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Modeling and analysis of transport in the mammary glands

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Abstract

The transport of three 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. This work 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 performed takes into account the unique transport mechanisms for each of the toxins. Our model predicts the movement of toxins and/or drugs within the mammary glands as well as their bioaccumulation in the tissues.

Keywords: breast cancer, breast milk, mammary glands, breastfeeding, porous media

(Some figures may appear in colour only in the online journal)

Nomenclature

C	Concentration [mol m^{-3}]
S	Source [mol $(m^3 s)^{-1}$]
ε	Porosity
D	Diffusion [m s^{-2}]
J	Flux [mol $(m^2 s)^{-1}$]
V	Electrical potential [V]
Z	Charge number
U_m	Mobility [s mol kg^{-1}]
T	Temperature [K]
F	Faraday constant [mol C^{-1}]
E_m	Membrane potential [V]
E	Electric field [V]
K_B	Boltzmann constant [kg m^2 $(s^2$ K$)^{-1}$]
T	Viscosity [Pa s]
r	Radius [m]

Subscripts

BM	Breast milk
PL	Plasma layer

1. Introduction

The female breast initiates development after the sixth week of gestation and reaches its complete development during pregnancy and childbirth [1]. 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' [2]. Mammals are the only ones that possess the capability of milk production and excretion after giving birth [2].

In comparison with other organs in the body, the mammary glands are constantly evolving throughout the different life stages of female mammals. The mammary gland of a newborn is limited to a primitive ductal system with limited endbuds that ultimately differentiate into epithelial cells once

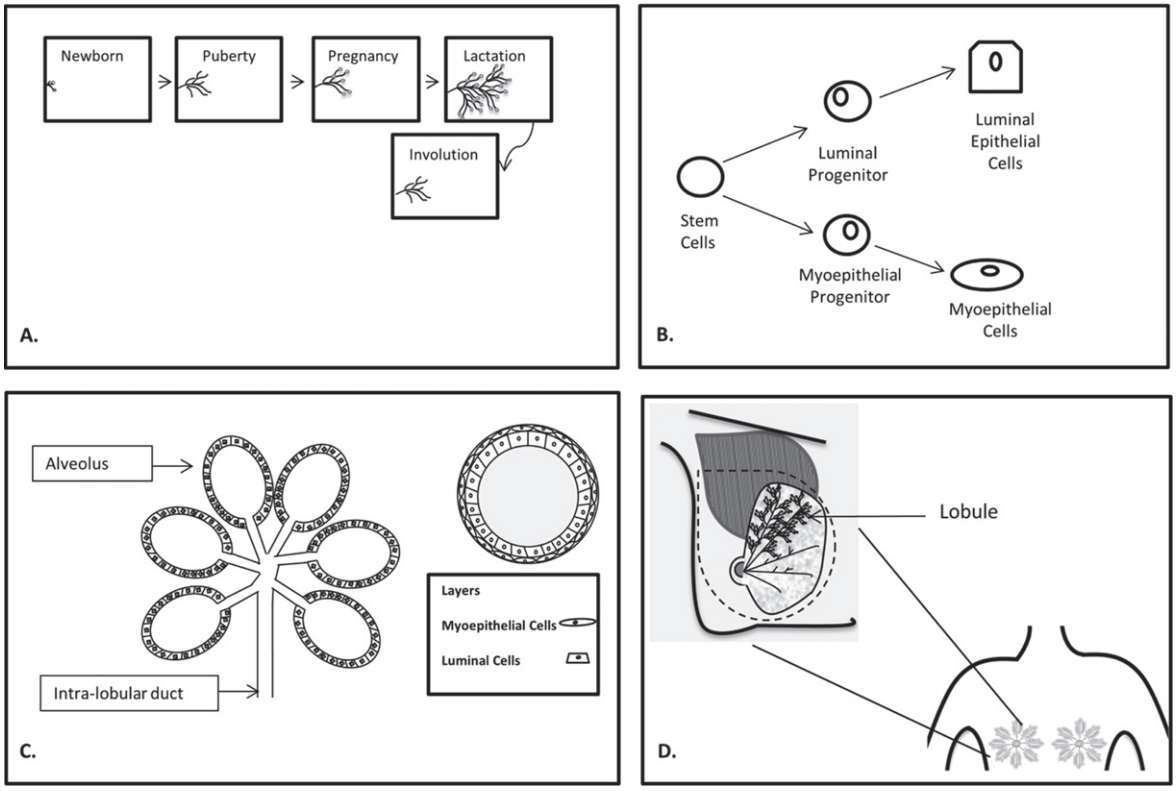

Figure 1. Mammary gland development. (A) Alveologenesis, (B) stem cell differentiation, (C) lobule and (D) breast structure.

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 send 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 [3].

In order for the new mother to fulfill the nutritional needs of the newborn, the mammary gland undergoes several changes, which include alveologenesis (figure 1(A)) 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 1(B)). These two cell types are in control of the contraction and secretion of breast milk during the lactation period [4].

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 1(C)) as well as an arterial system responsible for transferring immune factors, nutrients and some toxins into the breast milk [2]. 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. 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 [5].

The transport mechanism of breast milk initiates in the alveoli. A cluster of alveoli is called a lobule and a group of lobules is called a lobe (figure 1(D)). 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. Changes in diet and environmental factors affect the reliability of the milk transported through the milk ducts [6].

It is believed that an increased number of toxins present in the mammary tissue are responsible for the onset of the breast cancer [7]. 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 [8]. For this reason it is recommended that breast milk samples should be monitored in order to check and control persistent organic pollutants as well as other particles in different regions around the world [9]. It is critical to understand the effects of banned and regulated products on the breastfeeding population in comparison with previous studies [8].

Breast milk sampling has become one of the preferred testing methods. Many countries such as Mexico, Japan, Germany and China, among others, are continously

developing field studies in different regions of their countries in order to establish correlations between breast milk samples, lifestyle and incidence of breast cancer [10–12]. 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 [6].

Different lifestyles, environmental factors as well as a delay in starting a family are increasing the probability of being diagnosed with breast cancer [13]. In the United States, it is expected that one out of eight women will be diagnosed with breast cancer [14]. Research groups have established a relationship between breast milk samples with a higher presence of toxins and countries with higher rates of breast cancer [6, 15, 16].

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 [6, 15, 17, 18]. 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 [19]. 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 breast tumors initiate. Research groups have shown the importance of using mathematical models to address biological questions [20]. The quantitative analysis of the transport of toxins provides additional insight for 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 [21–24].

2. Formulation

2.1. Multi-layer model

Extensive experimental procedures performed in human mammary glands can be challenging and sometimes almost impossible [25]. 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 (M/P 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 [6, 8, 9, 11, 12, 25–30]. This 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 1–5. Our work demonstrates how the toxins enter various layers over a determined period of time, which makes it closer to physiological conditions.

Table 1. Solute properties.

Caffeine particle	
Particle radius	3.7×10^{-10} (m)
Molecular weight	194.1 (g)
DDT particle	
Particle radius	6.3×10^{-10} (m)
Molecular weight	354.9 (g)
Cimetidine particle	
Particle radius	5.5×10^{-10} (m)
Molecular weight	252.3 (g)

The transport mechanism in the mammary gland is shown schematically in figure 2. The arterial blood supplies the toxins, nutrients and immune factors through the luminal cells until it reaches the breast milk cavity [30]. As seen in figure 2, milk globules represent the breast milk produced. The transport occurs by diffusion, which can be active or passive diffusion. Active diffusion utilizes membrane potential or proteins to reach the alveolar cavity while 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 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.

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 the 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 [31–36].

In the epithelial cells, the fluid phase viscosity of the medium is not much higher from that estimated for water. In this case the viscosity used in our work is 1.1 cP for the epithelial layers [37]. To estimate the diffusion coefficient of the solute in a 'cell layer', we approximated it by utilizing the Stokes–Einstein equation (equation (1)) as a starting point. Using this equation we are able to consider the effect of particle size and the viscosity of the cells.

$$D = \frac{k_B T}{6\pi\eta r}, \tag{1}$$

where D is the diffusion coefficient, k_B is Boltzmann's constant $= 1.38 \times 10^{-23}$ m^2 kg (s^{2}K)$^{-1}$, T is the temperature $=$ 310 K, $\eta =$ viscosity of cells $= 1.1c$P, r is the radius of the molecule (cimetidine, caffeine or DDT). The diffusion coefficients obtained with the utilization of the Stokes–Einstein equation gave us a starting point to estimate the diffusion

Table 2. Physiological parameters used in the simulation of alveolar system.

Layers	Parameters	Value	Units	References
Plasma layer	Thickness	5×10^{-6}	[m]	[63]
	Density	1139	[kg m^{-3}]	[64]
	Dynamic viscosity	1.5×10^{-3}	[Pa s]	[65]
Myoepithelial layer	Thickness	8×10^{-6}	[m]	[66]
	Density	1060	[kg m^{-3}]	[67]
	Dynamic viscosity	1.1×10^{-3}	[Pa s]	[37]
	Porosity	5×10^{-4}	—	[22]
Luminal epithelial layer	Thickness	17×10^{-6}	[m]	[66]
	Density	1160	[kg m^{-3}]	[68]
	Dynamic viscosity	1.1×10^{-3}	[Pa s]	[37]
	Porosity	5×10^{-4}	—	[22]
Breast milk layer	Diameter	100×10^{-6}	[m]	[66]
	Density	1139	[kg m^{-3}]	[64, 69]
	Dynamic viscosity	14.7×10^{-3}	[Pa s]	[70, 71]

Table 3. Diffusion coefficients for the transport of caffeine into the breast milk. The diffusion coefficients were estimated using the Stokes–Einstein relationship.

Layers	Parameters	Value	Units
Plasma layer	Diffusion coefficient	4.0×10^{-10}	m s^{-2}
Myoepithelial layer	Diffusion coefficient	5.5×10^{-10}	m s^{-2}
Luminal epithelial layer	Diffusion coefficient	5.5×10^{-10}	m s^{-2}
Breast milk layer	Diffusion coefficient	4.1×10^{-11}	m s^{-2}

Table 4. Diffusion coefficients for the transport of DDT into the breast milk. The diffusion coefficients were estimated using the Stokes–Einstein relationship.

Layers	Parameters	Value	Units
Plasma layer	Diffusion coefficient	2.3×10^{-10}	m s^{-2}
Myoepithelial layer	Diffusion coefficient	3.2×10^{-10}	m s^{-2}
Luminal epithelial layer	Diffusion coefficient	3.2×10^{-10}	m s^{-2}
Breast milk layer	Diffusion coefficient	2.5×10^{-11}	m s^{-2}

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 [38]. Most of the experimental work performed 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 a solvent as discussed in other publications [39].

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 [21–23]. The effective diffusion coefficient of the epithelial cells layers can be estimated using equation (2).

$$D_{eff} = D\varepsilon, \tag{2}$$

where D is the diffusion coefficient, D_{eff} is the effective mass diffusion coefficient and ε is the porosity of the layer. The diffusion coefficients in the ductal epithelium of the mammary gland are not available. After in extensive literature search we were only able to 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 [35]. The solute movement in CACO cells is from the lumen into the bloodstream. Some of the parameters are shown in table 6. Given these parameters, we are able to make the appropriate assumptions for our model.

In order to assess the utilization of the Stokes–Einstein equation, we ran the model utilizing the diffusion coefficient of CACO epithelial cells versus our model of the mammary gland epithelial cells. As shown in figures 3 and 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 performed in the intestine layers, where drugs move from the lumen into the plasma. Other factors are also important in our model, such as porosity, which takes into consideration the transport across a porous membrane.

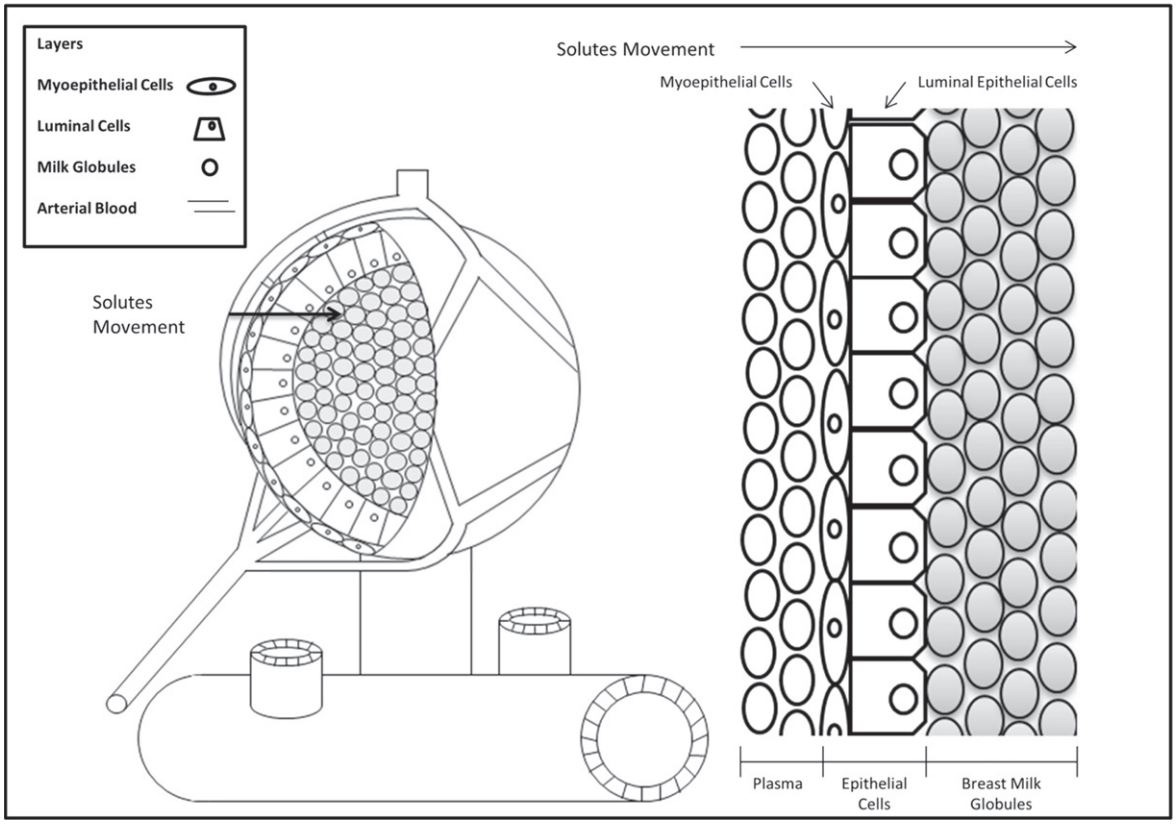

Figure 2. Transport of solutes into the breast milk layer.

Table 5. Physiological parameters used in the simulation of cimetidine in the alveolar system. The diffusion coefficients were estimated using the Stokes–Einstein relationship.

Layers	Parameters	Value	Units	References
Plasma layer	Diffusion coefficient	2.7×10^{-10}	[m s^{-2}]	
	Relative permittivity	0.2	[–]	[72]
	Electrical conductivity	1×10^{7}	[S m^{-1}]	[72]
Myoepithelial layer	Diffusion coefficient	3.6×10^{-10}	[m s^{-2}]	
	Relative permittivity	2.5×10^{7}	[–]	[72]
	Electrical conductivity	0.2	[S m^{-1}]	[72]
Luminal epithelial layer	Diffusion coefficient	3.6×10^{-10}	[m s^{-2}]	
	Relative permittivity	80	[–]	[73]
	Electrical conductivity	0.3	[S m^{-1}]	[74]
Breast milk layer	Diffusion coefficient	2.5×10^{-11}	[m s^{-2}]	
	Relative permittivity	96	[–]	[75]
	Electrical conductivity	740	[S m^{-1}]	[75]

Table 6. Comparison of the estimated diffusion coefficient of the epithelial cells with the available literature values.

Molecule	Porosity [22, 23]	Particle size [m]	Estimated diffusion coefficient ($m^2\ s^{-1}$)	Literature diffusion coefficient ($m^2\ s^{-1}$)	Estimated effective diffusion coefficient ($m^2\ s^{-1}$)[a]	Literature effective diffusion coefficient ($m^2\ s^{-1}$)[a]	References
Caffeine	5×10^{-4}	3.7×10^{-10}	5.5×10^{-10}	—	2.75×10^{-13}	3.75×10^{-13}	[58, 59]
DDT	5×10^{-4}	6.3×10^{-10}	3.2×10^{-10}	—	1.6×10^{-13}	—	[35, 58, 59]
Cimetidine	5×10^{-4}	5.5×10^{-10}	3.7×10^{-10}	7.7×10^{-10}	1.8×10^{-13}	0.72×10^{-13}	[35, 36, 58–60]

[a] Literature references available for epithelial cells surrounding the intestine layer (CACO cells).

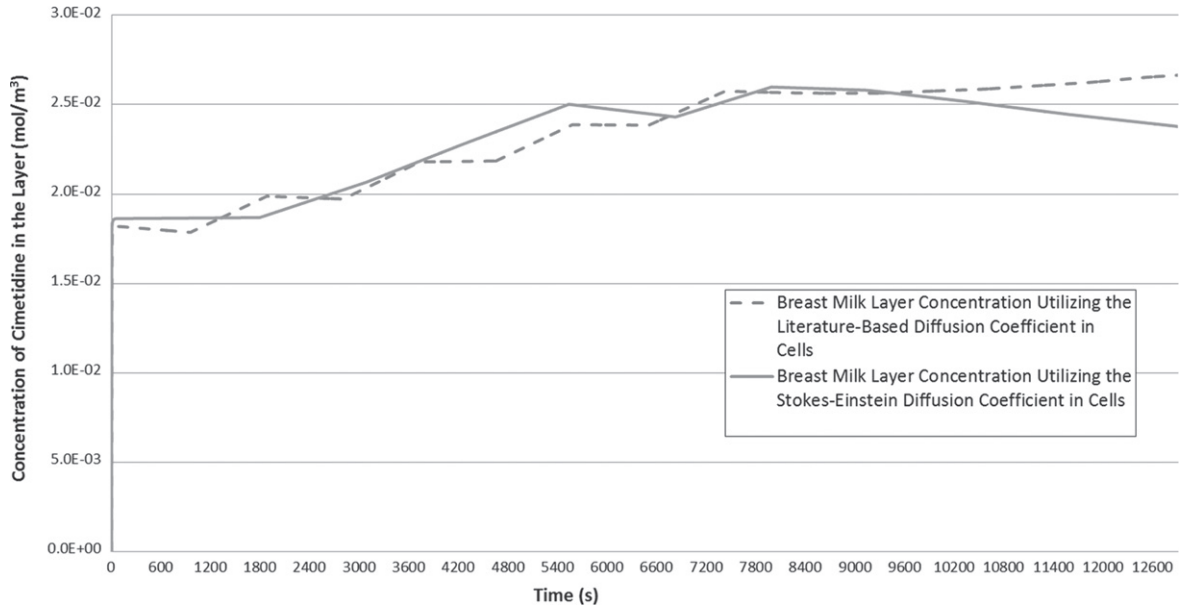

Figure 3. Diffusion coefficient comparison for cimetidine molecule.

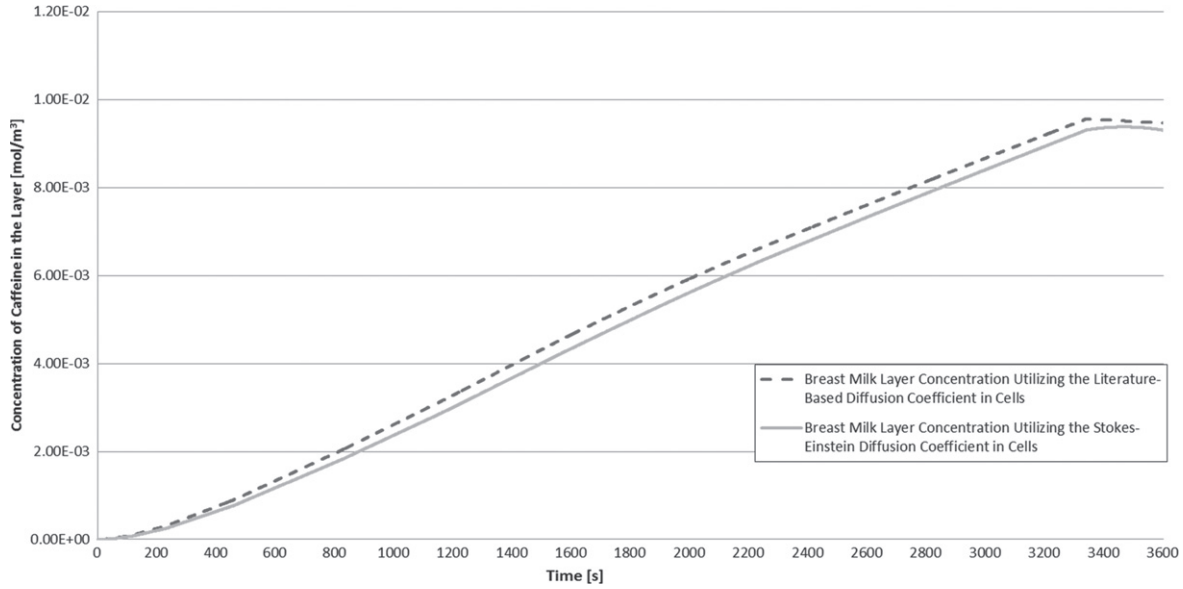

Figure 4. Diffusion coefficient comparison for caffeine molecule.

2.2. Passive diffusion

2.2.1. The transport mechanism of caffeine into breast milk.

Caffeine freely diffuses into the breast milk layer directly from the bloodstream [40–42]. The following two layers, the myoepithelial and luminal epithelial, serve as a thin diffusion barrier capable of blocking at least 30% of the caffeine concentration being transported into the breast milk [42, 43]. 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 [42]. 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 diffusion onto the second diffusion barrier called the 'luminal epithelial layer' [40]. In both of these layers the caffeine solute establishes its

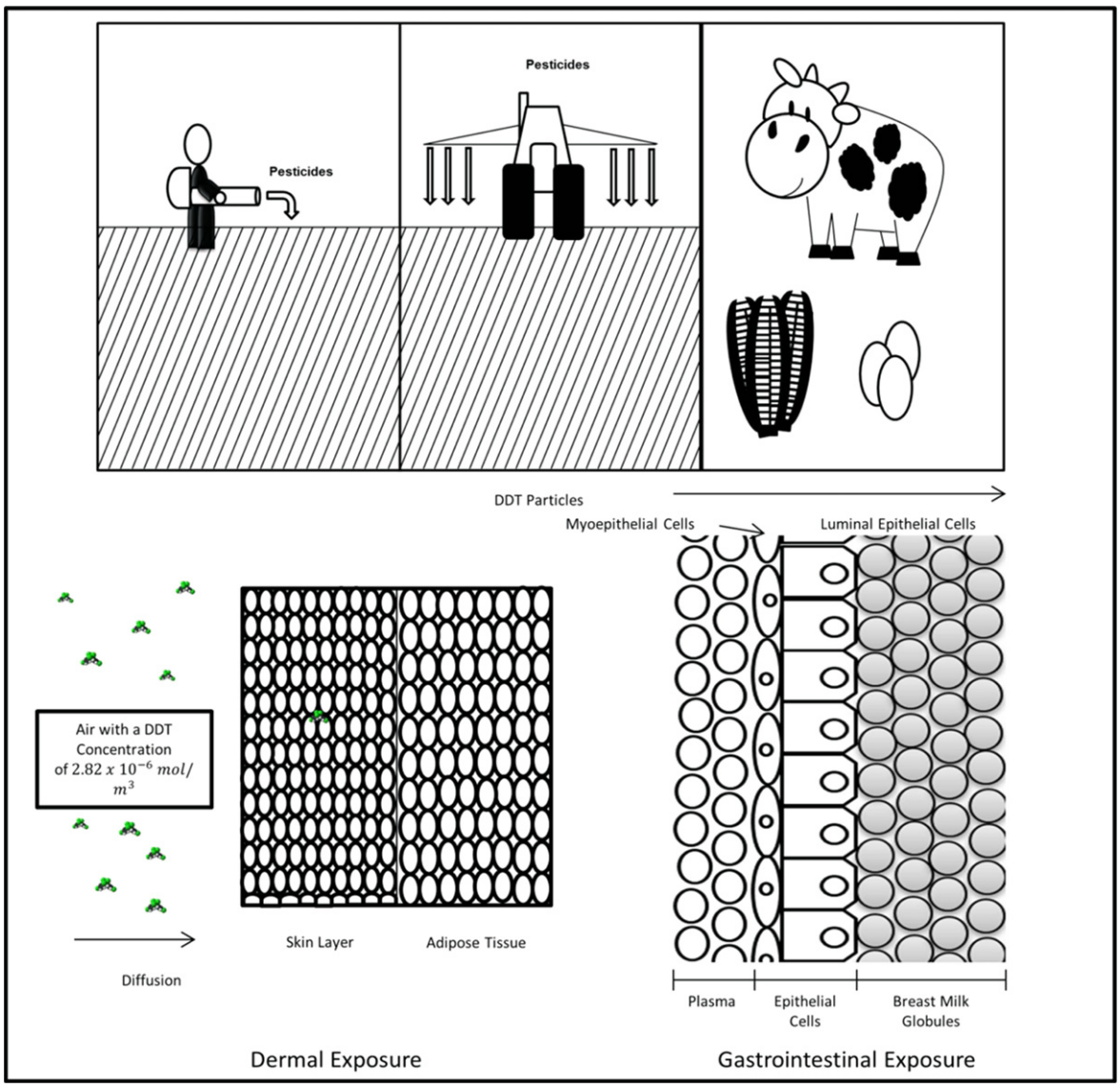

Figure 5. DDT dermal and gastrointestinal exposure.

presence to finally attempt to diffuse into the breast milk layer [43].

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

$$\varepsilon\frac{\partial c}{\partial t} + \nabla\cdot\left(-D\nabla\vec{c}\right) = S, \tag{3}$$

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.

2.2.2. The transport mechanism of DDT into breast milk. In several countries, 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 [44]. If DDT particles are sprayed by workers manually or are located close to a field recently sprayed, the regulations stipulate a maximum concentration of $2.8\,\mu\text{mol}\,\text{m}^{-3}$ or a flux of $1.3\times10^{-21}\,\text{mol}\,(\text{m}^2\text{s})^{-1}$ in the air as shown in figure 5 [45].

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 [46]. The contrast of the adsorption is due to the affinity of DDT to be stored in adipose tissues after the ingestion products contaminated with DDT particles such as fruits and vegetables. Unfortunately, DDT contamination is not limited to the air of, 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

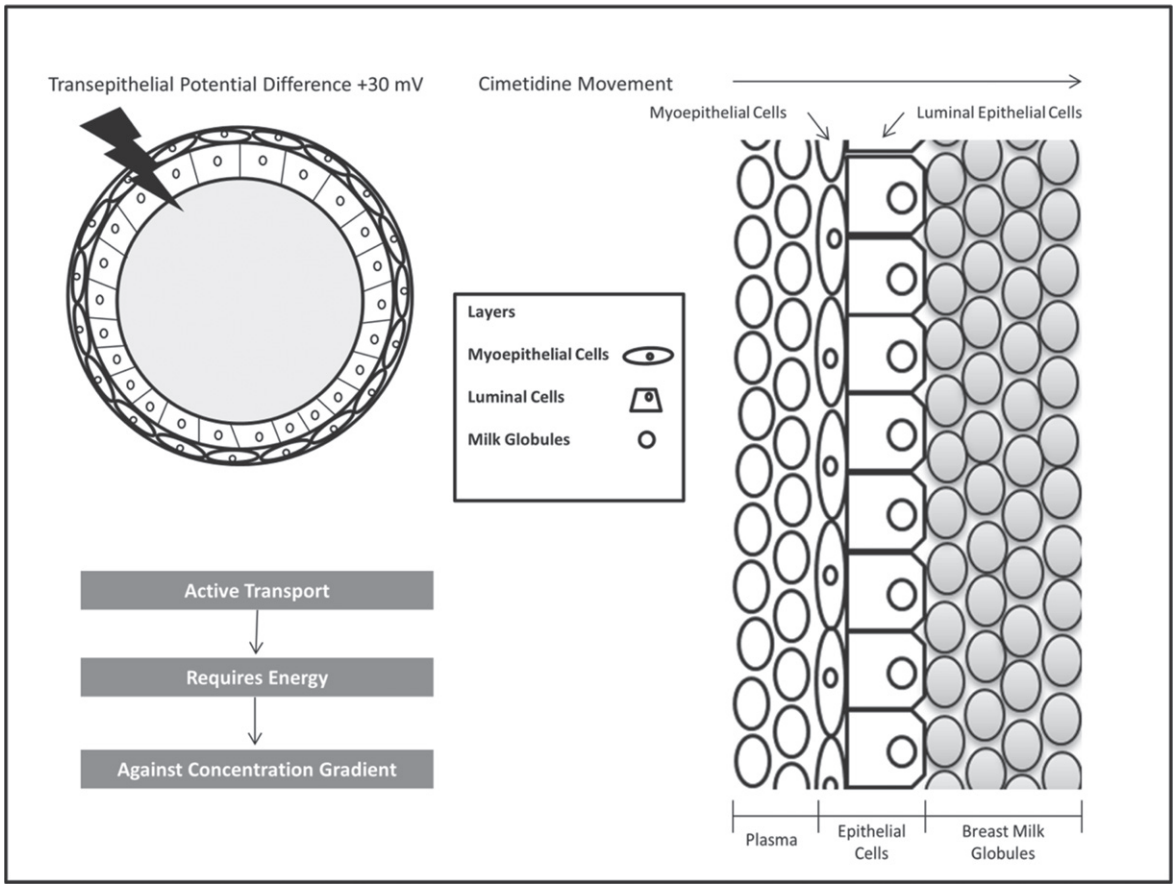

Figure 6. Active transport of cimetidine in the mammary gland.

Table 7. Literature milk to plasma ratios.

Particle	Literature expected M/P range	Model M/P range	References
Caffeine	0.7–0.9	0.8	[42, 43, 55]
DDT	0.5–2.6	1	[47]
Cimetidine	4.6–11.7	2.9–5.4	[35, 36]

decades ago, but the particle contamination is still present in studies performed on breast milk from women in the most affected areas [12].

We consider the scenario where the concentration of DDT is inside the bloodstream feeding the alveoli. Schecter *et al* [47] presented a study of the concentration of DDT in blood samples from women in northern Vietnam. Based on their experimental method, it was estimated that they possess an average concentration of 1.3 ± 0.2 ng-ml^{-1} and 4.6 ± 1.0 ng ml^{-1} in rural and urban zones, respectively [47]. In a separate study in Mexico, the blood serum DDT concentration was found to be around 1.8 ± 3.8 ng ml^{-1} [48].

The passive diffusion equation (1) was utilized in the analysis of all the multiple layers in the alveoli. The concentrations from the work of Schecter *et al* [47] 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 [47].

2.3. 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 to cure gastrointestinal ulcers [49]. 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 [28]. The M/P ranges from 4–12 in most of the predicted results [50].

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 actively diffusing cimetidine into the breast milk. The mammary glands have shown an electric potential ranging from −35 to −49 mV [51]. Researchers have demonstrated that normal human breast epithelium has a transepithelial potential difference of approximately +30 mV which is distributed across the cellular wall [52].

Table 8. Comparison of the calculated milk to plasma ratios of passive diffusion particles with the available literature values.

Time [s]	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})			
3600	Passive diffusion of caffeine into the breast milk	12.3×10^{-3}	11.5×10^{-3}	7.4×10^{-3}	9.3×10^{-3}	0.7–0.9	0.8	[27, 41]
	Passive diffusion of DDT into the breast milk	–	6.3×10^{-6}	–	6.3×10^{-6}	1	1	[32, 33, 39]

Table 9. Comparison of the calculated dermal adsorption of DDT pesticides with the available literature values.

Time [s]	Model	Experimental dermal adsorption	Model dermal adsorption	References
3600	Dermal exposure to a concentration of DDT	>3%	>1%	[57]

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

$$\frac{\partial c}{\partial t} + \nabla \cdot \left(-D\nabla c - zU_m Fc\nabla V\right) = S$$
$$E_m = -\nabla V$$
$$V = V_0, \tag{4}$$

where c is the concentration of cimetidine, D is the diffusion coefficient, z is the charge number, U_{m} is 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*'s [28] experimental work, where they observed an average M/P ratio of approximately 5.5 after single oral doses of 100, 600 and 1200 mg. Given these values, the membrane potential of the epithelial cells is estimated utilizing the Goldman–Hodgkin–Katz voltage equation.

$$\text{Milk to plasma ratio} = 5.5$$
$$E_m = 61.5\ \text{mV}\log\left(\frac{\text{Milk concentration}}{\text{Plasma concentration}}\right)$$
$$= 61.5\ \text{mV}\log\left(M/P\right) = 61.5\log\left(5.5\right)$$
$$= -45.5\ \text{mV}. \tag{5}$$

The transepithelial potential difference across the boundary layers, as shown in figure 6, considered for the model is +30 mV based on the suggested breast duct transepithelial potential by McCaig *et al* [52]. For this reason the voltage of the boundary layer of the epithelial layers was estimated to be −45 mV as estimated in equation (5) [52].

3. Results and discussion

3.1. Comparisons

The expected M/P 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 [53]. In most cases, the M/P ratio is considered only on single dosages and in a steady state environment and for simplicity an average ratio is calculated [54]. The literature expected M/P ratios of cimetidine, caffeine and DDT are presented in table 7.

For the passive diffusion of caffeine into the breast milk our mathematical model forecasted an M/P ratio equal to 0.8, which is in agreement with the average experimental results as illustrated in table 8 [42, 43, 55]. 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 [48]. To illustrate the DDT scenario table 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 M/P ratio reaches 1 [56]. Furthermore, we also estimated the dermal adsorption of DDT molecules after exposure to these chemicals. The results of our mathematical models shown in table 9 demonstrate that the percentage is lower than 1% compared with the average percentage presented by the health associations [57].

In contrast with the DDT and caffeine transport mechanisms, some particles such as cimetidine follow an active diffusion mechanism. Considering the unique properties of an active transport mechanism, table 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 M/P ratio is lower than unity. This mechanism demonstrates that in order to have a higher concentration of cimetidine in the breast milk than in the plasma, as shown in literature [28, 50], there is a need to include additional parameters as discussed earlier. The second scenario shown in table 10 considers an average concentration and M/P ratio. Our mathematical model is in agreement with the average experimental M/P. In the third case shown in table 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 [28, 50].

3.2. Effect of variations in the diffusion coefficient on the M/P and concentration distribution

Parameter selection is important for accurate model predictions. Some parameters are known in the literature as shown in tables (1)–(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 correct parameter selection, we compared it with literature-based diffusion coefficients available for intestinal epithelial cells as shown in figures 3 and 4 [35, 36, 58–60]. 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. Here, we perform an additional investigation regarding the variation of the diffusion coefficient by studying the impact of modifying the epithelial layers' diffusion coefficients by an order of magnitude from the value predicted by the Stokes–Einstein relationship, as shown in figure 7. The alveolus is a microstructure, making the transport sensitive to order of magnitude changes. The 150 mg caffeine oral dose remains constant in the comparisons, but the concentration levels of caffeine in the plasma and the breast milk experience mild variation as a result of the

Table 10. Comparison of the calculated milk to plasma ratios of active diffusion particles with the available literature values.

Time [s]	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})			
12 600	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	[35, 36]
	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	[35, 36]

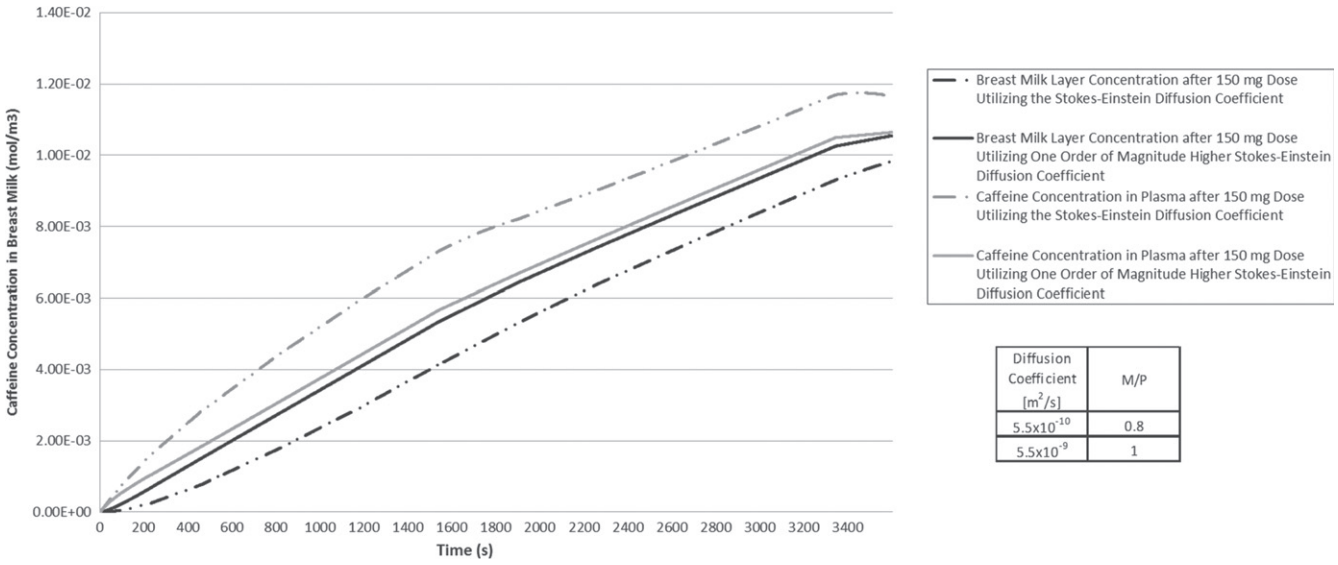

Figure 7. Impact of an order of magnitude increase in the diffusion coefficient on the caffeine levels in the breast milk.

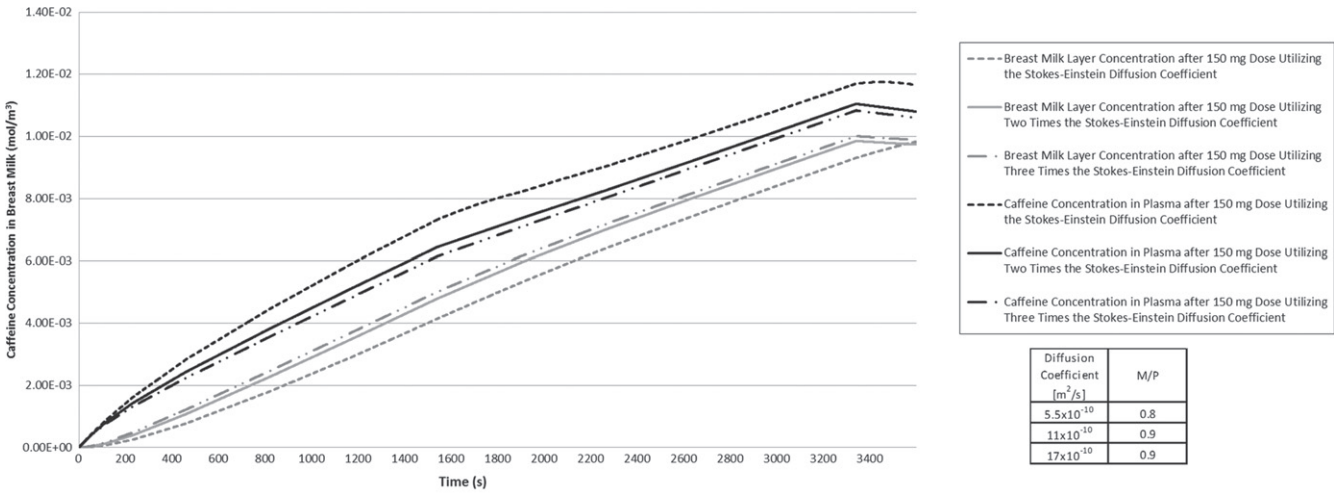

Figure 8. Impact of variations in the diffusion coefficient on the caffeine levels in the breast milk.

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 M/P ratio 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 figure 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 figure 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.

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 breast-feeding, making it easier to obtain experimental comparisons.

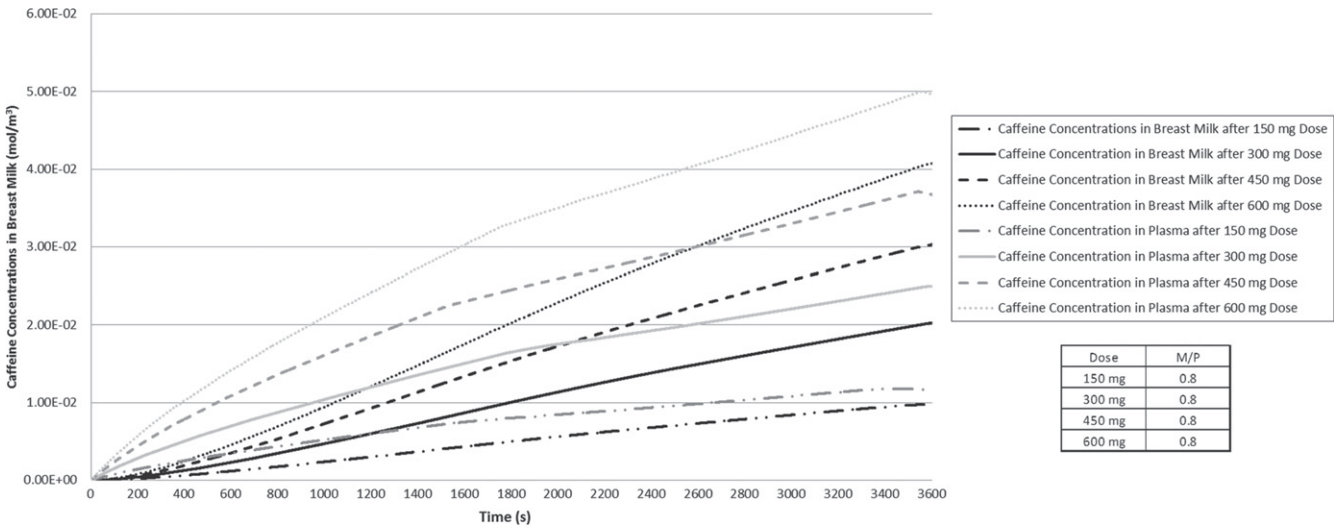

Figure 9. Relationship between oral dose and caffeine concentrations in breast milk.

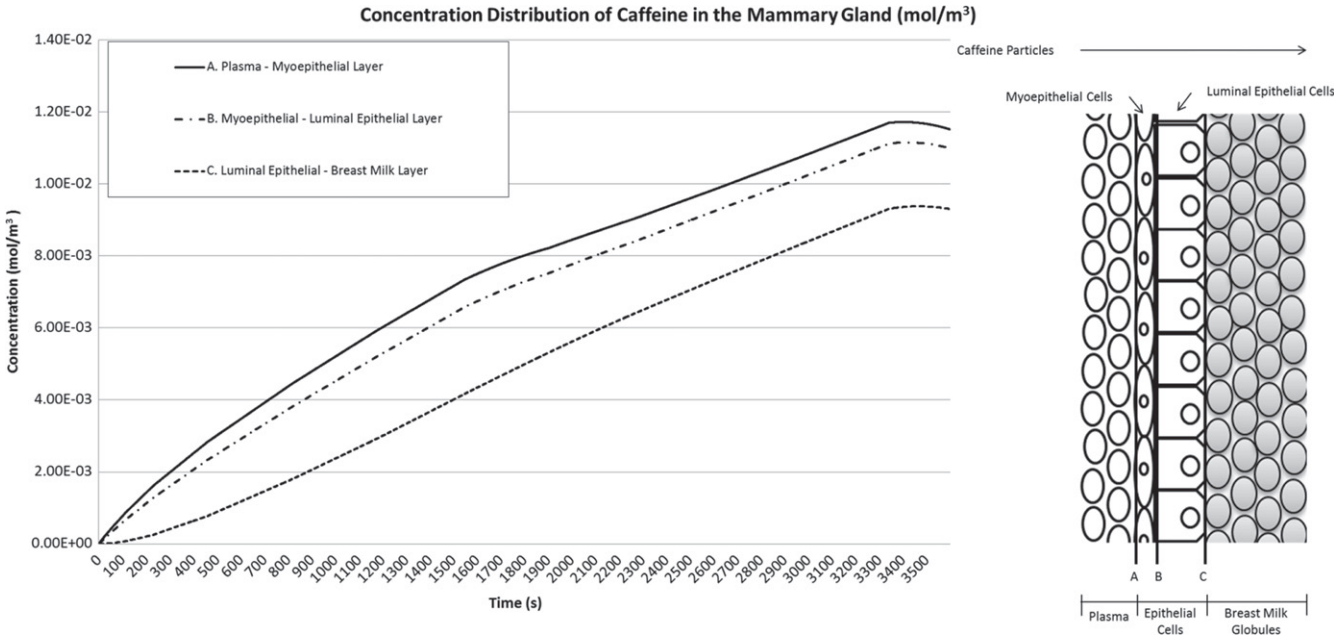

Figure 10. Passive diffusion of caffeine into the breast milk.

The relationship between the caffeine oral dose and bioavailability in breast milk have been demonstrated experimentally in prior work, [25, 40–42, 55, 61]. For this reason, the dosage has been studied and it has been experimentally demonstrated that when the oral dosage increases the M/P 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% of the drug trying to enter the breast milk layer (M/P = 0.8) [42, 43, 55].

In figure 9, we have demonstrated that our model also confirms the experimental results and that as the concentration of caffeine increases the M/P 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 [42]. Our model demonstrates how the concentration levels are affected by the barriers which the molecules face prior to entering the breast milk.

3.3. 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

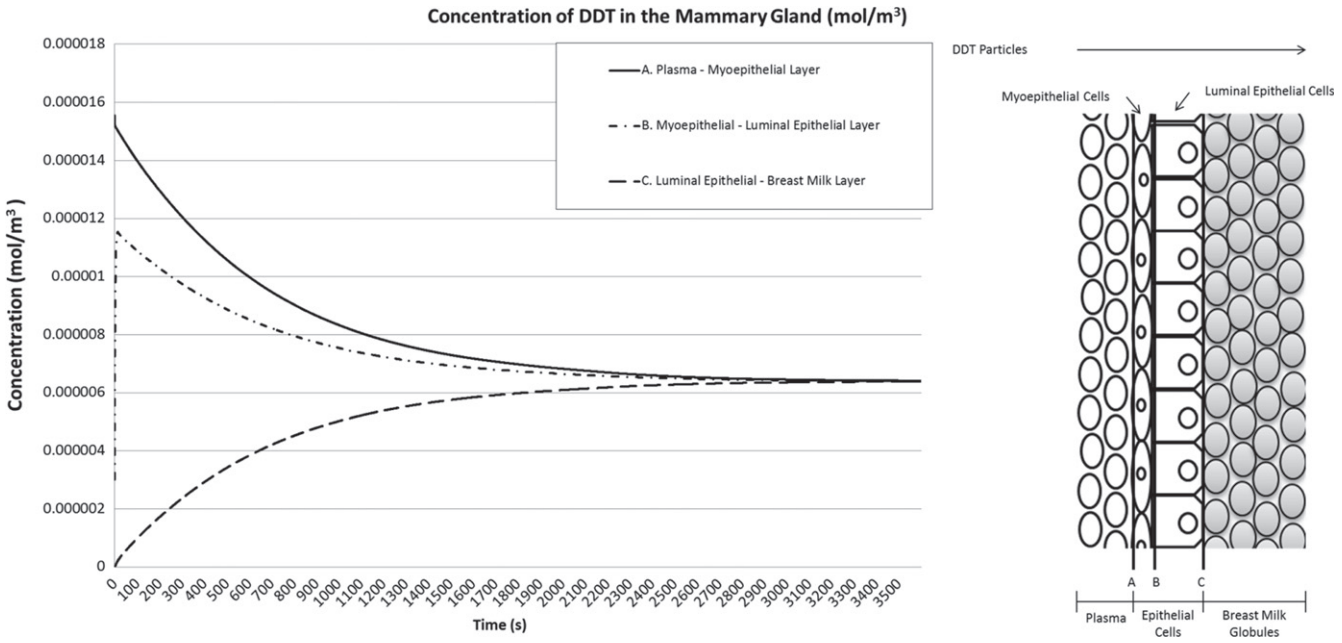

Figure 11. Passive diffusion of DDT into the breast milk.

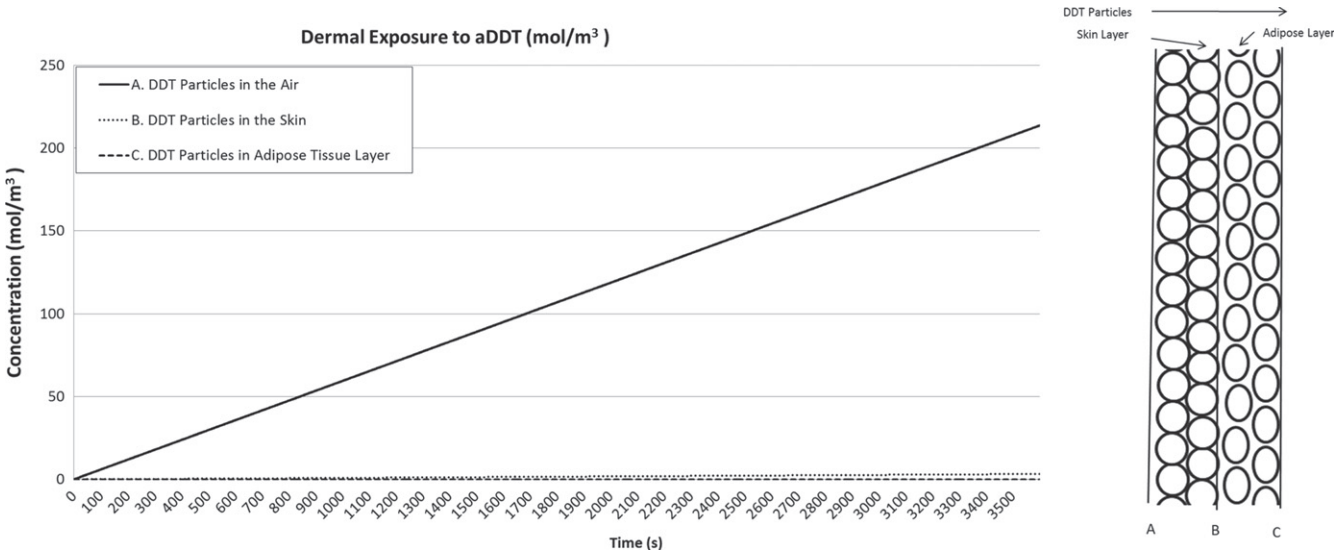

Figure 12. Passive diffusion of DDT within the skin and adipose tissue layers.

of caffeine in the bloodstream, based on the experimental work of Tyrala *et al* [42], 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} [42]. As can be seen in figure 10 the M/P ratio for this study is approximately 0.7, which is consistent with the experimental data which show that the average M/P ratio is between 0.7 and 0.8 [42, 43].

3.4. Study of the transport of DDT into breast milk

Organochloride pesticides are accumulated in food, soils, air and animals [44]. 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 this 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 [56].

Our results for the passive diffusion of DDT within the mammary gland are shown in figure 11. The M/P ratio of DDT has an average of 0.5–2.6, but if the comparison is performed in the fat levels the ratio reaches almost one [56]. Given these considerations, it is expected that at least the fat contained in the breast milk will contain a high percentage

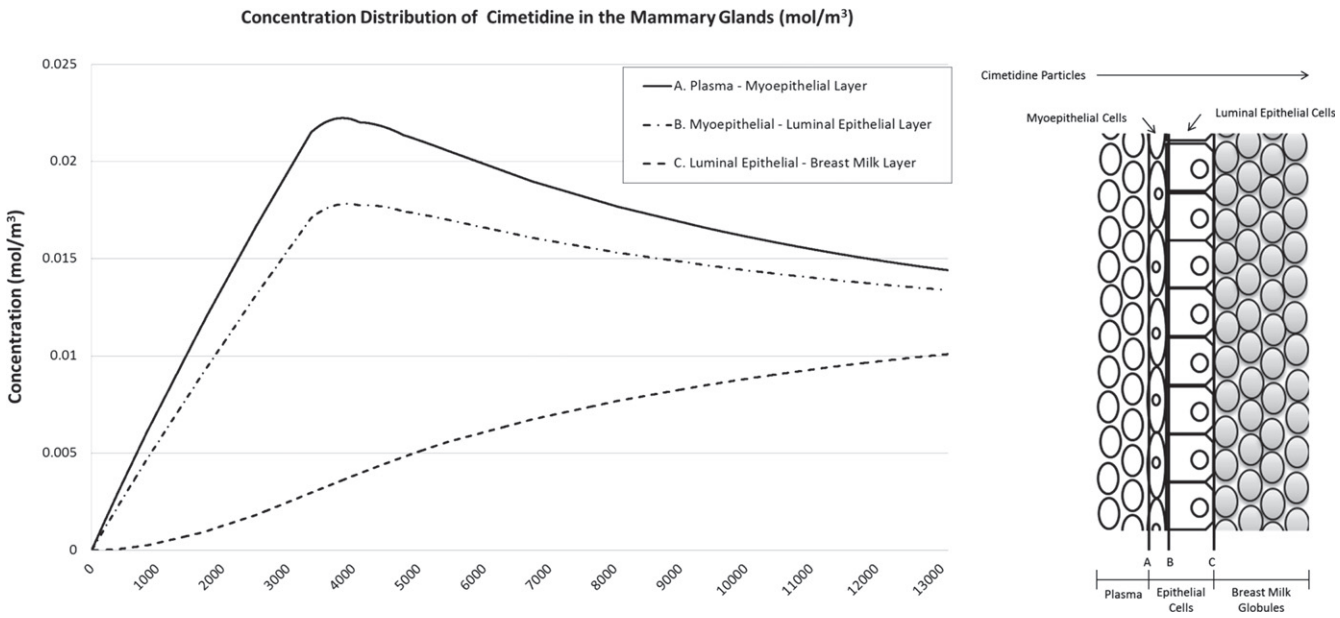

Figure 13. Passive diffusion of cimetidine into the breast milk.

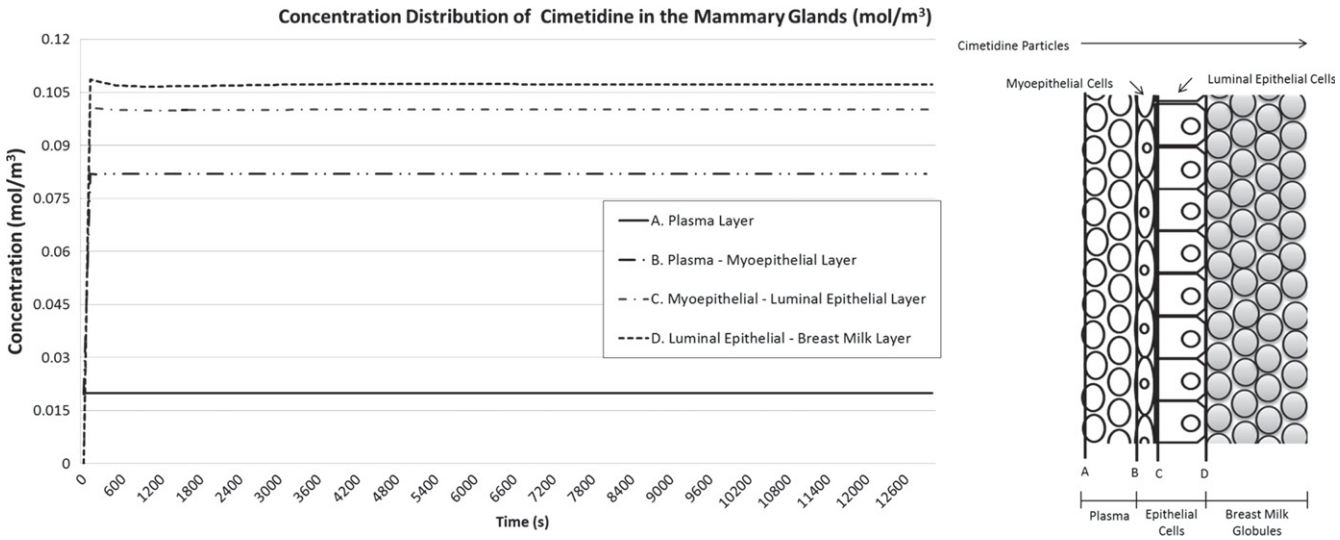

Figure 14. Active diffusion of cimetidine into the breast milk.

of DDT. Based on the experimental work from Waliszewski *et al* [48], an initial concentration of DDT of 33 μmol m^{-3} will result in a breast milk layer concentration of approximately 11.5 μmol m^{-3}. The results displayed in figure 11 demonstrate agreement with the experimental DDT concentrations and the available DDT concentration in, literature [10, 48, 56].

3.5. Study of dermal exposure to DDT

Dermal exposure to DDT and its distribution within the skin and the adipose tissue layers is displayed in figure 12. Figure 12 demonstrates that the DDT pesticides are able to penetrate and remain within the skin over a long period of time. Research performed on organochlorides has 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 [57]. 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.

3.6. 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 [62]. Figure 13 demonstrates that the concentration of cimetidine in a scenario consisting uniquely of only 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

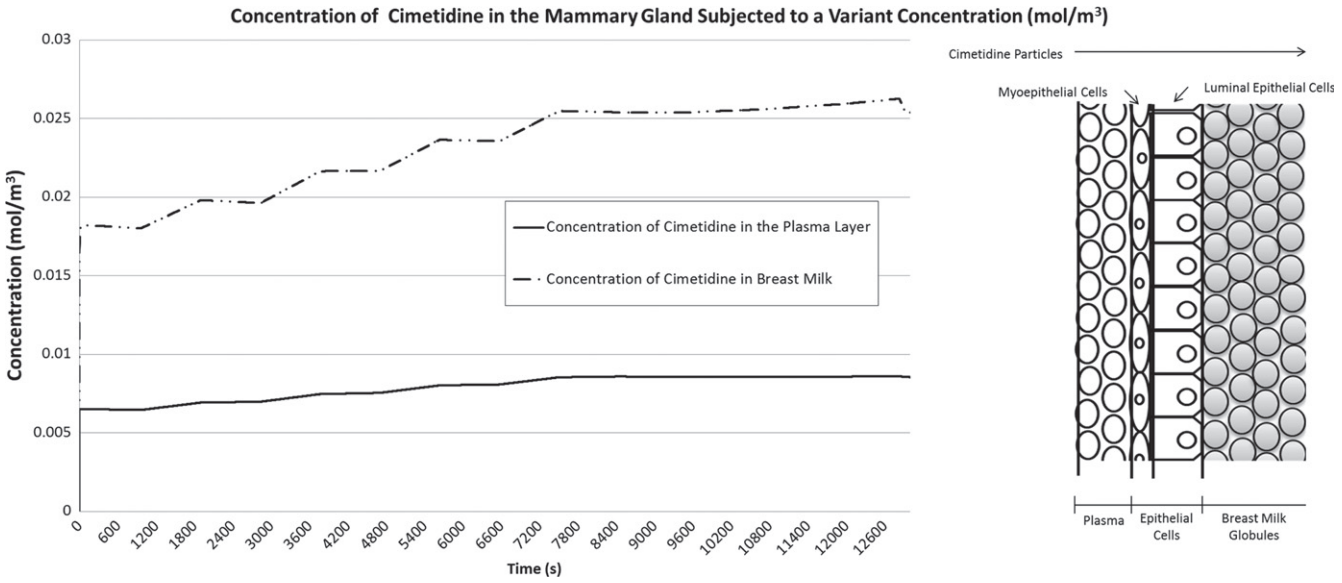

Figure 15. Active diffusion of cimetidine into the breast milk subject to a variant concentration.

concentration gradient, a differential potential is required to facilitate the movement.

Figure 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 (figure 6) to examine the impact of the electrical potential. Our work demonstrates that an M/P ratio of approximately five is attained, resulting in a higher concentration in the breast milk. This is consistent with the available experimental results [28, 49]. Figure 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 play 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.

4. 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 M/P ratio. 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 M/P ratio during the lactation period is still not fully understood in the pharmaceutical industry or in agencies dealing with toxicology assessment. We have been able to predict this ratio within our model.

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