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# Mathematical modeling of transient state transdermal drug delivery

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## ABSTRACT

## MATHEMATICAL MODELING OF TRANSIENT STATE TRANSDERMAL DRUG DELIVERY

**by**
**Alison Nickol Weltner**

In this work, a two-pathway mathematical model for transdermal drug delivery with iontophoresis is presented. The partial differential equations are described and then solved. An alternative, two-pathway, three-layer model is also presented, and the implications of the coefficients within the equation are discussed. Using Franz cell iontophoretic delivery data from three drug substances (amitriptyline HCl, clomipramine HCl, and nortriptyline HCl), the two-pathway model is regressed to determine the diffusion coefficient and the concentration within the skin at the drug reservoir interface. ANOVA analysis indicates a correlation between iontophoretic current and concentration of drug within the stratum corneum.

# MATHEMATICAL MODELING OF TRANSIENT STATE TRANSDERMAL DRUG DELIVERY

by
**Alison Nickol Weltner**

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To my parents, in deep gratitude for the support and encouragement they have given throughout every step of my education.

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# CHAPTER 1

# INTRODUCTION

Transdermal drug delivery is the practice of delivering a therapeutic chemical substance to the body via the skin. The transdermal route of drug administration is advantageous for a number of reasons. Entry into the general circulation through the skin circumvents hepatic metabolism, the process by which an orally administered drug may be chemically altered before reaching its target organ. Unlike delivery systems which require intermittent dosing, the transdermal route can circumvent fluctuating concentration levels within the blood. A transdermal delivery system can make continuous dosing feasible, thereby achieving steady drug concentrations within the body.

Additionally, transdermal systems offer great potential for enhanced patient compliance. Applying a transdermal patch is easy and painless. Once applied to the patient's skin, the patch remains in place for an extended period of time, during which a slow, controlled amount of drug is released to the body. The patient receives the needed dose in a manner convenient and non-disruptive to his or her normal activities. Issues resulting from a patient forgetting to take his or her medication or failing to take the medication as directed can be minimized.

While transdermal delivery systems offer great advantages to the consumer, such techniques present formidable challenges to their designers. The necessary development work is extensive. Transdermal drug delivery requires an understanding and application of both pharmacological principles and engineering principles as well. The device must

allow the drug to penetrate the barrier properties of the skin, while also achieving a release a rate that suits the patient's needs.

## 1.1 The Structure of the Skin

The barrier properties of the skin are innately difficult to overcome. The natural function of skin is to deter foreign substances from entering the body and to prevent necessary fluids and nutrients from escaping the body. To design an effective transdermal delivery system, these barrier properties must be addressed.

The most significant contributor to these barrier properties is the outer-most layer known as the stratum corneum (Bouwstra et al., 2001). The stratum corneum is composed of keratin-filled dead cells known as "corneocytes." The cell membrane of the corneocytes contains a dense, highly impermeable protein layer. Surrounding the corneocytes is a continuous, crystalline lipid bilayer. A common conceptual representation of the stratum corneum is the "brick and mortar" model, named for its similarity in appearance to individual bricks (i.e. the corneocytes) surrounded by mortar (i.e. the lipid regions) (Barry, 1983; Pieper et al., 2003). Although the structure of the lipid bilayer is not easily overcome, its continuity across the stratum corneum makes it a potential route for transport through the stratum corneum (Bouwstra et al., 2001).

Below the stratum corneum is the viable epidermis. The epidermis demonstrates fewer barrier properties than the stratum corneum (Lee et al., 1997). Unlike the stratum corneum, the viable epidermis contains enzymes which can have a metabolic function (Lee et al., 1997). Below the viable epidermis are the capillaries, which can readily

absorb substances from the epidermis into the bloodstream. Once in the bloodstream, the drug substance can be carried to the target organ.

## 1.2 Methods for Enhancing Drug Penetration Through the Skin

Due to the difficulty in penetrating the stratum corneum, various means and mechanisms for enhancing transdermal drug delivery have been pursued. Two such methods for overcoming the stratum corneum barrier properties are the use of chemical enhancers and the use of iontophoresis.

### 1.2.1 Drug Penetration Enhancement Through the Use of Chemical Enhancers

Chemical enhancers improve transdermal drug delivery by reducing the resistance of the stratum corneum to the drug molecules. Existing evidence suggests that chemical enhancers operate by disturbing the lipid organization in the stratum corneum. The mechanism(s) by which the enhancer disturbs the stratum corneum lipid organization depend on the enhancer's chemical structure and affinities. Bouwstra et al. (2001) have theorized several possible mechanisms for disturbing the stratum corneum lipid organization: (a) the enhancer molecules insert themselves sporadically into the lipid lamellae, (b) the enhancer inserts itself between the lamellae layers, thus disturbing the packing of the layers, and (c) the enhancer molecules form an organized, separate phase within the lamella. Additionally, other works have shown how specific types of chemical bonding, such as hydrogen bonding, can effect a change in drug penetration through skin (Hadgraft, 2001; Du Plessis et al., 2002).

Several methods have been used to incorporate chemical enhancers in transdermal drug delivery systems. One approach has been to incorporate the chemical enhancer into

the drug formation itself (Harrison et al.; 1996; Fang et al.; 1999; Essa et al., 2004). Another approach has been to incorporate the chemical enhancers as a pretreatment for the skin prior to the application of the drug vehicle itself (Graaff et al.; 2003, Nair and Panchagnula, 2003; Wang et al., 2003). *In vitro* observations show that skin samples treated with a fatty acid prior to application of the drug resulted in increased overall drug penetration (Wang et al., 2003; Nair and Panchagnula, 2003).

### 1.2.2 Drug Penetration Enhancement Through the Use of Iontophoresis

Another approach to improving transdermal drug delivery is a technique called iontophoresis. In iontophoretic delivery, a small electric current is used to drive the drug molecules across the skin barrier. Iontophoresis is a promising technology that has already received some regulatory approval. Iontocaine®, an iontophoretic transdermal system developed by Iomed Corporation (Salt Lake City, Utah), is currently marketed for the administration of lidocaine HCl, a local anesthetic. Vyteris, Inc. (Fairlawn, New Jersey), in partnership with B. Braun Medical, Inc., is awaiting approval of its own iontophoretic system for transdermal lidocaine delivery. Alza Corporation (Mountainview, California), a subsidiary of Johnson & Johnson, has developed an iontophoretic delivery system known as E-Trans® and has applied the E-Trans® technology to administer fentanyl HCl, an analgesic. The product is now in late-stage development.

Drugs delivered via iontophoresis are typically hydrophilic and ionic. Positively charged drugs are placed at the anode; negatively charged drugs are placed at the cathode. A small battery provides a voltage that drives the drug out of the donor

reservoir and into the skin. To complete the electric circuit, a buffer solution is placed in a return reservoir (Junginger, 2002). A diagram is given in Figure 1.1.

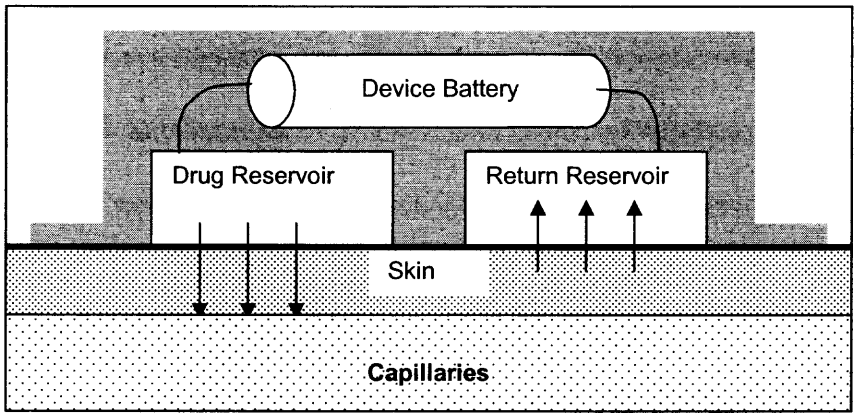



**Figure 1.1** Design of an iontophoretic delivery patch. The device consists of a drug reservoir and a return reservoir connected by a battery. The placement of the anode and cathode depends on the charge of the drug being delivered.

### 1.3 *In Vitro* Methods for Study and Analysis of Transdermal Drug Delivery

Studies of fundamental transdermal drug delivery principles are commonly conducted *in vitro*, rather than *in vivo*. *In vivo* analysis presents myriad complexities, complications, and restrictions that render it unsuitable for basic investigation into mass transfer principles. For example, ethics prohibit conducting *in vivo* studies in humans before sufficient knowledge regarding toxicity or side effects has been gathered. Furthermore, the interpretation of data from *in vivo* testing is more problematic, since other physiological variables may alter or mask the effect of the studied parameter.

*In vitro* studies of transdermal delivery principles are typically conducted in a Franz diffusion cell. A Franz cell consists of two chambers, one of which contains the drug substance and one of which contains the receptor fluid. A small, thin piece of skin

is placed between the compartments, thus allowing the drug substance to penetrate from the donor compartment across the skin to the receptor compartment. Periodically, the drug concentration in the receptor fluid is collected and assayed to determine the quantity of drug transported across the skin. A diagram is given in Figure 1.2.

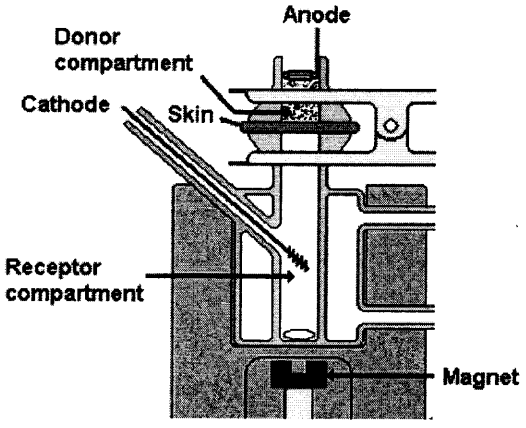



**Figure 1.2** Diagram of a Franz cell apparatus for iontophoretic delivery. The drug and its vehicle are loaded into the donor compartment. The drug diffuses through the skin and into the receptor compartment. In the iontophoretic system shown here, the anode is placed at the donor cell and the cathode at the receptor cell.

Franz cell data analysis is performed by calculating $Q$, the mass quantity of drug accumulated in the receptor cell over time, and then plotting $Q$ as a function of time ($t$). For most iontophoretic delivery, the $Q$ versus $t$ plot is nonlinear at initial $t$ values but becomes linear at large $t$ values. The slope of the linear region, divided by the surface area available for diffusion, represents the flux of the drug through the skin in units of mass per area per time.

Analysis by the graphical method is useful for quantifying and comparing results among individual studies. However, the graphical method alone cannot explain why differences or similarities among a given set of experiments exist. Employing a

mathematical model can enhance the analysis process by offering insight as to the mechanism(s) by which a particular outcome is achieved.

## 1.4 Applying Mathematical Models to Analyze Transdermal Drug Delivery Data

In this work, two new models for mathematical analysis of drug delivery through the skin are presented. The models are based on previous work available in the literature. The first model, discussed in Chapter 2, is a one-layer, two-pathway model which characterizes delivery through the stratum corneum. The second model (Chapter 3) is a three-layer, two-pathway model which encompasses delivery from the drug vehicle (i.e. the delivery device) itself to the stratum corneum, and then from the stratum corneum through the viable epidermis and finally to the capillaries.

In Chapter 2, the solutions to the partial differential equations for the one-layer, two-pathway model are presented. In Chapter 4, these solutions are applied to several existing sets of iontophoretic delivery data. Using nonlinear regression software, a series of coefficients corresponding to the diffusion coefficient and the concentration within the stratum corneum at the vehicle-device interface are established. The values for these coefficients are compared and discussed.

# CHAPTER 2

# TRANSIENT MODEL FOR A TWO-PATHWAY SYSTEM

A number of mathematical models have been presented in the literature as a means of describing transdermal delivery data. These models have discussed passive diffusion (Lee and Aldama, 1992; Lee et al., 1996; Mitragotri, 2002; Tezel and Mitragotri, 2003), diffusion assisted through chemical enhancers (Moser et al., 2001), diffusion of solvents through the skin (Pikal, 2001), and diffusion aided through iontophoresis (Kontturi and Murtomäki, 1996; Lim et al., 2002).

The model proposed by Kontturi and Murtomäki (1996) for iontophoretic systems is based on the assumption that parallel pathways are available for drug diffusion in the skin. These routes are referred to as the aqueous and lipid pathways. Drug flux through the skin is thus a function of the flux through each of the two pathways.

Kontturi and Murtomäki present equations to characterize steady flux through the stratum corneum for their two-pathway system. However, the transient state solutions for this model have not yet been developed. In this chapter, the two-pathway model is analyzed and solved for the transient state condition.

## 2.1 The Kontturi-Murtomäki Two-Pathway Model

The two-pathway model developed by Kontturi and Murtomäki (1996) considers drug penetration through the stratum corneum to be the product of transport across two separate pathways. These pathways exist in parallel; migration from one path to the other is not addressed.

The two pathways differ based on their composition: one pathway is lipophilic and will take up only neutral molecules. The second pathway is hydrophilic and will readily take up charged ions as well as neutral molecules.

An important assumption in Kontturi and Murtomäki's work is that enhancement of delivery due to iontophoresis impacts the aqueous pathway exclusively. The rationale for this assumption is that only the aqueous pathway can carry an electrical current. In the Kontturi-Murtomäki model, enhancement of flux through the aqueous pathway due to iontophoresis is mathematically represented by a factor of $E$. The value of $E$ increases with increased iontophoretic current.

As a general principle, the total flux through parallel pathways is equal to the sum of the fluxes through the individual paths, scaled by their surface areas. Thus, if $J_a$ and $J_l$ are defined as fluxes through the aqueous and lipid pathways, respectively, then the total flux across the skin is equal to the sum of these fluxes multiplied by the fraction of their respective surface areas.

In the presence of iontophoresis, the effective flux through the aqueous pathway is increased. The surface area fraction consisting of the aqueous pathway is defined as $\varepsilon$ (Kontturi and Murtomäki, 1996). It thus follows that the surface area fraction consisting of the lipid pathway is $1-\varepsilon$. As such, in terms of the enhancement factor $E$, the total flux in the skin due to iontophoresis ($J_{if}$) may be defined as:

$$J_{if} = E * \varepsilon * J_a + (1-\varepsilon)J_l \qquad (2.1)$$

Kontturi and Murtomäki's model applies to transport through the stratum corneum only. The boundary conditions assume that the drug is applied to the skin in a well-stirred reservoir. The bulk concentration of this reservoir, $C_b$, partitions into the skin

layer according to the partition coefficient $P$. Furthermore, Kontturi and Murtomäki's analysis is generalized such that bulk solution in the donor reservoir may contain a drug substance in both ionized and non-ionized forms. A parameter α, defined as the fraction of drug present in the non-ionized form, accounts for this possibility. A diagram of the system analyzed by the Kontturi-Murtomäki two-pathway model is given in Figure 2.1.

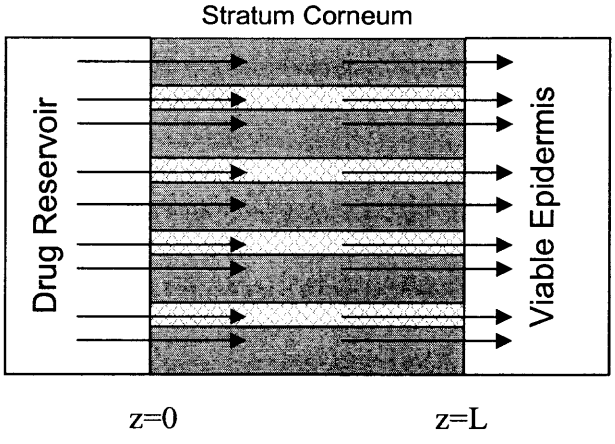



**Figure 2.1** Diagram of transport through the stratum corneum as characterized by the two-pathway model. The darker regions in the stratum corneum represent the lipid pathway; the lighter regions represent the aqueous pathway.

Through their analysis, Kontturi and Murtomäki show that drug flux through the lipid pathway of the stratum corneum may be represented as:

$$J_l = \frac{\alpha C_b k_D}{1 + \frac{k_D L}{D_l P}} \tag{2.2}$$

where $D_l$ is the diffusion coefficient for the drug in the lipid path and $L$ is the length of the diffusion pathway, taking into account both tortuosity and membrane thickness. Embedded in this result are two boundary conditions. The concentration of drug at the

drug reservoir-skin interface is dependent upon the lipid pathway partition coefficient, the concentration of drug in the bulk, and the fraction of the bulk drug in the non-ionized form. (The surface area fraction of the lipid pathway $(1-\varepsilon)$ is omitted from the final result as a simplification, since the quantity is taken to be very close to unity. Kontturi and Murtomäki use a value of $10^{-4}$ for $\varepsilon$.)

Hence, the steady state flux at this interface (i.e. at $z=0$) is represented as $D_l \alpha P C_b / L$. At the opposite end, transport out of the stratum corneum skin layer and into the viable epidermis is governed as a desorption process obeying first-order kinetics with a rate constant of $k_D$.

The flux through the aqueous layer is given as:

$$J_a = \varepsilon D_a \frac{C_b}{L} \tag{2.3}$$

where $D_a$ is the drug diffusion coefficient in the aqueous path. The concentration boundary condition of the aqueous layer at the reservoir-stratum corneum interface is equal to $C_b$. For the aqueous layer, the concentration boundary condition at the stratum corneum-epidermis interface is taken to be 0, due to the infinite sink approximation.

The boundary conditions for the lipid and aqueous pathways differ. Unlike the lipid layer, Kontturi and Murtomäki do not multiply the skin concentration for the aqueous pathway by the quantity α, because both the ionized and non-ionized forms of the drug are permitted to pass through the aqueous pathway.

The boundary conditions at $z=L$ (i.e. the stratum corneum/viable epidermis interface) differ for the two pathways as well. Kontturi and Murtomäki explain that the viable epidermis is characterized by polarity similar to that of water. Because the aqueous layer and the viable epidermis are alike with respect to polarity, desorption from

the aqueous pathway to the epidermis should have a time scale much faster than that of the diffusion process through the aqueous path itself. Thus, infinite sink conditions is an appropriate approximation for the aqueous path.

The difference in polarity between the lipid pathway and the viable epidermis, however, poses a problem to which the perfect sink approximation cannot be applied. Based on empirical observations (Kontturi and Murtomäki, 1996), Fickian diffusion alone cannot account for lipid pathway diffusion. To summarize Kontturi and Murtomäki's argument (1996), if the perfect sink condition applied to the stratum corneum-viable epidermis interface for the lipid path as well as the aqueous path, then partition from the lipid layer of the stratum corneum to the viable epidermis would increase or decrease only with a single partition coefficient. Furthermore, partition of a highly lipophilic drug into the lipid layer from the drug reservoir would have to be fast as well, due to the similar polarity between the drug and the lipophilic layer. As such, the only rate limiting parameter for the lipid pathway would be the diffusion coefficient through the pathway itself. Empirical data does not match this conclusion. Thus, a kinetic process must occur at the stratum corneum-viable epidermis interface to account for this problem. Kontturi and Murtomäki capture the effect of this transfer between skin layers in terms of a first order kinetics model with a rate constant of $k_D$.

## 2.2 Partial Differential Equations Describing the Two-Pathway System

The analysis performed by Kontturi and Murtomäki (1996) is based on Fickian diffusion and application of suitable boundary conditions. However, the flux equations in the Kontturi-Murtomäki analysis do not address the transient state conditions. Kontturi and

Murtomäki's results are only applicable to steady state, constant flux conditions, and thus only apply when the large time approximation is employed.

Understanding the drug concentration profile for transient conditions, however, is important for several reasons. For example, in the case of high potency drugs, the quantity delivered to the plasma prior to achieving steady state flux may lead to toxicity problems. In the delivery of rescue medications, the time required to achieve an adequate plasma concentration may be more critical than the time required for steady state flux through the skin. Additionally, studying the dynamics of the system prior to the steady state conditions can help explain the rate limiting steps in the delivery process. This knowledge, in turn, can be utilized to improve the system design.

As previously explained, diffusion through the aqueous layer is entirely due to Fickian diffusion. The general equation for Fickian diffusion is:

$$\frac{\partial C_a}{\partial t} = D_a \frac{\partial^2 C_a}{\partial z^2} \tag{2.4}$$

To this equation, the enhancement factor $E$ is added to account for iontophoresis, thus obtaining:

$$\frac{\partial C_a}{\partial t} = E * D_a \frac{\partial^2 C_a}{\partial z^2} \tag{2.5}$$

In this equation, $z$ represents the linear direction over which the diffused substance travels, and $C_a$ represents the concentration within the aqueous pathway of the stratum corneum at a given location $z$ and time $t$. The drug reservoir-delivery device interface is defined as $z = 0$, and the delivery device-viable epidermis interface is defined as $z = L$.

The boundary conditions for the aqueous layer, at any time $t$, are given as:

$$C_a(0,t) = C_b \tag{2.6}$$

$$C_a(L,t) = 0 \tag{2.7}$$

and the initial condition is given as:

$$C_a(z,0) = 0 \tag{2.8}$$

The boundary conditions assume fast diffusion in the delivery device as compared to the skin. Thus, the drug concentration on the drug reservoir side at $z = 0$ remains constant.

The diffusion through the lipid layer during iontophoresis is also modeled by Fickian diffusion. Since iontophoresis enhances only the aqueous pathway, the partial differential equation for the lipid layer is analogous to the equation describing passive diffusion except that it does not contain the enhancement factor. The concentration in the lipid layer is thus described as:

$$\frac{\partial C_l}{\partial t} = D_l \frac{\partial^2 C_l}{\partial z^2} \tag{2.9}$$

The initial condition for the lipid layer is analogous to initial condition for the aqueous layer (i.e. no drug is present in the stratum corneum at $t = 0$ ).

$$C_l(z,0) = 0 \tag{2.10}$$

Applying the boundary conditions established by Kontturi and Murtomäki (1996), as introduced in Section 2.1, the concentration of the lipid layer at the device-skin interface at any time:

$$C_l(0,t) = \alpha P C_b \tag{2.11}$$

Unlike the aqueous pathway, however, the boundary condition for the lipid layer at the stratum corneum/epidermis interface is treated as a function of first order kinetics, rather than as an infinite sink. Thus, the condition of continuity of flux is applied.

$$-D_l \frac{\partial C_l(z,t)}{\partial z}\bigg|_{z=L} = \frac{k_D}{P} C_l(L,t) \tag{2.12}$$

### 2.3 Solving the Partial Differential Equations for Flux and Accumulation

Generally, transdermal systems are characterized in terms of the flux they provide. Flux, represented by $J_a$ and $J_l$ for the aqueous layer and the lipid layer, respectively, is expressed in units of mass of drug per area per time. As defined by the equations, flux is a function of time. However, as noted in Section 1.3, steady state flux is achieved for substantially large $t$ values.

For each pathway, the quantity of drug accumulated in the receptor cell over time is a function of the diffusion coefficient, the surface area available for diffusion, and the concentration gradient at $z = L$. These equations for the aqueous and lipid layers, respectively, may be represented as:

$$\frac{dQ_a(t)}{dt} = -E\varepsilon A D_a \left(\frac{\partial C_a}{\partial z}\right)\bigg|_{z=L} \tag{2.13}$$

$$\frac{dQ_l(t)}{dt} = -(1-\varepsilon) A D_l \left(\frac{\partial C_l}{\partial z}\right)\bigg|_{z=L} \tag{2.14}$$

The total surface area of skin available for diffusion is $A$. As introduced in Section 2.1, the parameters $\varepsilon$ and $1-\varepsilon$ represents the fraction of the skin surface area that is composed of the aqueous and lipid layers, respectively.

When the above equations are normalized for area, the resulting expression is the flux in units of mass per area per time:

$$J_a(t) = -E * D_a \left( \frac{\partial C_a}{\partial z} \right) \Bigg|_{z=L} \tag{2.15}$$

$$J_l(t) = -D_l \left( \frac{\partial C_l}{\partial z} \right) \Bigg|_{z=L} \tag{2.16}$$

Solving Equation 2.15 for the aqueous layer, the cumulative amount of drug penetrated through the aqueous pathway in the skin per unit area, as a function of time, becomes:

$$Q_a(t) = \left[ \frac{E * D_a t}{L^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \left( \frac{\cos(n\pi)}{n^2} * \exp\left( \frac{-E * D_a n^2 \pi^2 t}{L^2} \right) \right) \right] * C_b * L * A * \varepsilon \tag{2.17}$$

At very large time values, the exponential term in this equation quickly decays. Thus, at large time values, this equation may be approximated as a linear equation. Rearranging the above equation, the large time approximation becomes:

$$Q_a(t) = \frac{C_b A \varepsilon E D_a t}{L} - \frac{C_b L A \varepsilon}{6} \tag{2.18}$$

By studying this linear equation, it is clear that when $Q_a$ is plotted as a function of $t$, the y-axis intercept equals $-C_b LA\varepsilon/6$. Additionally, the line has a slope of $C_b A \varepsilon E D_a / L$.

The diffusion in the lipid layer may be represented as the change in total quantity of drug transported through the lipid layer over time as well. When Equation 2.14 is normalized by the area of the lipid layer, the resulting expression is the flux $J_l(t)$ in units of mass per area per volume:

$$J_l(t) = -D_l \left( \frac{\partial C_l}{\partial z} \right) \tag{2.19}$$

To solve this partial differential equation for the lipid layer, a Laplace transform is used, hence obtaining:

$$C_l = l^{-1}\left\{\frac{C_b P\alpha\left(sP\cos\left((L-z)\sqrt{\frac{s}{D_l}}\right)+k_D\sqrt{\frac{s}{D_l}}\sin\left((L-z)\sqrt{\frac{s}{D_l}}\right)\right)}{s\left(sP\cos\left((L)\sqrt{\frac{s}{D_l}}\right)+k_D\sqrt{\frac{s}{D_l}}\sin\left((L)\sqrt{\frac{s}{D_l}}\right)\right)}\right\} \tag{2.20}$$

When this equation is changed from the Laplace domain to the $z$ domain, and the limit is taken for large $t$ values, the following result is obtained:

$$\lim_{t\to\infty}[C_l(z,t)] = \frac{C_b P\alpha[k_D(L-z)+PD_l]}{k_D L + PD_l} \tag{2.21}$$

Taking the partial derivative of this equation with respect to z, the following is obtained:

$$\frac{\partial C_l}{\partial z} = \frac{-C_b P\alpha k_d}{k_D L + PD_l} \tag{2.22}$$

Flux through the lipid layer at the stratum corneum-viable epidermis interface, $z = L$, can be represented as:

$$J_l = -D_l \left.\frac{\partial C_l}{\partial z}\right|_{z=L} \tag{2.23}$$

Substituting Equation 2.22 into Equation 2.23:

$$J_l = \frac{D_l C_b k_D P\alpha}{k_D L + PD_l} \tag{2.24}$$

When each term in the numerator and denominator of Equation 2.24 is divided by $D_l P$, the result is Equation 2.2, the steady state value obtained by Kontturi and Murtomäki (1996).

# CHAPTER 3

# MODEL FOR A THREE-LAYER, TWO-PATHWAY SYSTEM

The two-pathway model presented in Chapter 2 is suitable for modeling iontophoretic drug delivery through the stratum corneum. In other transdermal drug delivery systems, it may be desirable to model drug flux through not only the stratum corneum, but through the viable epidermis and the delivery vehicle itself as well.

While the model presented in Chapter 2 is suitable for characterizing transdermal delivery of a drug dissolved in a well-mixed solution, it is not suitable for situations in which the drug formulation or vehicle is intended to control the rate of delivery. Many examples of these types of systems exist, such as the contraceptive patch (Ortho Evra®, Ortho-McNeil Pharmaceuticals, Raritan, New Jersey), which utilizes a matrix system to control hormone delivery to the body (Burkman, 2004).

In this chapter, the two-pathway system is extended from a one-layer model encompassing only the stratum corneum to a three-layer model that encompasses the drug vehicle, the stratum corneum, and the viable epidermis as well. Work from Chapter 2 is further developed by incorporating not only mass transfer across the layer, but also between the aqueous and lipid pathways. Mass transfer between pathways has been addressed in previous developed partial differential equations (Lee et al., 1996), but a simultaneous analysis of both multiple pathways and multiple layers has not yet been performed.

## 3.1 Partial Differential Equations for the Three-Layer Model

The three layers represented by the model are the drug delivery vehicle, the stratum corneum, and the viable epidermis. The partial differential equations characterizing the drug delivery follow the same principles of Fickian diffusion as those applied to the two-pathway, iontophoretic model. For the three-layer model, several assumptions are made. Firstly, it is assumed that the aqueous and lipid pathways exist in the stratum corneum and viable epidermis layers. The one-layer, two-pathway model for the stratum corneum has been previously discussed in Chapter 2. For the three-layer model, the viable epidermis is considered as a continuation of these two pathways, although drug delivery through the viable epidermis is expected to differ greatly from the stratum corneum, since the viable epidermis is more aqueous than the stratum corneum. The drug delivery vehicle is homogeneous in content, and thus, is treated as a one-pathway region.

The three regions (i.e. vehicle, stratum corneum, and viable epidermis) are represented as I, II, and III, respectively. The diffusion length through each region is taken to be $L_I$, $L_{II}$, and $L_{III}$, respectively. See Figure 3.1.

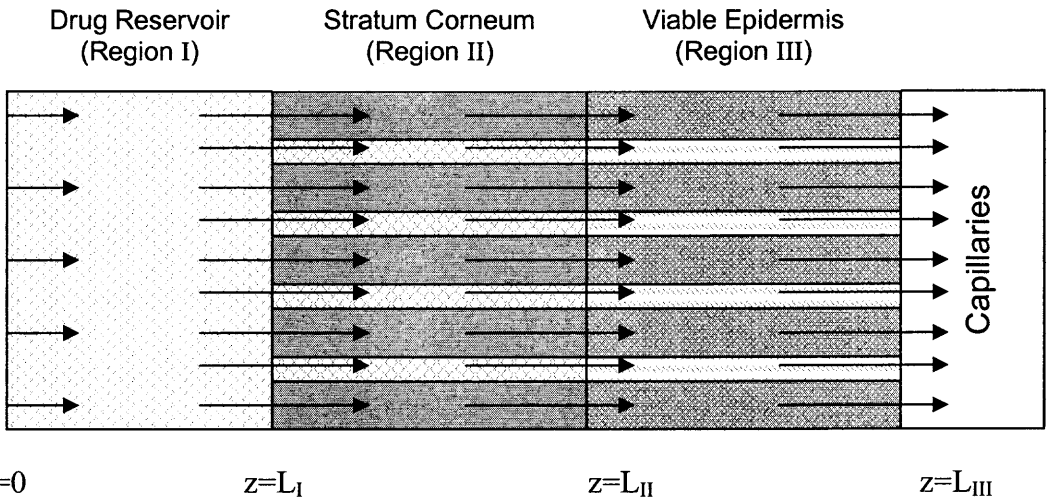



**Figure 3.1** Diagram of drug delivery through the vehicle, stratum corneum, and viable epidermis as characterized by the three-layer model. The darker regions in the stratum corneum and viable epidermis represent the lipid pathway; the lighter regions represent the aqueous pathway.

### 3.1.1 Characterization of Transport Through the Drug Vehicle

The equation for flow of the drug through the vehicle is:

$$\frac{\partial C_I}{\partial t} = D_I \frac{\partial^2 C_I}{\partial z^2} \tag{3.1}$$

The initial condition for this equation assumes uniform drug concentration $C_{Io}$ throughout the entire volume of the device at time 0. This is represented as:

$$C_I(z, t = 0) = C_{Io} \tag{3.2}$$

Furthermore, there are two boundary conditions for Equation 3.1, one to indicate continuity of flux at the vehicle-stratum corneum interface, and one to indicate no flux exists at the edge of the vehicle furthest from the skin (i.e. at $z = 0$). Symbolically, these conditions are represented as:

$$D_I \frac{\partial C_I}{\partial z}(L_I, t) = D_{II,A} \frac{\partial C_{II,A}}{\partial z}(L_I, t) \tag{3.3}$$

$$D_I \frac{\partial C_I}{\partial z}(L_I,t) = D_{II,L} \frac{\partial C_{II,L}}{\partial z}(L_I,t) \tag{3.4}$$

$$\frac{\partial C_I}{\partial z}(0,t) = 0 \tag{3.5}$$

In these equations, subscripts $A$ and $L$ designate the aqueous and lipid pathways, respectively.

### 3.1.2 Characterization of Transport Through the Stratum Corneum

Since the model assumes both lipid and aqueous pathways are available for drug delivery through the stratum corneum (Region II), separate equations and boundary conditions are written for each pathway.

For the aqueous pathway, the drug concentration profile in the stratum corneum over time is dependent upon three mass transfer mechanisms: 1) Fickian diffusion through the aqueous layer, 2) transfer from the lipid layer of the stratum corneum to the aqueous layer of the stratum corneum, and 3) transfer from the aqueous layer of the stratum corneum to the lipid layer of the stratum corneum. Diffusion is dependent upon the diffusion coefficient $D_{II,A}$ and $D_{II,L}$ for the drug in each pathway. The mass transfer between pathways depends on the concentration in each of the paths and a mass transfer coefficient. This model is represented as:

$$\frac{\partial C_{II,A}}{\partial t} = D_{II,A} \frac{\partial^2 C_{II,A}}{\partial z^2} + k_{II,A} C_{II,L} - k_{II,L} C_{II,A} \tag{3.6}$$

$$\frac{\partial C_{II,L}}{\partial t} = D_{II,L} \frac{\partial^2 C_{II,L}}{\partial z^2} + k_{II,L} C_{II,A} - k_{II,A} C_{II,L} \tag{3.7}$$

The coefficient for mass transfer to the aqueous layer from the lipid layer is $k_{II,L}$, and the coefficient for mass transfer to the lipid layer from the aqueous layer is $k_{II,A}$.

The initial conditions for these partial differential equations establish that at time 0, no drug is present in any part of the stratum corneum. Thus:

$$C_{II,A}(z,t=0)=0 \tag{3.8}$$

$$C_{II,L}(z,t=0)=0 \tag{3.9}$$

The stratum corneum boundary conditions establish that at the device-stratum corneum interface ($z=L_I$), the concentration in the stratum corneum equals the concentration in the device multiplied by the device/stratum corneum layer partition coefficient ($P_{II,A}$ and $P_{II,L}$ for the aqueous and lipid pathways, respectively).

$$C_{II,A}(L_I,t>0)=P_{II,A}C_I(L_I,t>0) \tag{3.10}$$

$$C_{II,L}(L_I,t>0)=P_{II,L}C_I(L_I,t>0) \tag{3.11}$$

Furthermore, at the opposite end of the stratum corneum ($z=L_I+L_{II}$), the flux continuity condition gives us the following relation between the stratum corneum and the viable epidermis:

$$D_{II,A}\frac{\partial C_{II,A}}{\partial z}(L_I+L_{II},t)=D_{III,A}\frac{\partial C_{III,A}}{\partial z}(L_I+L_{II},t) \tag{3.12}$$

$$D_{II,L}\frac{\partial C_{II,L}}{\partial z}(L_I+L_{II},t)=D_{III,L}\frac{\partial C_{III,L}}{\partial z}(L_I+L_{II},t) \tag{3.13}$$

### 3.1.3 Characterization of Transport Through the Viable Epidermis

Transport through the viable epidermis (Region III) may also be modeled as drug delivery through a set of two parallel pathways, one aqueous and the other lipid. As in the stratum corneum, mass transfer is modeled as the result of Fickian diffusion, transport from the aqueous to the lipid pathway, and transport from the lipid pathway to the aqueous pathway.

$$\frac{\partial C_{III,A}}{\partial t} = D_{III,A}\frac{\partial^2 C_{III,A}}{\partial z^2} + k_{III,A}C_{III,L} - k_{III,L}C_{III,A} \tag{3.14}$$

$$\frac{\partial C_{III,L}}{\partial t} = D_{III,L}\frac{\partial^2 C_{III,L}}{\partial z^2} + k_{III,L}C_{III,A} - k_{III,A}C_{III,L} \tag{3.15}$$

The initial condition of zero drug concentration at time 0 is also true for the viable epidermis.

$$C_{III,A}(z,t=0) = 0 \tag{3.16}$$

$$C_{III,L}(z,t=0) = 0 \tag{3.17}$$

The left boundary conditions (at $z = L_I + L_{II}$) for the viable epidermis state that concentration in each of the pathways is a function of concentration in the stratum corneum at the same point and the stratum corneum-viable epidermis partition coefficients ($P_{III,A}$ and $P_{III,L}$ for the aqueous and lipid pathways, respectively). The right boundary conditions for the viable epidermis layer, which are representative of the point of drug transfer to the capillaries, assume infinite sink conditions. These boundary conditions are defined as:

$$C_{III,A}(z = L_I + L_{II}, t > 0) = P_{III,A}C_{II}(z = L_I + L_{II}, t > 0) \tag{3.18}$$

$$C_{III,L}(z = L_I + L_{II}, t > 0) = P_{III,L}C_{II,L}(z = L_I + L_{II}, t > 0) \tag{3.19}$$

$$C_{III,A}(z = L_I + L_{II} + L_{III}, t > 0) = 0 \tag{3.20}$$

$$C_{III,L}(z = L_I + L_{II} + L_{III}, t > 0) = 0 \tag{3.21}$$

## 3.2 Potential Applications of the Three-Layer Model

The three-layer model for drug delivery through the skin is considerably more complex than the one-layer model presented in Chapter 2. The partial differential equations for the three-layer model would require additional attention in order to derive the analytical

equation. However, even in differential form, understanding the partial differential equations themselves can be useful in understanding transdermal delivery data and how certain variables will effect specific outcomes.

The three-layer model highlights the fact that a given drug substance, when applied to the skin, must be transported through three layers, each of which have very different characteristics. The rate-limiting mass transfer step for this three-layer system must be identified. Any attempt to speed or control the overall rate of drug delivery to the systemic circulation must include careful consideration of this rate-determining step.

Typically, the stratum corneum, by nature of its crystalline lipid structure, is the rate-limiting step for drug diffusion. This factor serves as justification for including only the stratum corneum layer in the model discussed in Chapter 2. However, for some transdermal systems, situations demanding exceptionally slow drug release are possible. In such situations, the diffusion coefficient for the drug through the drug vehicle ($D_l$) could be a critical parameter in ultimately controlling the rate of delivery to the systemic circulation.

Thus, even without solving the three-layer model partial differential equations, analyzing the diffusion coefficients relative to one another can provide insight into the transdermal delivery process. Such insight can then be utilized when designing experiments. For example, a study may need to quantify drug delivery through a series of differing drug vehicle formulations. Each formulation may contain varied concentrations of chemical enhancers, designed to speed the delivery process. Often, the time and resources required to test each modified formulation individually may be prohibitive. Analysis of the partial differential equation coefficients, however, can help

to narrow the number of potential formulations. For example, as discussed in Chapter 1, various chemical enhancers have been studied as a means of improving transdermal drug delivery. Analysis of the diffusion coefficients and partition coefficients can benefit such investigations by reducing the overall number of experiments run. If the diffusion and partition coefficient for a particular control group are known, then knowledge of the chemical properties of the skin and the drug substance may be used to plan and rationalize further trials. For instance, if partitioning of the drug into the skin is shown to be the rate-limiting step, then further studies can be designed to augment the partition coefficient rather than the diffusion coefficient.

As another example, assume that the diffusion, $D_{II}$, for a particular drug through the stratum corneum is known. Rather than testing each formulation for total effective diffusion through the skin, a small number of individual formulations could be tested to determine a set of baseline diffusions, $\{D^*\}$. Diffusion coefficients from this set can then be compared against one another and against $D_{II}$. This comparison would indicate if any significant differences in diffusion exist among the tested formulations, and if so, which candidate or candidates produce the most desirable results. Based on these preliminary results, an additional set of formulations could be prepared to optimize the formulation. Further comparison of diffusion coefficients could be performed among the subsequent set to further optimize, if necessary. The overall result from this approach would be determination of the desired drug vehicle formulation in a reduced number of empirical trials.

# CHAPTER 4

# DATA ANALYSIS USING THE TWO-PATHWAY MODEL

Thus far, two separate mathematical models have been presented as means of characterizing transdermal drug delivery phenomena. This section applies the transient model discussed in Chapter 2 to the analysis of *in vitro* transdermal drug delivery data. A simplification of this model is presented. The application of non-linear regression software for data analysis is then explained. Finally, the results of the analysis are discussed.

## 4.1 Analysis of Transdermal Iontophoretic Data for Three Drug Substances

A series of Franz diffusion cell experiments were conducted by Wang (2004) to study iontophoretic delivery of tricyclic antidepressants through human skin. The three drugs studied were amitriptyline HCl, clomipramine HCl, and nortriptyline HCl. Each of these molecules consists of three cyclical hydrocarbon chains and a subgroup chain extending from the center ring. The structure of the center ring and the subgroup chain differ slightly; this difference contributes to slight differences in molecular mass among the three drugs.

Wang's investigations consisted of varying the donor cell drug concentration (0.0032 M, 0.016 M, and 0.032 M) and varying the applied iontophoretic current density (0.1 $mA/cm^2$, 0.2 $mA/cm^2$, 0.3 $mA/cm^2$, and 0.4 $mA/cm^2$). For each specific set of the variables (that is, drug molecule, drug concentration, and current density), three individual determinations were performed. All three determinations for a given set of

concentration and current conditions were performed on human skin samples from the same donor. The large quantity of samples required for the study prohibited the use of the same donor for all studied conditions.

Human skin samples were placed in a Franz diffusion cell apparatus. The exposed surface area of each sample was 0.64 $cm^2$ and the skin thickness of each sample was 500±100 μm. At start up, each donor cell contained the drug substance dissolved in water to the desired molar concentration. Each receptor cell contained a phosphate saline solution. Analysis of the quantity of active in the receptor cell was performed by extracting samples from the receptor solution every hour for a total of 8 hours. Samples were analyzed for drug content via HPLC. Further details regarding the methods are given in Wang, 2004.

## 4.2 Simplification of the Two-Pathway Model

In Chapter 2, a two-pathway system describing transient drug transport through the stratum corneum was presented. These transient equations were based on the steady state equations developed by Kontturi and Murtomäki (1996).

One of the important assumptions of the Kontturi-Murtomäki model is that ionized drug molecules can only travel through the aqueous pathway of the stratum corneum. This assumption has an important impact on the analysis of the data set described in Section 4.1. All three selected drugs were salts, and thus, were highly soluble in the aqueous delivery vehicle. Having high solubilities, the quantity of non-ionized drug is essentially zero. Since the lipid pathway, as defined by Kontturi-Murtomäki, supports mass transfer of non-ionized drug only, the lipid pathway

characterized by this model cannot contribute to the flux of salts through the stratum corneum. Therefore, the analysis herein is performed using the aqueous pathway equation only.

Equation 2.17 established a transient model for iontophoretic drug delivery through the aqueous pathway of the stratum corneum. For the given system, however, several simplifications to this equation can be made.

In Equation 2.17, iontophoresis was shown to impact transdermal drug delivery through the enhancement factor $E$, which serves as a multiplier for the diffusion term $D_a$. To perform data analysis, the diffusion coefficient $D_a$ and the enhancement factor $E$ may be treated as a single, combined variable, defined here as $D'$.

The drug concentration in the aqueous pathway at the skin-reservoir interface can be considered as a function of the bulk drug concentration and a partition coefficient $P$. As such, the drug concentration within the skin at the interface is represented as $PC_b$. However, for the purpose of nonlinear regression modeling, this quantity may be treated as a combined variable, annotated here as $C'$.

Additionally, Equation 2.17 contained a term $\varepsilon$ which represented the surface area fraction belonging to the aqueous pathways. Although Kontturi and Murtomäki use a quantity of $10^{-4}$ for this parameter (1996), an exact value is not known for the analyzed system. This parameter must be included if separate fluxes through both the aqueous and lipid pathways are to be derived. However, because only aqueous pathway diffusion is assumed, the parameter $\varepsilon$ can be eliminated. The cumulative amount of drug through the lipid pathway is assumed to be 0 for all time points. Thus, the cumulative amount of drug through a skin sample of surface area $A$ is equal to the cumulative amount of drug

through the aqueous pathway surface area $A\varepsilon$. For analysis, therefore, the cumulative amount of drug, $Q_a(t)$, may be normalized by the total surface area $A$. The resulting quantity, defined as $M(t)$, is expressed in units of mass per surface area.

The analytical solution to the aqueous layer equation includes an exponential term embedded in an infinite summation series. Since the exponential portion of this series causes the summation to decay quickly, the summation may be truncated after only a few terms. For the analysis discussed herein, three terms (i.e. $n = 3$) were used.

Incorporating these simplifications, Equation 2.17 can be re-written as:

$$M(t) = \left[ \frac{D't}{L^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{3} \left( \frac{\cos(n\pi)}{n^2} * \exp\left( \frac{-D'n^2\pi^2 t}{L^2} \right) \right) \right] * C' * L \qquad (4.1)$$

As previously described in Chapter 2, $L$ is the length of the diffusion pathway and is considered to be equal to the thickness of the skin samples (500 μm).

### 4.3 Method of Data Analysis

For each Franz cell determination, the cumulative quantity of drug in the receptor cell was measured after every hour for a total of eight hours (Wang, 2004). To find the set of $M(t)$ values for a given set of conditions, the individual $M(t)$ values for each of the three determinations were averaged to find the mean quantity $\overline{M}$ at each hour.

For data analysis, NLREG nonlinear regression analysis software (Author: Phillip Sherrod, www.nlreg.com) was used on a Dell personal computer to provide a set of numerical solutions to Equation 4.1. The set of $\overline{M}$ values were entered into NLREG for each drug at each specified set of conditions. The software regressed the data and returned values for $D'$ and $C'$ for each set of data.

## 4.4 Resulting Diffusion and Partition Coefficients

After performing the nonlinear regression analysis, the data sets were analyzed to determine the existence of any correlation between either $D'$ or $C'$ and current density and/or donor cell drug concentration. Analysis was performed using analysis of variance (ANOVA) for each of the three drugs.

### 4.4.1 Relationship of Current Density to Drug Diffusion Coefficients

For each of the three drugs, results of ANOVA showed no statistically significant relationship between the current density and the diffusion coefficient $D'$. This result indicated that the diffusion coefficient is constant for a particular drug irrespective of iontophoretic current. The mean diffusion coefficient and standard deviation for each drug are given in Table 4.1.

**Table 4.1** Molar Mass and Calculated Diffusion Coefficients for Amitriptyline HCl, Clomipramine HCl, and Nortriptyline HCl

| Drug | Molar Mass [g/mol] | Diffusion Coefficient ($*10^{-4}$) [$cm^2/hr$] |
|---|---|---|
| Amitriptyline | 313.9 | $1.78 \pm 0.33$ |
| Clomipramine | 351.3 | $1.68 \pm 0.46$ |
| Nortriptyline | 299.8 | $1.05 \pm 0.14$ |

As presented in Equation 2.17, Kontturi and Murtomäki (1996) incorporated the effect of iontophoresis into their mathematical model through the use of the enhancement factor $E$. The magnitude of $E$ was taken to increase with current density. The results of the ANOVA analysis conducted herein are contrary to the findings of Kontturi and Murtomäki. As previously discussed, the diffusion coefficient and the enhancement

factor $E$ were treated as the lumped parameter $D'$. Based on Equation 2.17, a relationship between $D'$ and applied current would be expected. However, the constant value of $D'$ indicates that diffusivity is not dependent upon current for the drug substances studies at the range of currents studied.

### 4.4.2 ANOVA Analysis of Drug Partition Coefficients

Nonlinear analysis of Equation 4.1 included the parameter $C'$, which represents the concentration within the stratum corneum at the donor cell reservoir-skin interface (i.e. z=0). $C'$ is expressed in units of $\mu g/cm^3$. The concentration at this point is a function of both the bulk drug concentration in the applied delivery device ($C_b$) and the drug partition coefficient $P$. In Section 4.2, the value of $C'$ is taken to be the product of these two. Two ANOVA analyses were carried out. First, the values of $C'$ were analyzed for any correlation to iontophoretic current or to applied bulk concentration. Then, the same analysis was conducted after normalizing $C'$ by the known bulk concentration $C_b$. The results of the ANOVA analysis are given in Table 4.2.

**Table 4.2** Results of ANOVA Analysis for the Skin Concentration C′ and the Drug Partition Coefficient P

| Drug | Correlation to Iontophoretic Current | | | Correlation to the Bulk Concentration in the Vehicle | | |
|---|---|---|---|---|---|---|
| | $F_{critical}$ Value | Test Statistic from Analysis of C' | Test Statistic from Analysis of P | $F_{critical}$ Value | Test Statistic from Analysis of C' | Test Statistic from Analysis of P |
| Amitriptyline | 6.94 | 16.13 | 2.68 | 6.94 | 5.57 | 55.75 |
| Clomipramine | 5.14 | 14.06 | 4.90 | 4.76 | 6.41 | 57.02 |
| Nortriptyline | 5.14 | 6.80 | 2.66 | 4.76 | 0.77 | 28.86 |

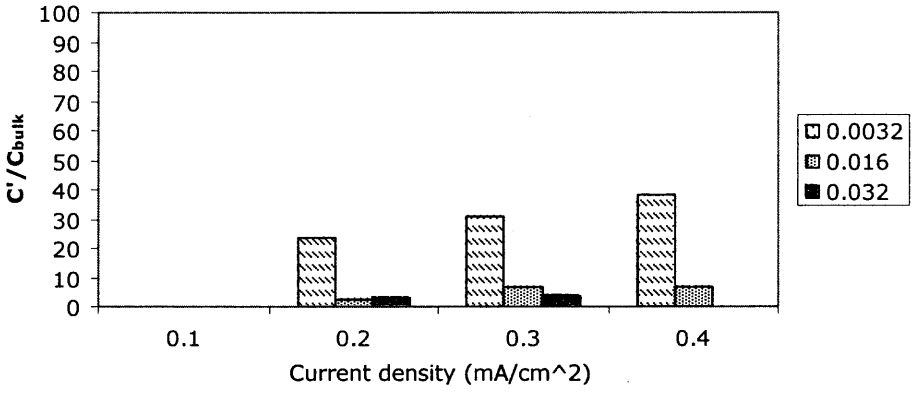
The ANOVA results showed that a statistically significant relationship exists between iontophoretic current and the skin-side concentration $C'$. However, when the partition coefficient is calculated by normalizing $C'$ for the bulk drug concentration within the vehicle, ANOVA analysis showed no statistically significant correlation between applied current the partition coefficient.

Analysis of a possible correlation to bulk concentration gave the opposite result. With the exception of clomipramine, no statistically significant relationship was noted between the skin-side concentration $C'$ and the bulk concentration $C_b$. After normalizing $C'$ by $C_b$ to obtain $P'$, a strong correlation was detected for all three drugs, as expected.

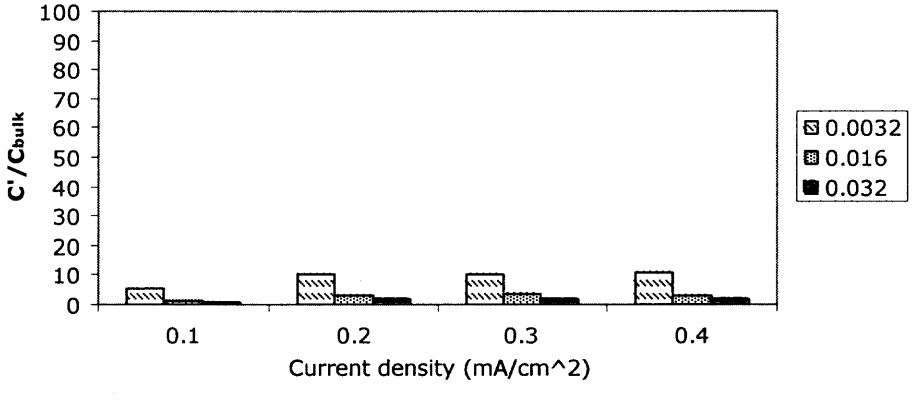
The positive correlation between the magnitude of applied iontophoretic current and the stratum corneum concentration at $z = 0$ indicates that iontophoresis enhances flux through the stratum corneum by increasing the concentration of drug penetrating the skin at the skin-device interface. However, the lack of correlation between $P'$ and applied current indicate that the mechanism for enhancement is not simply increasing the drug's partition coefficient.

The permeation from the bulk solution in the drug reservoir to the skin is greatly enhanced at lower concentrations. This trend was generally observed for all three of the drugs and indicates that as concentration increases, a lesser fraction of the drug in the donor cell is transmitted through the skin. This effect is shown in Figure 4.1

The results of the analysis indicate that other factors may be impacting upon the magnitude of stratum corneum concentration at the interface. One such factor that could impact upon $C'$ is the skin damage factor. The skin damage factor may change the available volume in which the drug concentrates within the skin.




(a)

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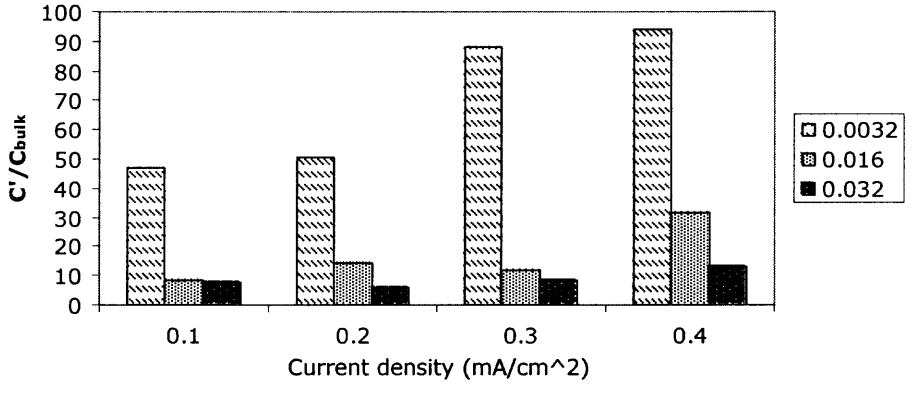



(c)

**Figure 4.1** Values of P for (a) amitriptyline HCl, (b) clomipramine HCl, and (c) nortriptyline HCl. Concentrations are in units of mol/L. Among trials run at equal current densities, P, calculated as the ratio of $C'/C_{bulk}$, decreased with increasing concentration.

### 4.5 Comparison of Flux from Nonlinear Regression and Graphical Analysis

Wang (2004) employed a graphical method for computing the flux. The cumulative amount through the skin versus time was plotted for each data set. The graph yielded a curve function that could be approximated as a linear function at large time values. From the linear region of the graph, the slope was determined and then normalized by the skin sample surface area in order to calculate the empirical flux.

As a means of assessing the results of the transient state equation versus the flux calculated by the conventional, graphical method, a parity plot (Figure 4.2) was constructed. The empirical flux values $J_g$ derived from graphing the data (Wang, 2004), was compared to the theoretical flux values $J_t$ computed from the transient state equation coefficients.

To calculate theoretical flux values $J_t$, the transient state equation and large time approximation were used. At large $t$ values, Equation 2.17 becomes linear. The slope of this equation is equal to the product of the diffusion coefficient and the concentration at the skin-device boundary divided by the diffusion pathway length. Thus, the flux as computed by the transient equation parameters ($J_t$), with units of mass per time area, can be calculated as

$$J_t = \frac{C'^{*}D'}{L} \tag{4.2}$$

For the given data sets, the exposed surface area in the Franz cell diffusion apparatus was 0.64 $cm^2$. The length of the diffusion pathway was considered equal to the thickness of the skin sample (i.e. 500±100 μm). Tortuosity of the path was considered negligible with respect to the entire thickness of the skin. A comparison of the flux

values calculated from the nonlinear regression parameters and applied iontophoretic current is shown in Figure 4.3.

The two sets of flux values $J_g$ and $J_t$ were in close agreement with each other. By plotting these two fluxes against one another and performing a linear fit, a regression parameter of 0.97 and a slope of nearly unity (0.97) were obtained. This result indicates that the two methods are in close agreement with each other.

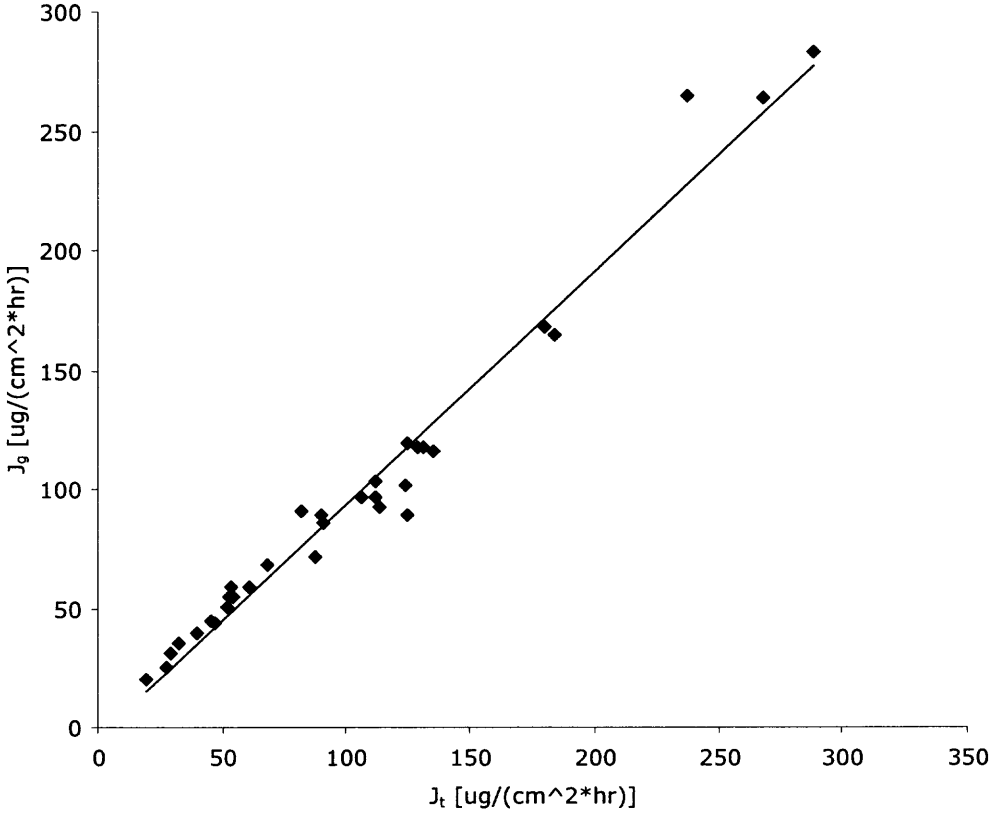
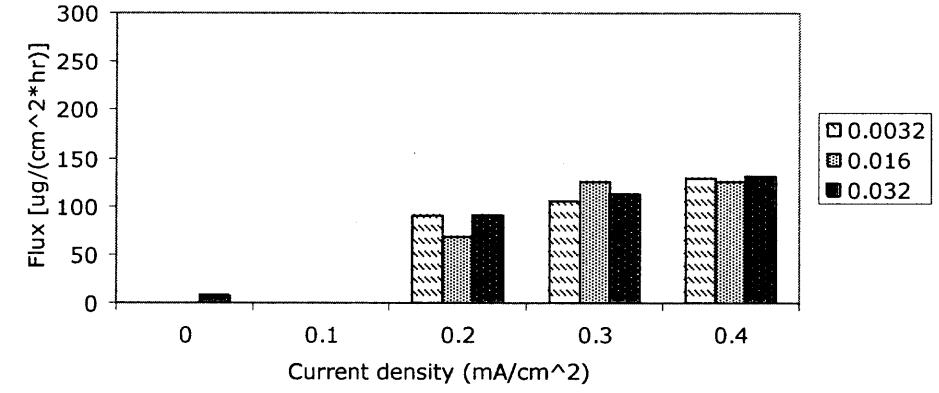
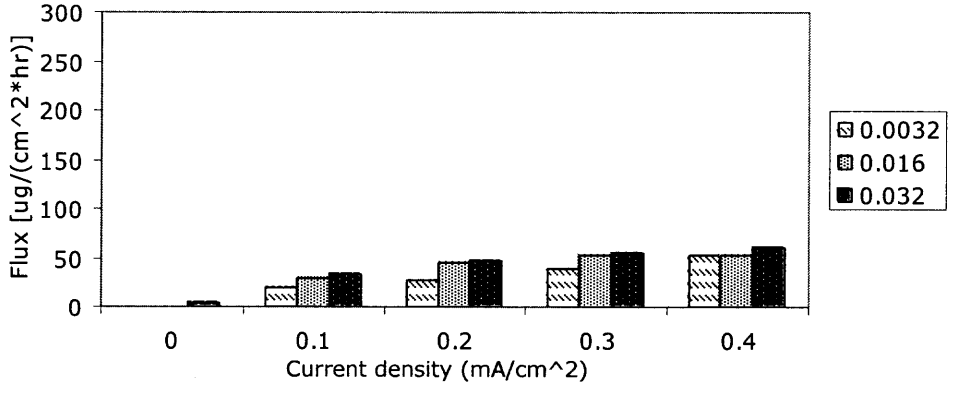


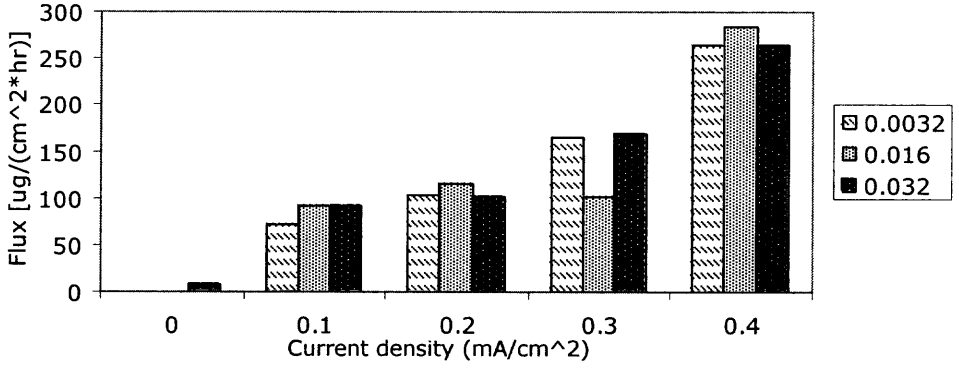

**Figure 4.2** Comparison of flux values obtained from the graphical interpretation of empirical data ($J_g$) and from theoretical calculation using the derived nonlinear regression parameters ($J_t$).




(a)




(b)




(c)

**Figure 4.3** Flux values $J_t$ calculated for (a) amitriptyline HCl, (b) clomipramine HCl, and (c) nortriptyline HCl. Concentrations are in units of mol/L. For the control group (i.e. no applied current), data for only one concentration (0.032 M) was available. For amitriptyline HCl, data for 0.1 mA/cm$^2$ was not available.

Flux determination using the conventional, graphical method is advantageous for several reasons. This method can be quickly and easily applied to Franz cell analysis. For characterizing iontophoretic data, the graphical approach is useful because flux through the skin during iontophoresis quickly becomes constant. Thus, the large time value approximation required by the graphical method can be applied relatively soon after drug device application. For example, most of the data points for the drugs analyzed herein fell within the linear range after the first hour. This minimized the number of data points omitted from the regression analysis. Among the disadvantages of the graphical method are: 1) the analysis does not capture the entire data set, 2) the selection of points in the linear portion of the curve can be subjective in nature, and 3) differing answers may be obtained based on the inclusion or omission of data points.

Use of the transient equation for delivery across the viable epidermis eliminates these concerns since all data points may be used. A more complete characterization of the data, even at small time values prior to reaching steady flux, may be achieved.

Historically, the major disadvantage of using a transient equation such as Equation 4.1 has been that it is nonlinear, and therefore, difficult to regress. However, by implementing nonlinear regression software, data sets can be very easily fit to an equation such as this.

More importantly, use of the transient equation to solve for flux enables one to determine the diffusion coefficient and to characterize the concentration within the stratum corneum at the skin-device interface. Such information can then be used to enhance the understanding of how a particular set of variables works to promote or hinder delivery through the skin.

### 4.6 Effect of $D'$ and $C'$ Parameters on Flux Through the Stratum Corneum

Typically, the key parameter of interest in transdermal drug delivery studies is flux. Since delivery through the stratum corneum is usually a rate-limiting step in the overall delivery of drug to the blood stream, control of this parameter, and understanding the options for enhancing it, are of great interest.

As the data shows, the calculated diffusion coefficients differed among drug substances but did not vary substantially with changes in current density or bulk concentration. As evidence of this, nortriptyline HCl produced the highest overall flux values, but the smallest diffusion coefficient of the three drugs analyzed.

# CHAPTER 5

# CONCLUSIONS

The close agreement between the flux values derived from the aqueous layer transient state equation and the graphical method demonstrate the suitability of the theoretical model for iontophoretic delivery. Additionally, the results act as further validation of the conventional, graphical method. The graphical method may be preferred as a suitable, simple means of flux analysis.

The transient model equations show that the delivery of amitriptyline HCl, clomipramine HCl, and nortriptyline HCl is dependent upon the diffusion coefficient parameter and the bulk drug concentration. The large time approximation for the accumulated drug penetration is helpful in understanding this effect. The large time approximation mathematically describes how these parameters impact overall drug delivery.

A major challenge in the designing of drugs for transdermal delivery is the establishment of an adequate and steady rate of delivery through the skin. The implications of the large time approximation equation can be readily applied in developing the correct system for the correct delivery strategy.

The large time approximation shows that drug delivery can be influenced by bulk concentration, the drug partition coefficient, the diffusion coefficient, and the diffusion pathway length. Bulk concentration is frequently the easiest of these to control, from a formulation standpoint. It is also a very important parameter because it impacts both the rate of drug transport and the overall quantity of drug transported.

Increasing the value of the diffusion coefficient will also increase drug delivery through the skin. Diffusivity is related to chemical or physical properties of the skin, the drug and the vehicle into which the drug is formulated. In order to utilize knowledge of the diffusion coefficient to augment overall drug permeation, an understanding of which factors increase diffusivity is required.

Typically for a biological system such as the skin, the diffusion pathway length is modeled as a constant of the skin itself. Many studies available in the literature, however, indicate that tortuosity of the stratum corneum can vary with hydration levels. Since the diffusion pathway is a lumped parameter that incorporates both membrane thickness and path tortuosity, empirical results suggest means of altering the diffusion pathway length do exist (Talereja et al., 2001). Furthermore, Tezel and Mitragotri note (2003) that the size of the diffused particles may change the effective tortuosity. The skin contains pores that extend both perpendicular to and parallel to the direction of drug transport. Due to their size, smaller particles may be able to diffuse through a greater number of paths, while large particles are more limited in the number of diffusion pathways through which they can pass. As such, diffusion of the smaller particles through the stratum corneum may follow a less direct path than diffusion of larger particles. This factor may be the reason why nortriptyline HCl, which had the smallest molar mass, also had the smallest diffusion coefficient.

A series of partial differential equations for a three-layer system were additionally presented. Studying the coefficients in this equation can also help to illuminate the mechanisms for drug delivery through this type of system.

In the development of drug delivery regimens, particularly those used for high potency drugs, it may be necessary to understand the delivery dynamics at the initial time points as well as after achieving steady flux. Additionally, particularly for the purposes of data collection and analysis, one may wish to know after what time point is the large time approximation appropriate. By using nonlinear regression, the dilemma of judging which data to include and which data to omit is circumvented. Furthermore, the nonlinear regression method allows one to calculate coefficients such as the diffusion coefficient and the partition coefficient. Utilizing this information, additional insight into the mechanisms of transdermal drug delivery can be achieved.

# APPENDIX

## NLREG PROGRAM RESULTS

This Appendix contains the results generated from nonlinear analysis with NLREG.

**Amitriptyline 0.5%, 0.2 mA/cm^2**

```
  1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;        // Diffusion coefficient in aqueous layer
   6: Parameter C1;    // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.

   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 262
Final sum of squared deviations = 1.7888542E+002
Final sum of deviations = 4.8031824E+000
Standard error of estimate = 5.0552
Average deviation = 3.8251
Maximum deviation for any observation = 7.77709
Proportion of variance explained (R^2) = 0.9985  (99.85%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9983  (99.83%)
Durbin-Watson test for autocorrelation = 1.593

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 16:36.  Runtime = 0.06 seconds.

             ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
-----------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0        334.5104        132.3387        120.9255


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
-----------------  -------------  ----------------
                Da              1    0.000188528046
                C1              1        15115.8932
```

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 116804.9 | 116804.9 | 4570.72 | 0.00001 |
| Error | 7 | 178.8854 | 25.55506 | | |
| Total | 8 | 116983.8 | | | |

## Amitriptyline 0.1%, 0.2 mA/cm^2

```
1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
Copyright (c) 1992-2004 Phillip H. Sherrod.
Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 267
Final sum of squared deviations = 2.6638562E+002
Final sum of deviations = 1.0011188E+001
Standard error of estimate = 6.16888
Average deviation = 3.70543
Maximum deviation for any observation = 13.9705
Proportion of variance explained (R^2) = 0.9966  (99.66%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9962  (99.62%)
Durbin-Watson test for autocorrelation = 1.793

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 16:40.  Runtime = 0.05 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 278.6589 | 117.0429 | 99.46896 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000254444822 |
| C1 | 1 | 8501.25776 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 78886.2 | 78886.2 | 2072.95 | 0.00001 |
| Error | 7 | 266.3856 | 38.05509 | | |
| Total | 8 | 79152.59 | | | |

**Amitriptyline 1%, 0.2 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1117.26)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (2513.84)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1090.08)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (756.387)
Stopped due to: Relative function convergence.

   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 250
Final sum of squared deviations = 1.3059280E+002
Final sum of deviations = -2.3224444E-001
Standard error of estimate = 4.31927
Average deviation = 3.10056
Maximum deviation for any observation = 7.27618
Proportion of variance explained (R^2) = 0.9987  (99.87%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9985  (99.85%)
Durbin-Watson test for autocorrelation = 1.818

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 16:46.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 309.4743 | 117.1628 | 112.7275 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000154599546 |
| C1 | 1 | 18583.1992 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 101529.3 | 101529.3 | 5442.14 | 0.00001 |
| Error | 7 | 130.5928 | 18.65611 | | |
| Total | 8 | 101659.9 | | | |

**Amitriptyline 0.5%, 0.3 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.

   ----  Final Results  ----
NLREG version 6.1
Copyright (c) 1992-2004 Phillip H. Sherrod.

Aqueous layer equation
Number of observations = 8
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 259
Final sum of squared deviations = 1.1991658E+002
Final sum of deviations = -5.5311663E+000
Standard error of estimate = 4.47058
Average deviation = 3.26535
Maximum deviation for any observation = 7.70795
Proportion of variance explained (R^2) = 0.9992  (99.92%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9990  (99.90%)
Durbin-Watson test for autocorrelation = 1.965

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 16:51.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4.1875 | 2.852787 |
| Qa | 0 | 371.8916 | 158.5584 | 142.2011 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000170814282 |
| C1 | 1 | 19685.9202 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 141428.2 | 141428.2 | 7076.33 | 0.00001 |
| Error | 6 | 119.9166 | 19.9861 | | |
| Total | 7 | 141548.1 | | | |

**Amitriptyline 0.1 %, 0.3 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (848.706)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1909.59)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (696.047)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (535.655)
Stopped due to: Both parameter and relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 8
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Both parameter and relative function convergence.
Number of iterations performed = 276
Final sum of squared deviations = 1.0768419E+002
Final sum of deviations = -3.0535445E+000
Standard error of estimate = 4.23643
Average deviation = 3.27239
Maximum deviation for any observation = 6.84825
Proportion of variance explained (R^2) = 0.9991  (99.91%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9990  (99.90%)
Durbin-Watson test for autocorrelation = 1.969

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 16:59.  Runtime = 0.06 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4.1875 | 2.852787 |
| Qa | 0 | 346.514 | 148.265 | 131.6869 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000176182512 |
| C1 | 1 | 17490.2645 |

```
                ----  Analysis of Variance  ----
  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        121282.4        121282.4    6757.67  0.00001
Error          6        107.6842        17.94736
Total          7          121390
```

**Amitriptyline 1%, 0.3 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 8
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 254
Final sum of squared deviations = 1.3849388E+002
Final sum of deviations = -7.8437033E+000
Standard error of estimate = 4.80441
Average deviation = 3.03441
Maximum deviation for any observation = 9.80597
Proportion of variance explained (R^2) = 0.9990  (99.90%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9988  (99.88%)
Durbin-Watson test for autocorrelation = 1.887

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 17:04.  Runtime = 0.06 seconds.


            ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8          4.1875        2.852787
                Qa               0        363.4999        148.5523        138.3594


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
------------------  -------------  ----------------
                Da              1    0.000142352593
                C1              1        25044.2122


                ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        133864.8        133864.8    5799.45  0.00001
Error          6        138.4939        23.08231
Total          7        134003.3
```

**Amitriptyline 0.5%, 0.4 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 8
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 242
Final sum of squared deviations = 1.7078124E+002
Final sum of deviations = -6.4273812E+000
Standard error of estimate = 5.33512
Average deviation = 3.69599
Maximum deviation for any observation = 9.53796
Proportion of variance explained (R^2) = 0.9992  (99.92%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9990  (99.90%)
Durbin-Watson test for autocorrelation = 2.140

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 17:09.  Runtime = 0.06 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4.1875 | 2.852787 |
| Qa | 0 | 451.6179 | 191.6727 | 172.2663 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000168335368 |
| C1 | 1 | 24359.7112 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 207558.9 | 207558.9 | 7292.10 | 0.00001 |
| Error | 6 | 170.7812 | 28.46354 | | |
| Total | 7 | 207729.6 | | | |

**Amitriptyline 0.1%, 0.4 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;    // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
Copyright (c) 1992-2004 Phillip H. Sherrod.

Aqueous layer equation
Number of observations = 8
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 240
Final sum of squared deviations = 2.5646125E+002
Final sum of deviations = -7.5247469E+000
Standard error of estimate = 6.53785
Average deviation = 5.2399
Maximum deviation for any observation = 8.44332
Proportion of variance explained (R^2) = 0.9988  (99.88%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9986  (99.86%)
Durbin-Watson test for autocorrelation = 1.916

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 17:14.  Runtime = 0.05 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4.1875 | 2.852787 |
| Qa | 0 | 448.3213 | 194.8525 | 172.7989 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000183746456 |
| C1 | 1 | 21564.0313 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 208759.8 | 208759.8 | 4884.01 | 0.00001 |
| Error | 6 | 256.4613 | 42.74354 | | |
| Total | 7 | 209016.2 | | | |

**Amitriptyline 1%, 0.4 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 8
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 242
Final sum of squared deviations = 2.9223007E+002
Final sum of deviations = -9.0032209E+000
Standard error of estimate = 6.9789
Average deviation = 5.07336
Maximum deviation for any observation = 10.4809
Proportion of variance explained (R^2) = 0.9986  (99.86%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9983  (99.83%)
Durbin-Watson test for autocorrelation = 2.022

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 17:17.  Runtime = 0.06 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4.1875 | 2.852787 |
| Qa | 0 | 440.6788 | 185.523 | 169.806 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000155975524 |
| C1 | 1 | 26767.9595 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 201546.4 | 201546.4 | 4138.10 | 0.00001 |
| Error | 6 | 292.2301 | 48.70501 | | |
| Total | 7 | 201838.7 | | | |

**Amitriptyline Control**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1950.97)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (4389.68)
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 132
Final sum of squared deviations = 2.9412881E+000
Final sum of deviations = -1.1868429E+000
Standard error of estimate = 0.648216
Average deviation = 0.488224
Maximum deviation for any observation = 1.12615
Proportion of variance explained (R^2) = 0.9964  (99.64%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9959  (99.59%)
Durbin-Watson test for autocorrelation = 2.558

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 16:06.  Runtime = 0.11 seconds.


             ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value    Maximum value     Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8        3.722222        3.011552
                Qa               0        26.20842        10.10091         10.1134


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
------------------  -------------  ----------------
                Da              1    0.000201656757
                C1              1        1094.28638


                  ----  Analysis of Variance  ----
  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        815.3049        815.3049    1940.35  0.00001
Error          7        2.941288        0.420184
Total          8        818.2462
```

**Clomipramine 0.5%, 0.1 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 252
Final sum of squared deviations = 9.7682850E+001
Final sum of deviations = 6.3080725E+000
Standard error of estimate = 3.7356
Average deviation = 2.4816
Maximum deviation for any observation = 7.91646
Proportion of variance explained (R^2) = 0.9930  (99.30%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9920  (99.20%)
Durbin-Watson test for autocorrelation = 2.005

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 17:33.  Runtime = 0.06 seconds.


             ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0         118.742        49.07861        41.67313


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
------------------  -------------  ----------------
                Da              1    0.000242031313
                C1              1        3810.58179


                  ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        13795.52        13795.52     988.59  0.00001
Error          7        97.68285        13.95469
Total          8         13893.2
```

**Clomipramine 0.1%, 0.1 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 208
Final sum of squared deviations = 1.2456287E+002
Final sum of deviations = 9.9853494E+000
Standard error of estimate = 4.21838
Average deviation = 3.10507
Maximum deviation for any observation = 6.99341
Proportion of variance explained (R^2) = 0.9714  (97.14%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9673  (96.73%)
Durbin-Watson test for autocorrelation = 0.995

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 17:38.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 68.91982 | 26.71473 | 23.34472 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000161268285 |
| C1 | 1 | 3781.61138 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 4235.244 | 4235.244 | 238.01 | 0.00001 |
| Error | 7 | 124.5629 | 17.7947 | | |
| Total | 8 | 4359.807 | | | |

**Clomipramine 1%, 0.1 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (979.203)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (529.692)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (991.656)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (2231.23)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (931.208)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (562.399)
Stopped due to: Relative function convergence.


   ----  Final Results  ----
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 271
Final sum of squared deviations = 9.7451427E+001
Final sum of deviations = 3.5233566E+000
Standard error of estimate = 3.73117
Average deviation = 2.74922
Maximum deviation for any observation = 6.65513
Proportion of variance explained (R^2) = 0.9948  (99.48%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9940  (99.40%)
Durbin-Watson test for autocorrelation = 1.220

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 18:27.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 136.61 | 56.54963 | 48.24946 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000259387173 |
| C1 | 1 | 4007.55573 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 18526.63 | 18526.63 | 1330.78 | 0.00001 |
| Error | 7 | 97.45143 | 13.92163 | | |
| Total | 8 | 18624.08 | | | |

**Clomipramine 0.5%, 0.2 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (705.543)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1348.46)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (3034.02)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (613.49)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1380.35)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (553.516)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (560.391)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1168.75)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (2629.69)
```

```
Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (555.096)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1248.97)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (558.608)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (640.285)
Stopped due to: Relative function convergence.


   ----  Final Results  ----

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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 235
Final sum of squared deviations = 1.8086043E+002
Final sum of deviations = 9.8590568E+000
Standard error of estimate = 5.08303
Average deviation = 3.41423
Maximum deviation for any observation = 9.74235
Proportion of variance explained (R^2) = 0.9912  (99.12%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9900  (99.00%)
Durbin-Watson test for autocorrelation = 1.531

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 18:32.  Runtime = 0.11 seconds.


             ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0        142.9518        53.09832          50.766


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
------------------  -------------  ----------------
                Da              1    0.000130961661
                C1              1        11041.4339


                  ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        20436.64        20436.64     790.98  0.00001
Error          7        180.8604         25.8372
Total          8         20617.5
```

**Clomipramine 0.1%, 0.2 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (639.326)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1438.48)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (603.632)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1358.17)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (934.034)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (912.722)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (2053.62)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (861.832)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (531.872)
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 188
Final sum of squared deviations = 7.8825876E+001
Final sum of deviations = 1.6649555E+000
Standard error of estimate = 3.35572
Average deviation = 2.67774
Maximum deviation for any observation = 4.50932
Proportion of variance explained (R^2) = 0.9885  (98.85%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9869  (98.69%)
Durbin-Watson test for autocorrelation = 2.538
```

```
Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 18:37.  Runtime = 0.11 seconds.

            ----  Descriptive Statistics for Variables  ----
      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0        78.39276        28.96193          29.278


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
------------------  -------------  ---------------
                Da              1    0.000118247768
                C1              1        7390.97932


                  ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        6778.782        6778.782     601.98  0.00001
Error          7        78.82588        11.26084
Total          8        6857.608
```

## Clomipramine 1%, 0.2 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 218
Final sum of squared deviations = 1.8471323E+002
Final sum of deviations = 8.3035381E+000
Standard error of estimate = 5.13689
Average deviation = 3.92323
Maximum deviation for any observation = 8.01804
Proportion of variance explained (R^2) = 0.9921  (99.21%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9910  (99.10%)
Durbin-Watson test for autocorrelation = 1.871

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 18:41.  Runtime = 0.05 seconds.
```

```
              ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0        153.8132        56.59293         54.0279


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
------------------  -------------  ----------------
                Da              1    0.000137392377
                C1              1        10841.2227


                  ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1         23167.4         23167.4     877.97  0.00001
Error          7        184.7132         26.3876
Total          8        23352.11
```

## Clomipramine 0.5%, 0.3 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 202
Final sum of squared deviations = 9.3550511E+001
Final sum of deviations = -2.6479496E-001
Standard error of estimate = 3.65573
Average deviation = 2.53801
Maximum deviation for any observation = 6.14425
Proportion of variance explained (R^2) = 0.9969  (99.69%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9964  (99.64%)
Durbin-Watson test for autocorrelation = 1.272

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 18:45.  Runtime = 0.11 seconds.


              ----  Descriptive Statistics for Variables  ----
```

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 167.5041 | 61.84164 | 61.11416 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000137540292 |
| C1 | 1 | 12025.6431 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 29785.97 | 29785.97 | 2228.76 | 0.00001 |
| Error | 7 | 93.55051 | 13.36436 | | |
| Total | 8 | 29879.52 | | | |

## Clomipramine 0.1 %, 0.3 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 224
Final sum of squared deviations = 6.4111178E+001
Final sum of deviations = 1.8142024E+000
Standard error of estimate = 3.02634
Average deviation = 2.33539
Maximum deviation for any observation = 4.98542
Proportion of variance explained (R^2) = 0.9970  (99.70%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9966  (99.66%)
Durbin-Watson test for autocorrelation = 2.092

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 18:51.  Runtime = 0.06 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 142.0597 | 55.18286 | 51.59176 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000170408637 |
| C1 | 1 | 7413.48622 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 21229.57 | 21229.57 | 2317.96 | 0.00001 |
| Error | 7 | 64.11118 | 9.15874 | | |
| Total | 8 | 21293.68 | | | |

**Clomipramine 1%, 0.3 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.
```

---- Final Results ----

```
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 218
Final sum of squared deviations = 1.6878953E+002
Final sum of deviations = 7.9779120E+000
Standard error of estimate = 4.91048
Average deviation = 3.58038
Maximum deviation for any observation = 8.95871
Proportion of variance explained (R^2) = 0.9947  (99.47%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9940  (99.40%)
Durbin-Watson test for autocorrelation = 1.634

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 18:57.  Runtime = 0.05 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 177.1643 | 65.8327 | 63.15223 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000137580909 |
| C1 | 1 | 12616.7123 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 31736.84 | 31736.84 | 1316.18 | 0.00001 |
| Error | 7 | 168.7895 | 24.11279 | | |
| Total | 8 | 31905.63 | | | |

## Clomipramine 0.5%, 0.4 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (559.349)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (883.083)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1986.94)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (761.438)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (594.758)
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 230
Final sum of squared deviations = 5.4892846E+001
Final sum of deviations = -4.9320779E-001
Standard error of estimate = 2.80033
Average deviation = 2.16673
Maximum deviation for any observation = 4.02908
Proportion of variance explained (R^2) = 0.9986  (99.86%)
```

```
Adjusted coefficient of multiple determination (Ra^2) = 0.9984  (99.84%)
Durbin-Watson test for autocorrelation = 1.526

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 22:24.  Runtime = 0.05 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 186.84 | 72.75818 | 69.0723 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000165603593 |
| C1 | 1 | 10290.1685 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 38112.97 | 38112.97 | 4860.21 | 0.00001 |
| Error | 7 | 54.89285 | 7.841835 | | |
| Total | 8 | 38167.86 | | | |

## Clomipramine 0.1%, 0.4 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (690.957)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1554.65)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (864.767)
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
```

```
Stopped due to: Relative function convergence.
Number of iterations performed = 253
Final sum of squared deviations = 4.3319788E+001
Final sum of deviations = -4.8491571E+000
Standard error of estimate = 2.48768
Average deviation = 1.70732
Maximum deviation for any observation = 4.57979
Proportion of variance explained (R^2) = 0.9990  (99.90%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9989  (99.89%)
Durbin-Watson test for autocorrelation = 1.523

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 22:26.  Runtime = 0.06 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 200.4261 | 81.93548 | 74.97452 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000211502927 |
| C1 | 1 | 7924.27242 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 44926.11 | 44926.11 | 7259.56 | 0.00001 |
| Error | 7 | 43.31979 | 6.188541 | | |
| Total | 8 | 44969.43 | | | |

## Clomipramine 1%, 0.4 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.
```

---- Final Results ----

```
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 245
Final sum of squared deviations = 9.0199496E+001
Final sum of deviations = -4.3002940E+000
```

```
Standard error of estimate = 3.58966
Average deviation = 2.60861
Maximum deviation for any observation = 5.49494
Proportion of variance explained (R^2) = 0.9978  (99.78%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9975  (99.75%)
Durbin-Watson test for autocorrelation = 1.387

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 22:29.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 198.9717 | 72.29846 | 72.28478 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000138151316 |
| C1 | 1 | 14033.9368 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 41710.52 | 41710.52 | 3236.98 | 0.00001 |
| Error | 7 | 90.1995 | 12.88564 | | |
| Total | 8 | 41800.72 | | | |

## Clomipramine Control

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (837.928)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (3351.71)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (7541.36)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (559.447)
Stopped due to: Relative function convergence.
```

```
   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 112
Final sum of squared deviations = 7.7923479E+000
Final sum of deviations = -1.6335173E+000
Standard error of estimate = 1.05508
Average deviation = 0.66161
Maximum deviation for any observation = 2.11871
Proportion of variance explained (R^2) = 0.9700  (97.00%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9657  (96.57%)
Durbin-Watson test for autocorrelation = 1.627

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 22:31.  Runtime = 0.06 seconds.
```

**---- Descriptive Statistics for Variables ----**

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 14.37897 | 5.357302 | 5.70016 |

**---- Calculated Parameter Values ----**

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000143145969 |
| C1 | 1 | 1003.10942 |

**---- Analysis of Variance ----**

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 252.1422 | 252.1422 | 226.50 | 0.00001 |
| Error | 7 | 7.792348 | 1.113193 | | |
| Total | 8 | 259.9346 | | | |

## Nortriptyline 0.5%, 0.1 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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Aqueous layer equation
Number of observations = 10
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
```

```
Number of iterations performed = 201
Final sum of squared deviations = 7.8049756E+002
Final sum of deviations = 1.1125482E+000
Standard error of estimate = 9.87736
Average deviation = 7.89258
Maximum deviation for any observation = 12.892
Proportion of variance explained (R^2) = 0.9857  (98.57%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9839  (98.39%)
Durbin-Watson test for autocorrelation = 1.201

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 22:11.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 3.6 | 2.875181 |
| Qa | 0 | 227.4917 | 69.02103 | 77.91361 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000103182878 |
| C1 | 1 | 25338.4834 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 53854.28 | 53854.28 | 552.00 | 0.00001 |
| Error | 8 | 780.4976 | 97.56219 | | |
| Total | 9 | 54634.78 | | | |

## Nortriptyline 0.1%, 0.1 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (695.429)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1564.71)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (601.323)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
```

```
Error: Argument to exp function is too large (598.137)
Stopped due to: Both parameter and relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Both parameter and relative function convergence.
Number of iterations performed = 200
Final sum of squared deviations = 5.0375094E+002
Final sum of deviations = 4.7724518E+000
Standard error of estimate = 8.48318
Average deviation = 6.49554
Maximum deviation for any observation = 13.2956
Proportion of variance explained (R^2) = 0.9903  (99.03%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9889  (98.89%)
Durbin-Watson test for autocorrelation = 1.641

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 22:15.  Runtime = 0.06 seconds.


            ----  Descriptive Statistics for Variables  ----
      Variable       Minimum value    Maximum value     Mean value      Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0        226.8062        76.61281        80.58367

  ----  Calculated Parameter Values  ----
     Parameter      Initial guess   Final estimate
------------------  -------------  ----------------
                Da              1   9.76163141E-005
                C1              1        28629.1712

                  ----  Analysis of Variance  ----
  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        51446.07        51446.07     714.88  0.00001
Error          7        503.7509        71.96442
Total          8        51949.82
```

## Nortriptyline 1%, 0.1 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (515.978)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1160.95)
```

```
Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (581.856)
Stopped due to: Relative function convergence.


   ----  Final Results  ----
NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 238
Final sum of squared deviations = 1.0158489E+003
Final sum of deviations = -9.5771445E+000
Standard error of estimate = 12.0466
Average deviation = 8.60352
Maximum deviation for any observation = 19.0787
Proportion of variance explained (R^2) = 0.9805  (98.05%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9777  (97.77%)
Durbin-Watson test for autocorrelation = 1.720

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 14-Mar-2004 22:16.  Runtime = 0.05 seconds.


            ----  Descriptive Statistics for Variables  ----
     Variable       Minimum value   Maximum value    Mean value     Standard dev.
-----------------  --------------  --------------  --------------  --------------
             time               0               8               4        2.738613
               Qa               0        230.4989        69.35096        80.64051


  ----  Calculated Parameter Values  ----
    Parameter      Initial guess   Final estimate
-----------------  -------------  ----------------
               Da              1   7.41886477E-005
               C1              1        48726.5052


                ----  Analysis of Variance  ----
  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        51007.28        51007.28     351.48  0.00001
Error          7        1015.849        145.1213
Total          8        52023.13
```

**Nortriptyline 0.5%, 0.2 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;          // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.
```

```
   ----  Final Results  ----

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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 242
Final sum of squared deviations = 8.6092709E+002
Final sum of deviations = -4.3780221E+000
Standard error of estimate = 11.0901
Average deviation = 8.64707
Maximum deviation for any observation = 16.8351
Proportion of variance explained (R^2) = 0.9934  (99.34%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9925  (99.25%)
Durbin-Watson test for autocorrelation = 1.975

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 20-Mar-2004 23:57.  Runtime = 0.11 seconds.


             ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0         345.752        119.6963        127.8532


  ----  Calculated Parameter Values  ----

     Parameter      Initial guess   Final estimate
------------------  -------------  ----------------
                Da              1    0.000100028247
                C1              1        42996.3147


                  ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        129910.6        129910.6    1056.27  0.00001
Error          7        860.9271        122.9896
Total          8        130771.5
```

## Nortriptyline 0.1%, 0.2 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
```

Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 238
Final sum of squared deviations = 3.8024210E+002
Final sum of deviations = -9.1110479E-001
Standard error of estimate = 7.37023
Average deviation = 5.43786
Maximum deviation for any observation = 12.1438
Proportion of variance explained (R^2) = 0.9965 (99.65%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9960 (99.60%)
Durbin-Watson test for autocorrelation = 1.766

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 21-Mar-2004 00:07. Runtime = 0.05 seconds.

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 325.6373 | 113.5885 | 116.9231 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000114590793 |
| C1 | 1 | 31015.0152 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 108987.8 | 108987.8 | 2006.39 | 0.00001 |
| Error | 7 | 380.2421 | 54.3203 | | |
| Total | 8 | 109368.1 | | | |

## Nortriptyline 1%, 0.2 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;          // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;
```

Beginning computation...
Stopped due to: Relative function convergence.

---- Final Results ----

NLREG version 6.1


Aqueous layer equation

```
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 124
Final sum of squared deviations = 2.0129181E+003
Final sum of deviations = -3.9763705E+000
Standard error of estimate = 16.9576
Average deviation = 13.0857
Maximum deviation for any observation = 29.6808
Proportion of variance explained (R^2) = 0.9834  (98.34%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9811  (98.11%)
Durbin-Watson test for autocorrelation = 2.340

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 20-Mar-2004 23:59.  Runtime = 0.06 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 350.959 | 116.2612 | 123.2301 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000105997218 |
| C1 | 1 | 37116.2575 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 119472.3 | 119472.3 | 415.47 | 0.00001 |
| Error | 7 | 2012.918 | 287.5597 | | |
| Total | 8 | 121485.3 | | | |

## Nortriptyline 0.5%, 0.3 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (889.988)
Stopped due to: Relative function convergence.
```

---- Final Results ----

```
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```

```
Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 229
Final sum of squared deviations = 1.3082240E+003
Final sum of deviations = -3.5035557E+000
Standard error of estimate = 13.6707
Average deviation = 11.2092
Maximum deviation for any observation = 17.6665
Proportion of variance explained (R^2) = 0.9953  (99.53%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9946  (99.46%)
Durbin-Watson test for autocorrelation = 1.423

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 21-Mar-2004 00:00.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 522.735 | 182.158 | 186.0137 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000120663135 |
| C1 | 1 | 45119.8302 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 275500.7 | 275500.7 | 1474.14 | 0.00001 |
| Error | 7 | 1308.224 | 186.8891 | | |
| Total | 8 | 276808.9 | | | |

## Nortriptyline 0.1 %, 0.3 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (844.623)
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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```

```
Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 210
Final sum of squared deviations = 1.5331897E+003
Final sum of deviations = 5.4305597E-001
Standard error of estimate = 14.7996
Average deviation = 11.9369
Maximum deviation for any observation = 21.0218
Proportion of variance explained (R^2) = 0.9944  (99.44%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9936  (99.36%)
Durbin-Watson test for autocorrelation = 1.272

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 21-Mar-2004 00:01.  Runtime = 0.11 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 523.341 | 178.2979 | 185.2808 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000108929453 |
| C1 | 1 | 53689.2724 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 273098.6 | 273098.6 | 1246.87 | 0.00001 |
| Error | 7 | 1533.19 | 219.0271 | | |
| Total | 8 | 274631.8 | | | |

## Nortriptyline 1%, 0.3 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (588.569)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1324.28)
```

```
Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (591.184)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1136.37)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (2556.83)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1048.64)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (589.087)
Stopped due to: Relative function convergence.


   ----  Final Results  ----
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 218
Final sum of squared deviations = 1.8814887E+003
Final sum of deviations = 6.7507443E+000
Standard error of estimate = 16.3946
Average deviation = 12.4069
Maximum deviation for any observation = 25.6267
Proportion of variance explained (R^2) = 0.9933  (99.33%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9923  (99.23%)
Durbin-Watson test for autocorrelation = 1.265

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 21-Mar-2004 00:09.  Runtime = 0.06 seconds.

            ----  Descriptive Statistics for Variables  ----
      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0         523.754        182.6759        186.7263

  ----  Calculated Parameter Values  ----
     Parameter      Initial guess   Final estimate
-----------------  -------------  ----------------
                Da              1    0.000113679205
                C1              1        50403.8657


                  ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        277052.3        277052.3    1030.76  0.00001
Error          7        1881.489        268.7841
Total          8        278933.8
```

**Nortriptyline 0.5%, 0.4 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1512.35)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (3402.78)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (559.665)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1259.25)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (562.443)
Stopped due to: Relative function convergence.


   ----  Final Results  ----
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 262
Final sum of squared deviations = 1.0941712E+004
Final sum of deviations = 4.5631002E+001
Standard error of estimate = 39.5361
Average deviation = 32.6294
Maximum deviation for any observation = 52.3435
Proportion of variance explained (R^2) = 0.9792  (97.92%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9762  (97.62%)
Durbin-Watson test for autocorrelation = 1.242

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 21-Mar-2004 00:10.  Runtime = 0.11 seconds.


             ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
------------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0          758.62        247.8367        256.3604


  ----  Calculated Parameter Values  ----
```

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 9.50196378E-005 |
| C1 | 1 | 96656.8082 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 514823.7 | 514823.7 | 329.36 | 0.00001 |
| Error | 7 | 10941.71 | 1563.102 | | |
| Total | 8 | 525765.4 | | | |

**Nortriptyline 0.1%, 0.4 mA/cm^2**

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1099.17)
Stopped due to: Relative function convergence.


   ----  Final Results  ----

NLREG version 6.1
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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 298
Final sum of squared deviations = 8.8309564E+003
Final sum of deviations = 4.9968661E+001
Standard error of estimate = 35.5185
Average deviation = 29.6055
Maximum deviation for any observation = 47.0195
Proportion of variance explained (R^2) = 0.9845  (98.45%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9823  (98.23%)
Durbin-Watson test for autocorrelation = 1.335

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 21-Mar-2004 00:12.  Runtime = 0.06 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 775.71 | 278.4834 | 267.1757 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|
| Da | 1 | 0.000131827005 |
| C1 | 1 | 57259.4572 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 562231.9 | 562231.9 | 445.66 | 0.00001 |
| Error | 7 | 8830.956 | 1261.565 | | |
| Total | 8 | 571062.8 | | | |

## Nortriptyline 1%, 0.4 mA/cm^2

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;       // Diffusion coefficient in aqueous layer
   6: Parameter C1;   // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...
Stopped due to: Relative function convergence.


   ----  Final Results  ----

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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 272
Final sum of squared deviations = 4.8650395E+003
Final sum of deviations = 3.3538052E+001
Standard error of estimate = 26.363
Average deviation = 20.9638
Maximum deviation for any observation = 34.8249
Proportion of variance explained (R^2) = 0.9912  (99.12%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9900  (99.00%)
Durbin-Watson test for autocorrelation = 1.284

Warning: Covariance matrix could not be computed because
the finite-difference Hessian was indefinite.
Analysis completed 21-Mar-2004 00:13.  Runtime = 0.05 seconds.
```

---- Descriptive Statistics for Variables ----

| Variable | Minimum value | Maximum value | Mean value | Standard dev. |
|---|---|---|---|---|
| time | 0 | 8 | 4 | 2.738613 |
| Qa | 0 | 748.444 | 259.4433 | 263.2319 |

---- Calculated Parameter Values ----

| Parameter | Initial guess | Final estimate |
|---|---|---|

```
Da                1     0.000107058764
C1                1         79724.3864
```

```
                ----  Analysis of Variance  ----

  Source     DF   Sum of Squares    Mean Square    F value   Prob(F)
----------  ----  --------------  --------------  ---------  -------
Regression     1        549463.3        549463.3     790.59  0.00001
Error          7        4865.039        695.0056
Total          8        554328.3
```

## Nortriptyline Control

```
   1: Title "Aqueous layer equation";
   2: Variable time;          // Cooling time in hr
   3: Variable Qa;  // Quantity thru skin in micrograms
   4: Constant L=500*10^-4;           // Skin thickness in cm
   5: Parameter Da;        // Diffusion coefficient in aqueous layer
   6: Parameter C1;    // Bulk concentration
   7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L^2)+cos(pi*2)/4*exp(-Da*4*pi^2*time/L^2)+cos(pi*3)/9*exp(-
Da*9*pi^2*time/L^2)));
   8: Plot xlabel="Time (hr)",ylabel="Drug permeated (micrograms)";
   9: Data;

Beginning computation...

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (1315.22)

Error executing line 7: Function Qa= C1*L*(Da*time/L^2-1/6-2/pi^2*(cos(pi)*exp(-
Da*pi^2*time/L
Error: Argument to exp function is too large (2959.25)
Stopped due to: Relative function convergence.


   ----  Final Results  ----

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Aqueous layer equation
Number of observations = 9
Maximum allowed number of iterations = 500
Convergence tolerance factor = 1.000000E-010
Stopped due to: Relative function convergence.
Number of iterations performed = 184
Final sum of squared deviations = 1.0144198E+001
Final sum of deviations = 6.1097103E-001
Standard error of estimate = 1.20382
Average deviation = 0.909294
Maximum deviation for any observation = 1.74446
Proportion of variance explained (R^2) = 0.9890  (98.90%)
Adjusted coefficient of multiple determination (Ra^2) = 0.9875  (98.75%)
Durbin-Watson test for autocorrelation = 0.943
Analysis completed 21-Mar-2004 00:15.  Runtime = 0.06 seconds.
```

```
             ----  Descriptive Statistics for Variables  ----

      Variable       Minimum value   Maximum value    Mean value     Standard dev.
-----------------  --------------  --------------  --------------  --------------
              time               0               8               4        2.738613
                Qa               0         31.6256        14.10991        10.74584
```

```
                ----  Calculated Parameter Values  ----
```

| Parameter | Initial guess | Final estimate | Standard error | t | Prob(t) |
|---|---|---|---|---|---|
| Da | 1 | 0.000584820878 | 0.0001994853 | 2.93 | 0.02197 |
| C1 | 1 | 355.080926 | 137.9424 | 2.57 | 0.03679 |

---- Analysis of Variance ----

| Source | DF | Sum of Squares | Mean Square | F value | Prob(F) |
|---|---|---|---|---|---|
| Regression | 1 | 913.6409 | 913.6409 | 630.46 | 0.00001 |
| Error | 7 | 10.1442 | 1.449171 | | |
| Total | 8 | 923.7851 | | | |

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