work from Waliszewski *et al.* [69] an initial concentration of DDT of $33\,\mu mol/m^3$ will result in a breast milk layer concentration of approximately $11.5\,\mu mol/m^3$. The present work displayed in Fig. 2.11 demonstrates an agreement with the experimental DDT concentrations in which the available DDT concentration in literature [19, 69, 77].

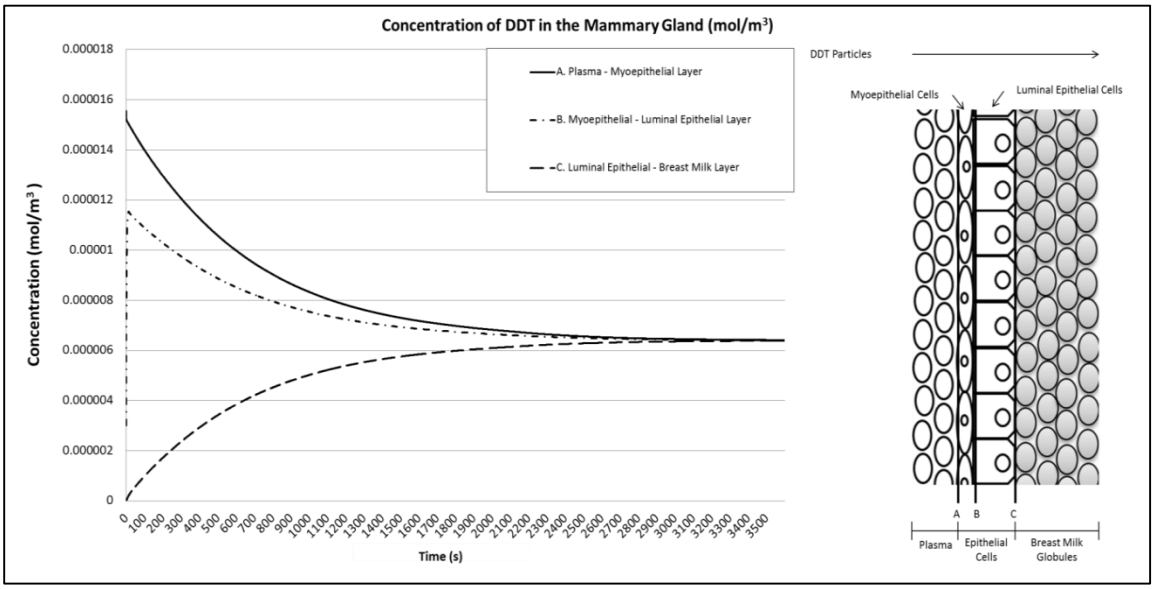

FIGURE 2.11 PASSIVE DIFFUSION OF DDT INTO THE BREAST MILK

STUDY OF THE DERMAL EXPOSURE TO DDT

Dermal exposure to DDT and its distribution within the skin and the adipose tissue layers is displayed in Fig. 2.12. Fig. 2.12 demonstrates that the DDT pesticides are able to penetrate and remain within the skin over a long period of time. Research done in organochlorides have demonstrated that approximately 3% of the pesticides are able to remain

on the skin after exposure but if the exposed skin is cleaned most of these particles are removed [78]. Our results are congruent showing a dermal adsorption fraction of 0.01, demonstrating the fact that the skin serves as a blockage for particles with sizes greater than 40 nm. Our models show that more than 97% of the DDT particles were blocked.

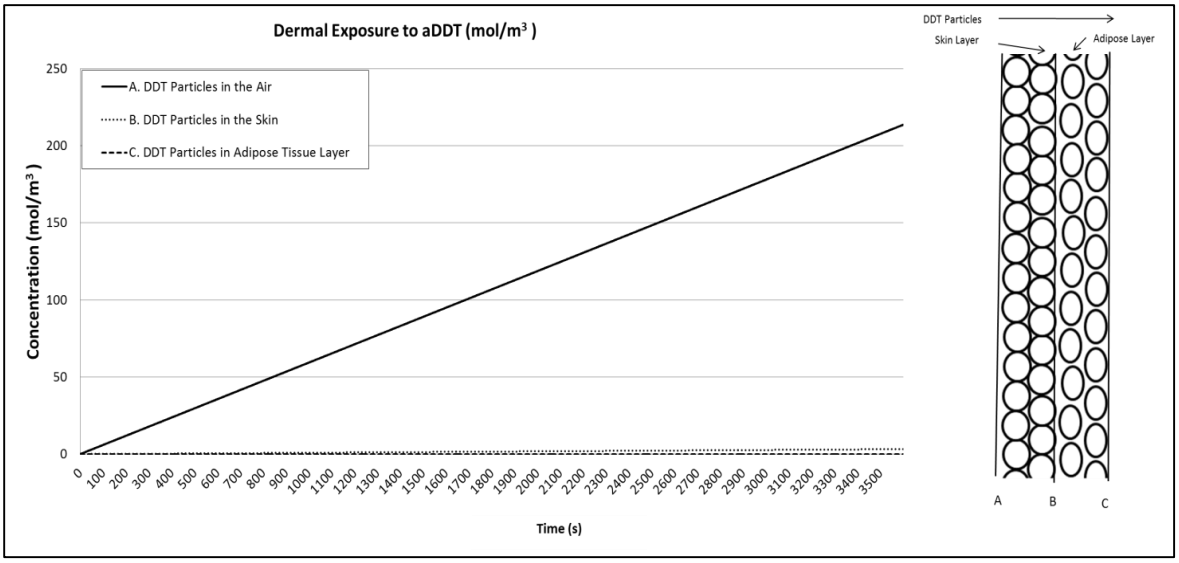

FIGURE 2.12 PASSIVE DIFFUSION OF DDT WITHIN THE SKIN AND ADIPOSE TISSUE LAYERS

STUDY OF THE TRANSPORT OF CIMETIDINE INTO THE BREAST MILK

Experimental results have consistently demonstrated a larger presence of cimetidine in the breast milk as compared to the plasma layer [80]. Figure 2.13 demonstrates that the concentration of cimetidine in a scenario consisting uniquely of passive diffusion will be higher in the plasma layer than in the breast milk.

As such in order for the cimetidine particles to move into the breast milk layer in lieu of an adverse concentration gradient, a differential potential is required to facilitate the movement.

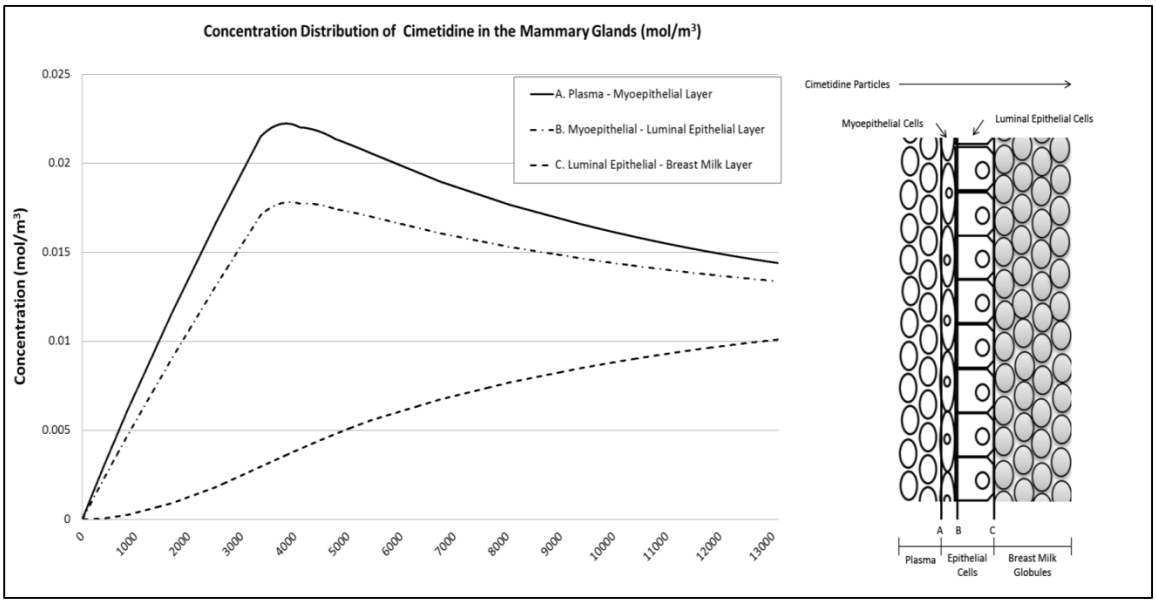

FIGURE 2.13 PASSIVE DIFFUSION OF CIMETIDINE INTO THE BREAST MILK

Figure 2.14 represents active diffusion of cimetidine into the breast milk. It demonstrates the role that the voltage plays in transporting drugs into the breast milk against the concentration gradient. As such our model incorporates a voltage applied to the epithelial cells boundary layers (Fig. 2.6) to examine the impact of the electrical potential. Our work demonstrates that an M/P ratio of approximately 5 is attained resulting in a higher concentration in the breast milk. This is consistent with the available experimental results [34, 70]. Fig. 2.15 displays the effect of a voltage on the same boundary layers when subjected to a variant concentration. It can be seen that along a variant concentration of cimetidine the layers

are playing a key role in transporting the particles into the breast milk. In contrast with the previous scenarios of passive diffusion, the epithelial layers have a high concentration of cimetidine in their domains demonstrating their active role in the movement of cimetidine.

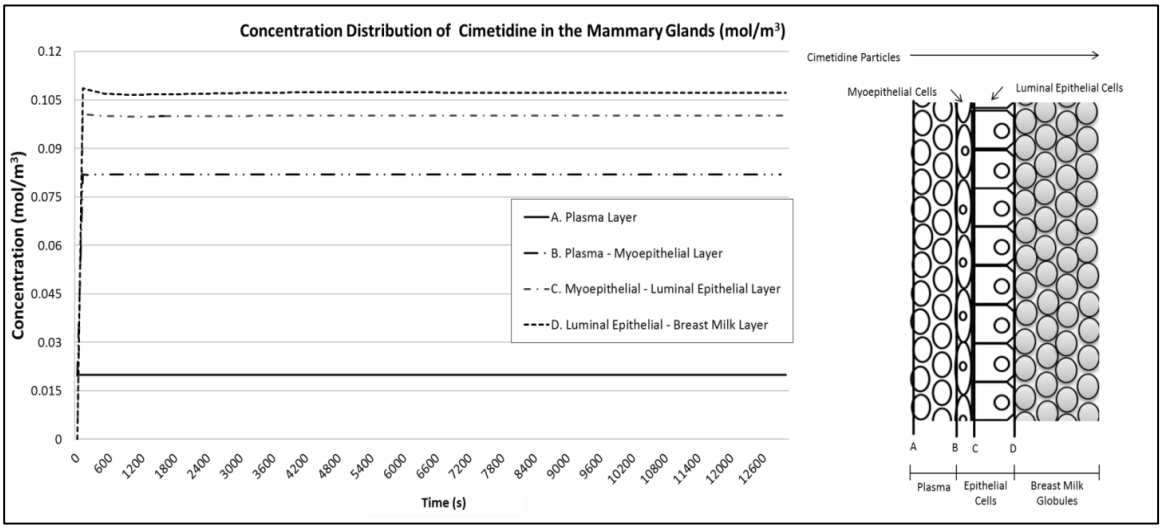

FIGURE 2.14 ACTIVE DIFFUSION OF CIMETIDINE INTO THE BREAST MILK

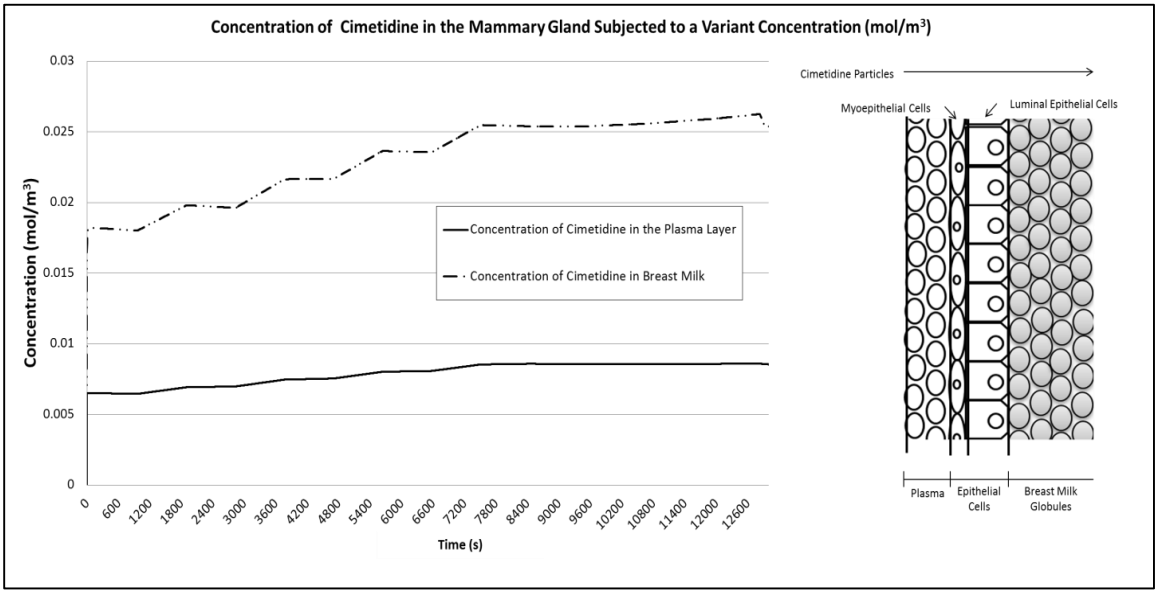

FIGURE 2.15 ACTIVE DIFFUSION OF CIMETIDINE INTO THE BREAST MILK SUBJECT TO A VARIANT CONCENTRATION

CONCLUSIONS

Transport within mammary glands is analyzed in this work. A comprehensive multi-layer model incorporating the plasma, myoepithelial cells, luminal epithelial cells and breast milk globules is constructed. The dermal exposure through the skin layer and the adipose tissue is also analyzed in this work. The results match and confirm the known experimental measurements with respect to the concentration trends and values as well as the milk to plasma ratio (M/P). The utilization of porous media to represent different layers takes into account the presence of blood vessels and interstitial space.

Our model predicts the transport of toxins in the mammary glands. We have been able to predict the bioaccumulation of toxins in the tissues as well as the toxicity level present in the breast milk. The prediction of the milk to plasma ratio during the lactation period is still not fully understood in the pharmaceutical as well as agencies dealing with toxicology assessment. We have been able to predict this ratio within our model.

3. CHAPTER 3. THERMAL EFFECTS ON TRANSPORT IN THE RESTING MAMMARY GLANDS

INTRODUCTION

Scientific Research has shown evidence of the relationship between exposure to toxins and the high risk of developing breast cancer [81]. Industrialized countries have higher exposure compared to non-industrialized nations. Immigration to industrialized countries and adopting a western lifestyle appears to increase the risk of developing breast cancer due to food additives or contaminants present in the diet and a higher exposure to environmental pollutants [22, 24, 25, 81]. Less than ten percent of the carcinogens we are exposed to have been tested and new chemicals are constantly found every year [82]. Tougher regulations from the United States have helped reduce breast cancer rates. Lately, most of the chemicals we are exposed to are being banned or controlled such as polycyclic aromatic hydrocarbons (PAHs), second hand smoke and other air pollutants. The impact of these regulations can be significant in reducing breast cancer risk [81].

Compared to men, women have a higher presence of toxins stored in the adipose tissue [81]. This has been corroborated through tissue and breast fluid sampling done in women, in which traces of carcinogens have been found [83]. Testing done on young girls and infants also has shown detectable levels of toxins caused by the early exposure to chemicals through the maternal serum, placenta and breast milk [81]. Timing plays a

key role in the environmental impact and the increased risk of breast cancer, research has shown that early exposure during breast development from prenatal to puberty increases the risk compared to late exposure [84].

Breast cancer diagnoses are increasing annually in the United States. Most of the cases develop in the ductal epithelium layers [85]. Women with abnormal epithelial cells in the mammary gland have a higher risk of developing breast cancer [86]. The development of breast cancer due to this abnormal growth is linked to morphological changes in the duct lining [86]. The type of breast cancer where malignant cells are confined in the ductal epithelium is called Ductal Carcinoma in Situ (DCIS) [87]. Less than half of the patients diagnosed with DCIS will develop invasive cancer [87]. A hypothesis for the development of breast cancer in the ductal epithelium is the accumulation of toxins in the area causing a carcinogenic microenvironment that would eventually disrupt cells [31]. A promising approach to detect the conditions of the epithelial cells lining the ducts is to test the breast fluids through minimally invasive methods.

To minimize the invasiveness clinicians are turning their attention to ductal lavage. Ductal lavage allows them to collect epithelial cells for cytologic evaluation [86]. Intraductal lavage also gives clinicians a chance to diagnose, treat and analyze DCIS [88]. An investigation of breast fluids in the ducts could detect the presence of breast cancer [89]. Nipple aspiration technique has also proven the potential to examine the conditions of the fluid. Experimental analysis done in a cohort of women demonstrated

a relationship between cytological epithelial in Nipple Aspirate Fluid (NAF) and breast cancer incidence [90]. Detection of abnormal epithelial cells after performing NAF has shown a strong relationship with breast cancer [86]. In the case of Ductal Carcinoma in Situ, being able to locally treat the cancer by introducing therapies through the nipple in order to reach the ductal epithelium will improve the chances of avoiding mastectomy, this technique could also be useful for drug delivery therapies[91].

In a Pre-surgery study done by Mahoney et al. they corroborated the feasibility of instilling localized therapies in the breast ductal system [87]. Different therapies are utilized to eradicate breast tumors. A useful technique is the utilization of heat sources to focus a direct heat flux on the breast cancerous site. The heat sources commonly used are ultrasound, laser-induced heat or radio wave radiation [92]. Hyperthermia in cancer treatment facilitates drug delivery, tumor eradication and it could eventually cause tumor regression if nanotubes are introduced along with thermal therapy [93, 94]. The utilization of heat to treat superficial tumors was described more than 5 millenniums ago in an Egyptian papyrus [95]. In current times the application of heat has proven to be an effective method combined with radiation to control breast cancer recurrences on the long term basis [95].

Drugs and toxins can be introduced into the bloodstream through dermal patches, ingestion or injection among other methods. Several publications have demonstrated the significant effect increasing the temperature has on the properties of the layers. As

the temperature rises, there is an increase in the permeability affecting directly the diffusion coefficient causing an increase in drug transport [96-99]. A better knowledge of the combination of drug therapies and hyperthermia is needed to analyze the new technologies to treat breast cancer. The aim of this research is the development of a comprehensive multi-layer mass transfer model of the resting mammary glands. Our main focus is in the breast ductal area where most of the cancer develops. Our computational model will provide a quantitative and qualitative analysis of the transport of toxins into the ducts and a unique thermal analysis is also discussed in this paper. The utilization of computational models to predict and analyze the transport in the mammary glands and arteries has been established previously by our research group [27-29, 97, 100, 101]. Other research groups have also stated the importance of analyzing mathematically drug delivery [102-104].

FORMULATION

MULTI-LAYER MODEL

Experimental procedures done in the resting human mammary gland to detect toxicity levels are challenging and invasive [31, 87]. Considering these limitations, research has shown that ductal lavage could provide an assessment of the breast cells conditions without being too invasive. In this work we present a theoretical approach to analyze the transport in the mammary glands. A multi-layer model is proposed considering the transport of toxins in the skin and in the bloodstream. The present work

takes into consideration the particle size and properties of the layers. The transport takes into consideration the properties of each of the layers. The movement of the particles across the layers occurs by diffusion, it can be passive or by facilitated diffusion. In this case the facilitated diffusion utilizes heat to improve the transport of particles into the breast ducts. In the passive or free diffusion the movements occurs due to the high to low concentration gradient. All the layers with the exception of the breast duct cavities are acting as diffusion barriers for the particles. The dimensions and properties of the layers utilized on the simulations are shown in Tables 3.1-3.3.

TABLE 3.11. SOLUTE PROPERTIES

Caffeine Particle		**References**
Particle Radius	$3.7x10^{-10}$[m]	[100]
Molecular Weight	194.1[g]	
Cimetidine Particle		
Particle Radius	$5.5x10^{-10}$[m]	[100]
Molecular Weight	252.3[g]	
Nicotine		
Particle Radius	$3.2x10^{-14}$[m]	[100, 105]
Molecular Weight	162.2[g]	
Aspirin		
Particle Radius	$2.2x10^{-10}$[m]	[100, 106]
Molecular Weight	180.1[g]	

TABLE 3.12. PHYSIOLOGICAL DATA USED IN THE SIMULATION OF THE RESTING MAMMARY GLAND

Layers	Parameters	Value	Units	References
Epidermis	Thickness	$500\ x10^{-6}$	$[m]$	[107]
	Density	1125	$[kg/m^3]$	[108]
	Dynamic Viscosity	15	$[Pa\ s]$	[109]
	Porosity	0.2	[1]	[110]
Dermis	Thickness	$1000x10^{-6}$	$[m]$	[107]
	Density	1047	$[kg/m^3]$	[108]
	Dynamic Viscosity	15	$[Pa\ s]$	[109]
	Porosity	0.2	[1]	[110]
Basement Membrane	Thickness	$1\ x10^{-6}$	$[m]$	[111]
	Density	1190	$[kg/m^3]$	[112]
	Dynamic Viscosity	48	$[Pa\ s]$	[113]
	Porosity	0.02	[1]	[114]
Endothelial Cells	Thickness	$1x10^{-6}$	$[m]$	[37]
	Density	1057	$[kg/m^3]$	[28]
	Dynamic Viscosity	$0.72x10^{-3}$	$[Pa\ s]$	[28]
	Porosity	$5x10^{-4}$	[1]	[28]
Plasma Layer	Thickness	$3x10^{-6}$	$[m]$	[37]
	Density	1139	$[kg/m^3]$	[38]
	Dynamic Viscosity	$1.5x10^{-3}$	$[Pa\ s]$	[39]
Adipose Tissue	Thickness	$600x10^{-6}$	$[m]$	
	Density	900	$[kg/m^3]$	[111]
	Dynamic Viscosity	$40x10^{-3}$	$[Pa\ s]$	[111]
	Porosity	0.17	[1]	[115]
Epithelial Cells	Thickness	$17x10^{-6}$	$[m]$	[40]
	Density	1160	$[kg/m^3]$	[43]
	Dynamic Viscosity	$1.1x10^{-3}$	$[Pa\ s]$	[42]
	Porosity	$5x10^{-4}$	[1]	[28]

TABLE 3.13. THERMOPHYSICAL DATA USED IN THE SIMULATION OF THE RESTING MAMMARY GLAND

Layers	Parameters	Value	Units	References
Epidermis	Heat Capacity	3437	$[J/(kg*K)]$	[101, 108]
	Thermal Conductivity	0.35	$[W/(m*K)]$	[101, 108]
	Metabolic Heat Source	1620	[W/m^3]	[101, 108]
	Blood Perfusion Rate	0.02	[1/s]	[101, 108]
Dermis	Heat Capacity	3437	$[J/(kg*K)]$	[101, 108]
	Thermal Conductivity	0.35	$[W/(m*K)]$	[101, 108]
	Metabolic Heat Source	1620	[W/m^3]	[101, 108]
	Blood Perfusion Rate	0.02	[1/s]	[101, 108]
Basement Membrane	Heat Capacity	3437	$[J/(kg*K)]$	[101, 108]
	Thermal Conductivity	0.35	$[W/(m*K)]$	[101, 108]
	Metabolic Heat Source	1620	[W/m^3]	[101, 108]
	Blood Perfusion Rate	0.02	[1/s]	[101, 108]
Endothelial Cells	Heat Capacity	3500	$[J/(kg*K)]$	[101, 108]
	Thermal Conductivity	0.6	$[W/(m*K)]$	[101, 108]
	Metabolic Heat Source	9500	[W/m^3]	[101, 108]
	Blood Perfusion Rate	1.39x10^{-2}	[1/s]	[101, 108]
Plasma Layer	Heat Capacity	3960	$[J/(kg*K)]$	[101, 108]
	Thermal Conductivity	0.45	$[W/(m*K)]$	[101, 108]
Adipose Tissue	Heat Capacity	2300	$[J/(kg*K)]$	[101, 108]
	Thermal Conductivity	0.22	$[W/(m*K)]$	[101, 108]
	Metabolic Heat Source	300	[W/m^3]	[101, 108]
	Blood Perfusion Rate	4.58x10^{-4}	[1/s]	[101, 108]
Epithelial Cells	Heat Capacity	3500	$[J/(kg*K)]$	[101, 108]
	Thermal Conductivity	0.6	$[W/(m*K)]$	[101, 108]
	Metabolic Heat Source	9500	[W/m^3]	[101, 108]
	Blood Perfusion Rate	1.39x10^{-2}	[1/s]	[101, 108]

The present work takes into consideration the particle size and properties of the layers. These properties affect the ratio of toxins that are able to reach the breast fluid. Our work demonstrates how the toxins enter various layers over a determined period of time making it physiologically pertinent for drug delivery. Some limitations do exist for our parameter estimation due to the invasiveness of developing experimental work in the breast tissue in order to estimate diffusion coefficients. For this reason, the diffusion coefficients for different layers were obtained using the Stokes-Einstein equation [100]. The Stokes-Einstein relationship has proven to be useful to estimate the diffusion coefficients of proteins, sugar and other small molecules in prior works [51-56, 100, 116].

PASSIVE DIFFUSION OF CAFFEINE, CIMETIDINE, ASPIRIN AND NICOTINE INTO THE BREAST DUCTS

The caffeine, cimetidine and aspirin transport mechanism initiates with the intake of these drugs as medication or in beverages and food. The substances enter the bloodstream gradually until they reach the peak concentration in the blood and start showing traces in the breast ducts that can be detected after performing a ductal lavage procedure [31, 87, 88]. Caffeine, aspirin, cimetidine and nicotine passively diffuse into the breast duct cavities. In contrast with the lactating mammary glands the transport of cimetidine in the resting mammary gland is not active as it moves through the mammary gland by passive diffusion. To estimate the particle properties we used

Stokes-Einstein radius. Stokes-Einstein radius is the radius of the diffusive molecule in the solvent and it is also commonly referred as hydrodynamic radius. The Stokes-Einstein radius is utilized when the Stokes-Einstein equation is considered to estimate diffusion coefficients [100]. Nicotine has a smaller stokes radius compared with caffeine, aspirin and cimetidine giving it a faster and higher diffusion rate. The concentration will be zero at the start but its presence in the bloodstream will increase over time. After time zero the concentration will start increasing over a period of time. The time selected for each molecule is dependent on the time it requires to reach the peak concentration before the body starts clearing the toxins from the bloodstream.

The passive transport mechanism of the particles can be represented by:

$$\varepsilon \frac{\partial c}{\partial t} + \nabla \bullet (-D\nabla \vec{c}) = S \qquad (3.1)$$

where c is the concentration of caffeine, cimetidine, aspirin or nicotine, D the diffusion coefficient, ε the porosity and S is the source term. Each diffusion barrier has a characteristic porosity, diffusion coefficient and an initial concentration to initiate the transport mechanism. A source term is also incorporated in the governing equation to accommodate the physiological mechanism of the particles entering the bloodstream after ingestion.

DERMAL DIFFUSION OF ASPIRIN AND NICOTINE INTO THE BREAST DUCTS

In the dermal diffusion scenario we introduce the epidermis and dermis layer. The thickness of these layers is specified in Table 3.2. The diffusion moves from the boundary of the epidermis and gets introduced by passive diffusion into the bloodstream until it reaches the breast ducts. The ratio of concentration expected to get into the breast duct is comparable to the movement from the bloodstream into the breast ducts shown in the previous section. Nicotine and aspirin patches provide a controlled drug dosage. To simulate this process we introduce a Drug Flux at the epidermis layer. The drug has to pass through several diffusion barriers before reaching the breast ducts. To encompass a generalized scenario the present work is considering the average properties of the mammary glands which incorporates different breast densities or ethnicities of the participants in breast cancer studies [25, 117-120]. The dermal transport can be represented by equation 3.1.

IMPACT ON THE DIFFUSION OF PARTICLES DUE TO HEAT LOAD

For this scenario we introduce an imposed heat load on the biological tissue. The thermophysical properties of different layers are given in Table 3.3. We utilize the Stokes-Einstein equation (Equation 3.2) which incorporates the thermal effect on mass diffusion.

$$D = \frac{k_B T}{6\pi\eta r} \qquad (3.2)$$

where D is the diffusion coefficient, k_B is the Boltzmann Constant ($1.38x10^{-23}$ m^2kg/s^2K), η is the viscosity of the layer and r is the radius of the molecule and T is the temperature at each of the layers which will be affected by the heat flux applied at the epidermis upper boundary layer. The Stokes-Einstein equation was used for all the layers except the dermis and epidermis. In these layers we utilized diffusion coefficients available in the literature and incorporated the temperature effect based on enhancement factors for the increase in temperature [121, 122].

The temperature distribution is governed by the energy equation while incorporating the biological heat generation:

$$\rho c_p \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + Q_{BIO}$$
$$Q_{BIO} = \rho_B c_B \omega_B (T_b - T) + Q_{MET} \qquad (3.3)$$

where ρ is the density, T the temperature, c_P the heat capacity, and k is the thermal conductivity of each of the layers. Q_{BIO} is dependent on the blood properties in which ρ_B represents the density, C_B the heat capacity, ω_B is the blood perfusion rate and T_B is the blood temperature. The value of Q_{MET} was obtained from the literature [108].

These equations provide a feasible way to model the heat transfer in different layers and the tissues. Biological tissues have heat limitations, for this reason we will expose the mammary gland only for 6 minutes to a heat flux of 500, 1000 and 2000 watts per meter square [101, 108].

RESULTS AND DISCUSSION

Diffusion of Caffeine and Cimetidine into the Breast Ducts

During the breastfeeding phase the mammary glands are fully developed to facilitate the diffusion of nutrients, immune factors as well as toxins as demonstrated in a prior work [100]. In contrast to this scenario the resting mammary gland does not facilitate the diffusion of toxins into the breast ducts and the structure is completely different as shown in the histology published by anatomy atlases [123]. In the upper right side of Fig. 3.1 the layers involved in the transport of toxins from the bloodstream into the breast ducts are illustrated. Caffeine and Cimetidine have stokes radius in the angstrom scale facilitating the movement through several layers surrounding the breast ducts. In Fig. 3.1 the concentration of cimetidine and caffeine entering the bloodstream is shown and the percentage that is able to cross through the diffusion barriers before reaching the breast ducts is displayed.

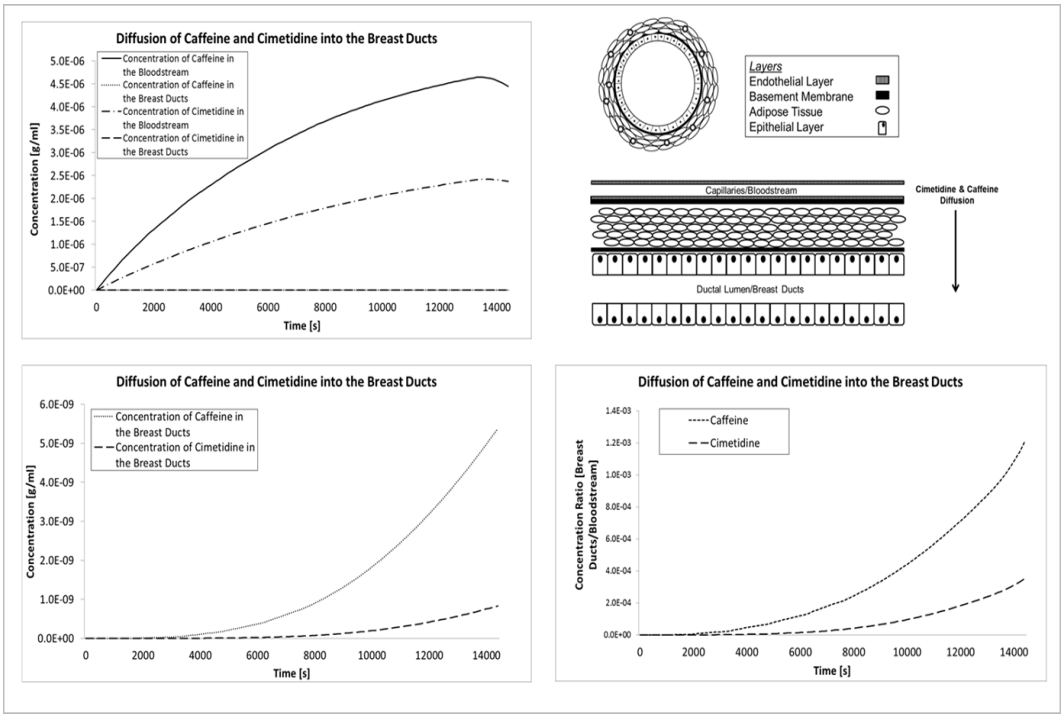

FIGURE 3.1 PASSIVE DIFFUSION OF CAFFEINE AND CIMETIDINE INTO THE BREAST DUCTS

Since the concentration entering the breast ducts is at least three orders of magnitude smaller we have enlarged the plot on the lower left side of Fig. 3.1 to illustrate the distribution in more detail. In what follows we demonstrate that the results from our computational simulation of the transport of cimetidine and caffeine are in agreement with the experimental data.

In order to verify the results, we compared our computational model results with the experimental work done by the Doctor Susan Love Research Foundation (DSLRF). In their experimental work they tested and analyzed the levels of toxins for caffeine and cimetidine found in the blood and in the breast fluid after the ingestion of caffeine and cimetidine. The breast fluid was extracted from the breast ducts through a ductal lavage

procedure. Based on the experimental data the percentage which diffused into the breast ducts is less than 0.01 percent. Detecting toxins in the duct lining is disquieting because it represents a bioaccumulation of toxins that could potentially cause DNA damage leading to DCIS [31, 87, 88]. In the lower right side of Fig. 3.1 we demonstrate that as in the ductal lavages performed by the DSLRF the concentration entering the breast ducts after a period of time is at least three orders of magnitude smaller. The comparison between our model results and the available literature concentration values, which also include nicotine and aspirin, is shown in detail in Table 3.4. As can be seen overall, quite a good agreement can be seen.

TABLE 3.14. COMPARISON OF THE CONCENTRATIONS OBTAINED BY THE PRESENT WORK OUR MODEL WITH THE AVAILABLE EXPERIMENTAL CONCENTRATION VALUES IN THE LITERATURE.

Time [sec]	Model	Bloodstream		Breast Tissue		Diffusion Percentage		References
		Experimental Concentration [ng/ml]	Model Concentration [ng/ml]	Experimental Concentration [ng/ml]	Model Concentration [ng/ml]	Experimental	Model	
14400	Passive Diffusion of Caffeine into the Breast Tissue	4500	4450	6	5	0.1	0.1	[31]
	Passive Diffusion of Cimetidine into the Breast Tissue	2500	2372	4	1	0.1	.04	[31]
1800	Passive Diffusion of Nicotine into the Breast Tissue	31-50	44	40-56	38	**85**	**86**	**[124]**
7200	Passive Diffusion of Aspirin into the Breast Tissue	105000	106436	NA	1	**NA**	**0.06**	**[125]**

DIFFUSION OF ASPIRIN AND NICOTINE INTO THE BREAST DUCTS

Aspirin is a common drug ingested in small dosages. Aspirin stokes radius is in the angstrom scale. Considering the particle size one would expect it to follow the same behavior as cimetidine and caffeine. Testing of these molecules has not been done, but it is a molecule that could be considered for future ductal lavage procedures. Considering these factors, we investigated the diffusion of this particle into the breast

ducts. As expected the diffusion of this molecule was three orders of magnitude lower than the concentration present in the bloodstream.

Another molecule that has been constantly tested in breast fluids is nicotine. Nicotine has demonstrated an ability to enable the abnormal growth of cells [126]. High levels of nicotine are found in the breast fluids after a ductal lavage. Most of the concentration in the bloodstream is detected in the breast fluids after a short period of time. Given these conditions, we have investigated the transport of nicotine into the breast fluid and have compared the results with experimental data available in the literature [124, 127].

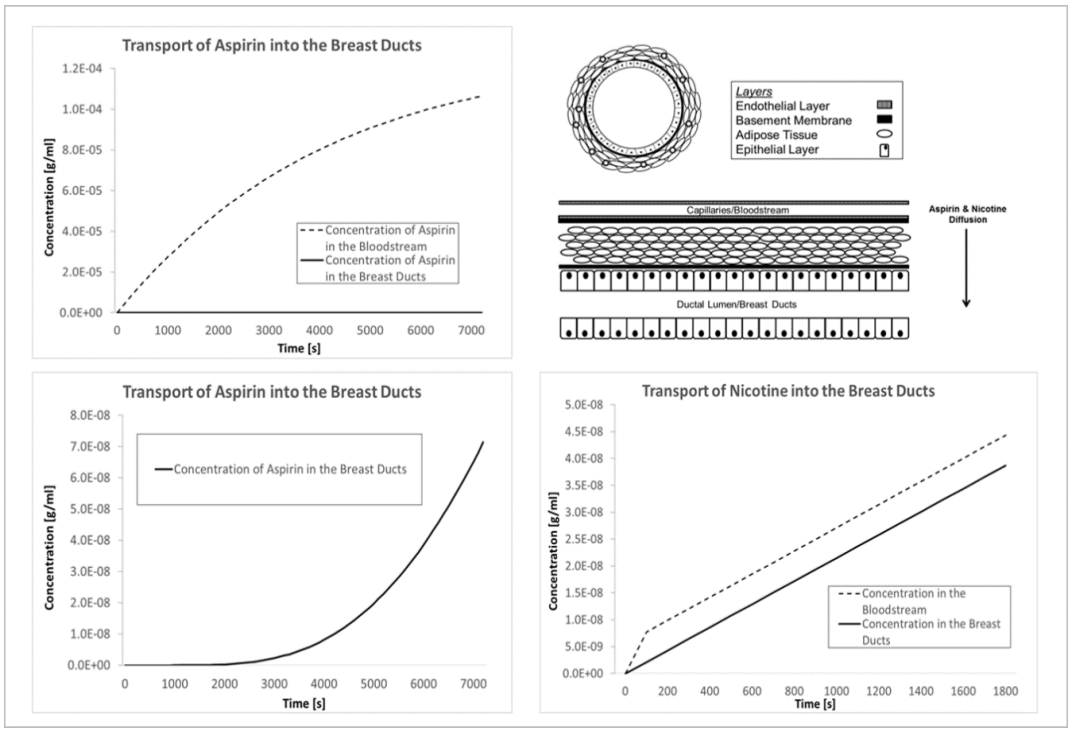

FIGURE 3.2 PASSIVE DIFFUSION OF ASPIRIN AND NICOTINE INTO THE BREAST DUCTS

In Fig. 3.2 we can visualize that at least 85 percent of the concentration in the bloodstream enters the breast ducts. The diffusion is facilitated by the reduced particle stokes radius which can easily penetrate through the diffusion barriers lining the breast ducts. In Table 3.4 we show the comparison between our model and the experimental results, demonstrating a very good agreement.

DERMAL DIFFUSION OF ASPIRIN AND NICOTINE INTO THE BREAST DUCTS

Pharmaceutical companies are constantly innovating and seeking the best drug delivery methods for their customers. In order to facilitate and control the dosage of medication some drugs are delivered dermally through patches. This convenient drug delivery method is becoming popular due to its simple utilization and its advantages. To simulate dermal diffusion we introduce two additional diffusion barriers: the epidermis and dermis. The particles need to be able to trespass these two barriers before reaching the capillaries. Once the drug enters the bloodstream, a percentage of these molecules are transported into the breast ducts as shown in Fig. 3.3.

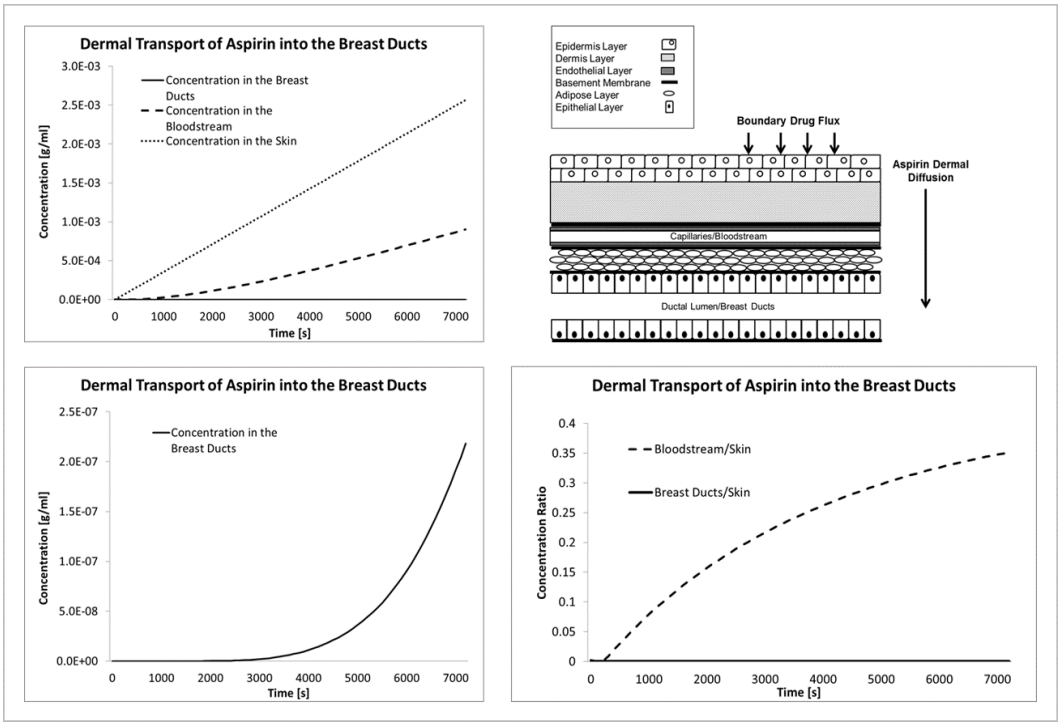

FIGURE 3.3 DERMAL TRANSPORT OF ASPIRIN INTO THE BREAST DUCTS

For the dermal transport of aspirin, experimental work has demonstrated that at least 32 percent of the concentration will diffuse into the bloodstream and that at least 0.02 percent will be introduced into the breast ducts [128]. Our computational simulation predicts the experimental results shown in literature as shown in Table 3.5.

TABLE 3.15. COMPARISON OF OUR RESULTS FOR THE DERMAL DIFFUSION OF ASPIRIN AND NICOTINE WITH AVAILABLE EXPERIMENTAL RESULTS

Time [sec]	**Model**	**Percentage Diffused into the Bloodstream**		**Percentage Diffused into the Breast Ducts**		**References**
		Experimental	**Model**	**Experimental**	**Model**	
7200	Passive Diffusion of Aspirin into the Breast Tissue	32%	35%	NA	0.02%	[128]
1800	Passive Diffusion of Nicotine into the Breast Tissue	100%	100%	100%	100%	[124]

Nicotine patches are popular to reduce addictions. For this reason in this scenario we also modelled the dermal transport of nicotine until it reaches the breast ducts. Figure 3.4 demonstrates that nicotine can easily penetrate into the breast ducts. Most of the nicotine entering the bloodstream from the skin layers is transported into the breast ducts as expected. In Table 3.5 a comparison between our model and the experimental data from the available literature is shown. It can be seen that overall a very good agreement exists between our results and the experimental data.

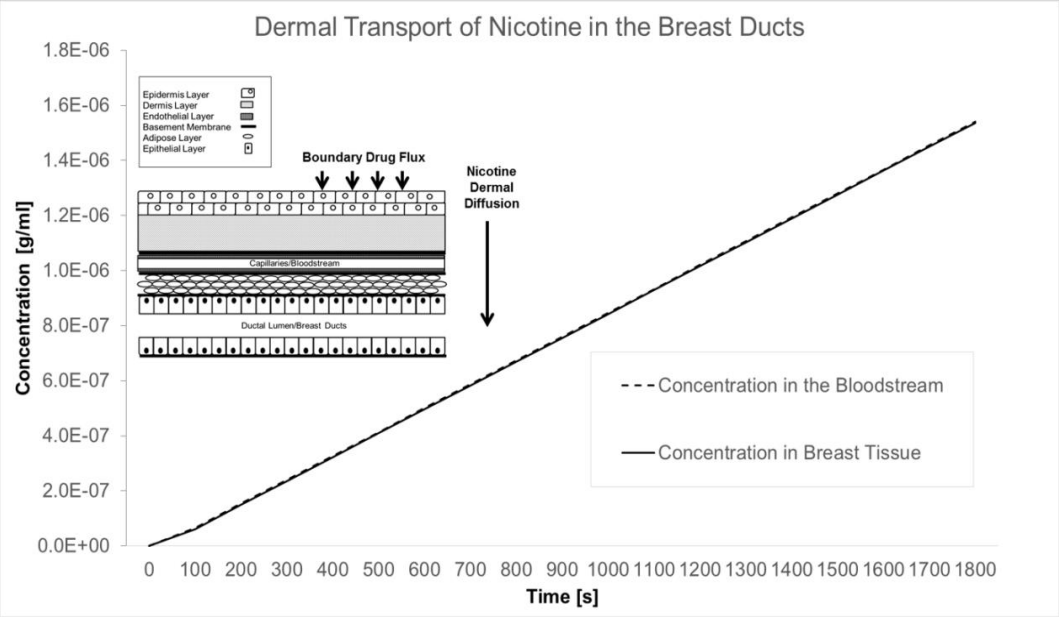

FIGURE 3.4 DERMAL TRANSPORT OF NICOTINE INTO THE BREAST DUCTS

THE EFFECT OF DERMAL HEAT EXPOSURE IN THE DIFFUSION OF CAFFEINE, CIMETIDINE AND ASPIRIN INTO THE BREAST DUCTS

Our model is able to analyze the bioheat transport in a micro and macro scale mostly focusing on thermal cancer therapies [97, 99, 129, 130]. Increasing the tissue and capillaries temperature can show a significant impact in the drug delivery considering that the temperatures in the organs, tissues and blood vessels are not the same after heat exposure [131, 132]. Understanding temperature distributions makes possible the effectiveness of thermal therapies for cancer [97]. Mathematical models are helpful in analyzing temperature distributions when exploring imposing high heat fluxes for short periods of times [133]. Irreversible damage can occur when temperatures increase

abruptly, considering these factors we considered prolonged exposure to lower heat fluxes [134].

In our earlier presentation of the results we had shown that caffeine, cimetidine and aspirin are not present in the breast ducts at high concentrations. To study the effects of hyperthermia on the transport of the cited substances the epidermis is subjected to a constant heat flux. The fluxes selected were 500, 1000 and 2000 w/m^2 [108]. These three fluxes were applied to the skin. As expected the applied heat flux would increase the temperature in each of the layers. The temperature in the breast ducts and the bloodstream is shown in Fig. 3.5.

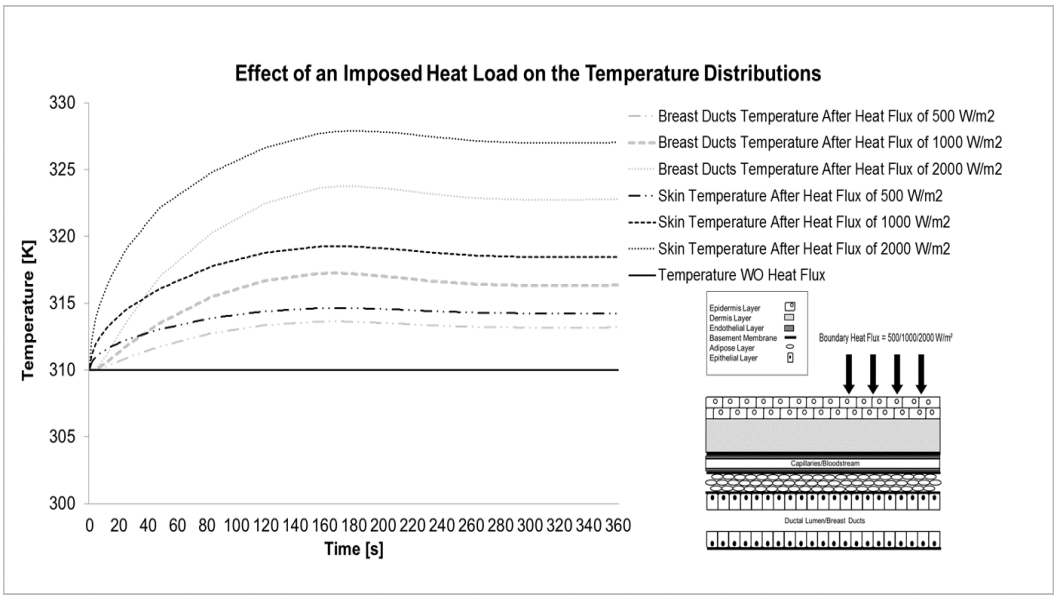

FIGURE 3.5. THE EFFECT OF AN IMPOSED HEAT LOAD ON THE TEMPERATURE DISTRIBUTIONS WITHIN DIFFERENT LAYERS

The initial temperature considered is 310 K in all the layers [135]. The maximum temperature reached in the bloodstream was 327 K (54C) and the minimum is 314 K (41C) which are comparable to hyperthermia therapies [92, 99, 121]. Fig 3.5 also shows the reduced influence of the heat flux effect based on the distance separating the layers from the heat source as has been demonstrated in prior publications [97, 99, 136]. Hyperthermia takes into account temperatures ranging from 42 to 50 C. The treatment is only done for several minutes before a significant part of the tissue undergoes severe necrosis [137]. Figure 6 shows the effect of an imposed heat load on the diffusion of caffeine, cimetidine and aspirin. The imposed heat flux increases the transport of the cited substances by 10 to 25 percent as shown in Table 3.6.

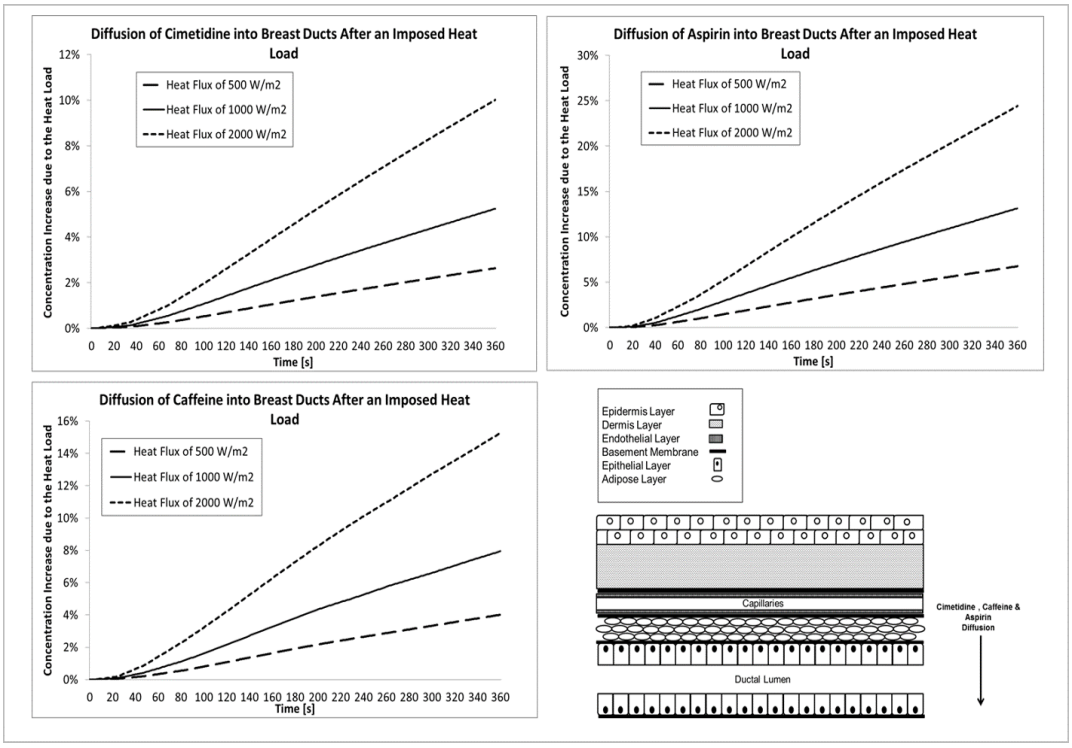

FIGURE 3.6. THE EFFECT OF HYPERTHERMIA ON THE CONCENTRATION DISTRIBUTION OF CIMETIDINE, ASPIRIN AND CAFFEINE.

TABLE 3.16. THE EFFECT OF HEAT LOAD ON THE CONCENTRATION ENHANCEMENT OF CAFFEINE, CIMETIDINE AND ASPIRIN

Time [sec]	Model	Maximum Concentration Enhancement at the Epithelial/Breast Duct Interface	References
360	Diffusion of Caffeine into the Breast Ducts	15%	[121]
360	Diffusion of Cimetidine into the Breast Ducts	10%	[121]
360	Diffusion of Aspirin into the Breast Ducts	25%	[121]

CONCLUSIONS

The thermal effects on the transport of a number of common substances such as caffeine, cimetidine, aspirin and nicotine are analyzed in this work. The utilization of a comprehensive multi-layer model incorporating the breast ducts and the dermal layers provided results in agreement with the experimental data. The biological tissues and the vascular system metabolic heat generation, blood-tissue convective heat exchange and the imposed heat load were taken into account. Our model was able to predict the transport of toxins into the breast ducts. The inclusion of a heat flux on the skin has a substantial effect on the diffusion of toxins into the breast ducts. The enhancement of particle diffusion due to heat load could potentially help in future drug delivery prescription methods.

DISSERTATION CONCLUSION

The transport of a number of common toxins such as caffeine, cimetidine, aspirin and nicotine are analyzed in this dissertation. The introduction of a theoretical approach is unique in the area. On this dissertation we presented the advantages of utilizing a comprehensive multi-layer model incorporating the properties of the mammary glands in both stages: lactating and resting. Our unique approach demonstrated agreement with the available experimental data. Our models are able to predict the transport of toxins into the breast ducts and breast milk by incorporating the properties of the biological tissues. The present work could potentially help in future drug delivery developments.

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