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Mathematical techniques for the estimation of the diffusion coefficient and elimination constant of agents in subcutaneous tissue

Lawrence T. Hersh

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Mathematical Techniques for the Estimation of the Diffusion Coefficient and Elimination Constant of Agents in Subcutaneous Tissue

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
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Mathematical Techniques for the Estimation of the Diffusion Coefficient and Elimination Constant of Agents in Subcutaneous Tissue

Lawrence T. Hersh

ABSTRACT

The purpose of this work was to develop methods to estimate the diffusion coefficient and elimination constant for dexamethasone in subcutaneous tissue. Solutions to the diffusion equation were found for different conditions relevant to implantation and injection. These solutions were then used as models for measured autoradiography data where the unknown model parameters were the diffusion coefficient and the elimination constant. The diffusion coefficient and elimination constant were then estimated by curve fitting the measured data to these models. Having these estimates would be of practical importance since inflammation surrounding implantable glucose sensors may be controlled through local release of dexamethasone at the site of implantation. Derivation of the appropriate model, how the model was used to estimate D and k, and various specific profile examples were investigated in detail.

Osmotic pumps containing $[^3]$H- dexamethasone were implanted into the subcutaneous tissue of rats. Digital autoradiography was used to measure the distribution of the $[^3]$H-dexamethasone within the subcutaneous tissue at 6, 24, and 60 hours after implantation. Measured concentration profiles, near the catheter tip through which the agent was released, were compared to solutions of the diffusion equation in order to characterize drug diffusion coefficients and elimination constants. There was good
agreement between the experimental data and the mathematical model used for
estimation. The diffusion coefficient for dexamethasone in subcutaneous tissue was
found to be \( D = 4.11 \pm 1.77 \times 10^{-10} \text{ m}^2/\text{s} \), and the elimination rate constant was found to
be \( k = 3.65 \pm 2.24 \times 10^{-5} \text{ s}^{-1} \).

Additionally, \( [^3\text{H}] \)-dexamethasone was injected into the subcutaneous tissue of
rats. Digital autoradiography was again used to measure the distribution of the \( [^3\text{H}] \)-
dexamethasone within the subcutaneous tissue at 2.5 and 20 minutes after injection.
Measured concentration profiles were again compared to a mathematical model of drug
diffusion for injection. There was good agreement between the experimental data and the
mathematical model. The diffusion coefficient found using this simple injection method
was \( 4.01 \pm 2.01 \times 10^{-10} \text{ m}^2/\text{s} \). The simple method given here for the determination of the
diffusion coefficient is general enough to be applied to other substances and tissues as
well.
Introduction

Several recent reports suggest that controlled local release of dexamethasone may be useful for preventing inflammation around an implantable glucose sensor (1-3). This decrease in inflammation is expected to increase glucose sensor function and lifetime. Local drug delivery may be achieved using biodegradable polymer implants (4), hydrogels (5), and osmotic pumps (6). Local delivery of dexamethasone would permit high interstitial drug concentrations at the site of glucose sensor implantation without producing high systemic drug levels. For successful local treatment, dexamethasone must be released and penetrate through the tissue surrounding the implanted glucose sensor. Additionally, the concentration of dexamethasone in the subcutaneous tissue surrounding the implanted glucose sensor must be high enough to prevent inflammation caused by an implant. In a previous study using dexamethasone to suppress inflammation due to an implant, local distribution of the drug in subcutaneous tissue was not determined (1). Although dexamethasone is a commonly used anti-inflammatory agent, its local concentration, diffusion coefficient, and rate of elimination have not been reported following subcutaneous release. The ability of dexamethasone to penetrate subcutaneous tissue can be measured, quantified, and compared to mathematical models (4). Therefore, the controlled delivery of dexamethasone in normal rat subcutaneous tissue was used in order to help develop a fundamental understanding of how the drug is transported in the subcutaneous tissue. Because the efficacy of controlled interstitial delivery depends on the distance the drug can penetrate into the tissue surrounding the
implantable glucose sensor, $[^3H]\text{-dexamethasone}$ was delivered from osmotic pumps implanted into the subcutaneous tissue of rats. Digital autoradiographic imaging was used to quantify the spatial distribution of radioactivity in the subcutaneous tissue at 6, 24, and 60 hours after subcutaneous implantation. Both the extent of penetration of dexamethasone and the effectiveness of simple transport models for quantification of penetration were investigated.

Additionally, many transport experiments are based on the injection of a finite volume of substance into the tissue of interest which then diffuses away. Some examples of injection-based diffusion experiments are the determination of the diffusion coefficient of small molecules in the brain (7), the determination of the diffusion coefficient of growth factors in the brain (8), and the determination of the diffusion coefficient of drugs in tumors(9, 10). Knowledge of the diffusion of a substance of interest in the tissue of interest is important for treatment efficacy. Therefore, it is also important to develop a method in which the diffusion coefficient of an injected substance in tissue can be determined in a relatively simple manner. Such a technique is investigated in this work by finding the diffusion coefficient of $[^3H]\text{-dexamethasone}$ in rat subcutaneous slices after an injection.

The purpose of this project was to develop a technique to estimate the diffusion coefficient and the rate of elimination of dexamethasone in subcutaneous tissue. The technique includes finding the solutions to relevant forms of the diffusion equation, and then using these solutions as models with unknown parameters: the diffusion coefficient and the elimination constant. Curve fitting measured autoradiography data to the models was then undertaken to estimate the diffusion coefficient and the elimination constant. In
this way, this newly developed technique aids in providing an understanding of the
diffusion of dexamethasone in subcutaneous tissue.

All animal experiments were performed under the approval of the University of South
Florida Animal Care and Use Program.
Experimental Methods

Materials

$[^3H]$-Dexamethasone (392.46 MW), specifically $[1,2,4,6,7-^3H]$-dexamethasone, was obtained from Amersham Biosciences Corp. (Piscataway, NJ). The specific activity was 88.0 Ci/mmol. Alzet osmotic pumps (1003D model) were obtained from Durect Corp. (Cupertino, CA).

Subcutaneous Implantation

Six male Sprague Dawley rats (Harlan, Indianapolis, IN, 375-399 g) were used. The rats were initially anesthetized by placing each rat in an induction chamber filled with a 5% mixture of isoflurane in oxygen. During surgery anesthetization of the rats was maintained using a 2.5% mixture of isoflurane in oxygen. Two pumps containing radiolabeled dexamethasone were implanted subcutaneously on either side of the shoulders of the rat. A 3-4 cm incision was made between the shoulder blades. A hemostat was inserted into the incision on the lateral aspect. By opening and closing the jaws of the hemostat, a pocket in the subcutaneous tissue just large enough for the pump was created. A tunnel to insert the tubing was made using a blunt probe. Excess bleeding was removed with sterile cotton gauze. The osmotic pump was implanted tubing end first. The wound was closed with 4-6 surgical staples. Two rats were sacrificed at 6, 24, and 60 hours after implantation. The rats were euthanized using CO$_2$. The tissue around the tip of the catheter was removed, quickly frozen on dry ice, and
stored at -80 °C to immobilize the tracers within the tissue sample. The frozen tissue samples were mounted on a cryostat chuck and cut in 10 μm sections. Sections taken at every 200 μm were used for autoradiographic imaging.

Preparation of Osmotic Pumps

A solution of [3H]-dexamethasone and sterile 0.9% (w/v) saline was loaded into the osmotic pumps (total volume 114 μL) using the protocol provided by the manufacturer. Each pump contained a total activity of 127 μCi. The pumps provided a controlled delivery at a rate of 1.0 μL/hour. To prevent the pump from causing a tissue reaction at the site of drug delivery, a 4 cm length of polyethylene tubing was connected to the body of the pump.

Subcutaneous Injection

Three male Sprague Dawley rats (Harlan, Indianapolis, IN; 375-399 g) were used. Again, the rats were euthanized using CO2 prior to the experiment. It took an average of 86 seconds for the tissue sections to freeze and is included in the total time of 2.5 minutes or 20 minutes. All tissue samples were then stored at -80 °C to immobilize the tracers within the tissue sample. As before, the frozen tissue samples were mounted on a cryostat chuck and cut in 10 μm thick sections. Sections taken at every 200 μm were used for autoradiographic imaging.

The 20 Minute Experiment

Three 0.04 mL solutions of [3H]-dexamethasone in sterile 0.9% (w/v) saline were used. Injections using insulin syringes were made into subcutaneous tissue on the backs of the rats. The approximate duration of the injection was 1 second. Each solution contained a total activity of 0.65 μCi. The tissue around the injection site (1 cm x 1 cm x
0.5 cm) was removed and frozen on dry ice to immobilize the $[^3]H$-dexamethasone in the tissue. The average time from injection to when the tissue froze, as measured using a surface thermometer (Mannix Testing & Measurement, Lynbrook, NY), was approximately 20 minutes after injection.

The 2.5 Minute Experiment

Three subcutaneous sections were harvested (1 cm x 1 cm x 0.5 cm) from the backs of the rats. Each section was injected with a 0.04 mL solution of $[^3]H$-dexamethasone in sterile 0.9% (w/v) saline using an insulin syringe and then frozen on dry ice. The injection duration was approximately 1 second. The average time from injection to when the tissue froze was approximately 2.5 minutes after injection.

Autoradiographic Imaging and Analysis

Autoradiographic images of the tissue sections were obtained using a recently developed real-time digital radioactivity-detection system, the Micro-Imager (Biospace Mesures, Paris, France) (11, 12). With the Micro-Imager, acquisition of events can be visualized in real-time on a monitor screen. Each event is individually analyzed by the computer. An event is a radioactivity decay event (11). The acquisition of events needs only to proceed for as long as is necessary to obtain a good image. In the osmotic pump implantation case, autoradiographic images with between 380,715 to 686,390 events were acquired over 24-45 hours to obtain good images. An optical image of the same tissue sample using the Micro-Imager was also obtained. The spatial variation in drug concentration from the osmotic pumps was quantified in the following way. The areas of subcutaneous tissue were identified on the optical image and then superimposed onto the corresponding autoradiographic image. The concentration profiles in the subcutaneous
tissue surrounding the catheter tip were determined directly from the autoradiographic images using Beta Vision+ software (Biospace Mesures, Paris, France). A line profile tool (1 mm wide) was used from the center of the catheter tip to the periphery of the subcutaneous tissue to obtain a number of events versus distance profile. The background number of events was subtracted from the number of events acquired. A number of events versus distance profiles were performed at 15° increments around the catheter tip on each section selected for analysis. The number of events at the catheter tip opening was calibrated to the known concentration of the agent in the pump to obtain concentration versus distance profiles at 6, 24, and 60 hours after implantation. An example implantation autoradiographic image is shown in Figure 1. Similar processing was done for the injection experiments providing concentration versus distance profiles at 2.5 minutes and 20 minutes. For the injection experiments, autoradiographic images with between 1,593,815 and 1,918,869 events were acquired over 71 hours 32 minutes to 72 hours 43 minutes to obtain good images. Example injection autoradiographic images are shown in Figure 2.
Figure 1. Example Autoradiographic Image, Implantation. This figure shows an autoradiographic image from rat subcutaneous tissue obtained using the Micro-Imager after implantation of an osmotic pump containing $[^3]H$-dexamethasone for 6 hours. The arrow shows the location and direction of the catheter tip. Each dot represents a radioactivity decay event. Lighter shades indicate higher activity. The bar represents a distance of 1 mm.
Figure 2. Example Autoradiographic Image, Injection. Panels a and b show a typical autoradiographic image obtained using the Micro-Imager 2.5 minutes and 20 minutes after injection of $[^3]H$-dexamethasone. Lighter areas indicate higher activity. The bar represents a distance of 2 mm.
Mathematical Formulation

In the diffusion equation, the diffusion coefficient (D) and the elimination constant (k) are parameters. Using the concentration versus distance profile information, estimated values for these parameters can be found by using a non-linear curve fitting technique. Essentially, the measured concentration profiles can be fit to the appropriate solution to the diffusion equation to optimally estimate D and k. The Marquardt-Levenberg algorithm was chosen for the curve fitting due to its simplicity of programming, stability in searching, and rapid convergence to a good solution.

The development of the required relevant solutions to the diffusion equation for implantation and injection results in three major forms, which are detailed below. First, the steady state solution for implantation is needed. This solution essential requires that the time derivative be set to zero and that there is a constant concentration surface. Elimination is included for the steady-state case. Additionally, the steady-state solution will be useful for expected and theoretical examination of concentration profiles. Also, the steady-state solution is essential as an intermediate step in deriving the full transient solution for the implantation experimental model. The steady-state solution was found using a standard series technique. Secondly, the full solution to the diffusion equation with elimination is needed for modeling the implantation experiments. This solution is quite involved and requires Laplace transform techniques, which are also detailed below. Third, the full transient solution with no elimination but with a given initial concentration in a confined volume is needed for modeling the injection experiments. Armed with
these solutions, curve fitting can be undertaken to estimate the diffusion coefficients and elimination constants for the various experiments in this work.

Mathematical Formulation, Implantation

The concentration profiles of $[^3H]$-dexamethasone obtained using the Micro-Imager were compared to mathematical models of drug diffusion and elimination. The model assumed constant drug concentration at the catheter tip/tissue interface, first-order elimination of drug; homogeneous and isotropic diffusion transport of drug through the subcutaneous tissue, negligible fluid convection, and spherical symmetry. The governing equation for the diffusion and elimination of a drug in subcutaneous tissue is:

$$\frac{\partial C}{\partial t} = D \left( \frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C}{\partial r} \right) - k \cdot C. \quad (1)$$

$C$ is the concentration of the drug in the subcutaneous tissue; $D$ is the diffusion coefficient of the drug in subcutaneous tissue; $r$ is the radial distance from the center of the catheter tip; $k$ is the first-order elimination constant for the drug from the subcutaneous tissue; and $t$ is the time after implantation. The boundary and initial conditions are:

$$C = 0, t = 0, r \geq a,$$
$$C = C_0, t > 0, r = a,$$
$$C = 0, t > 0, r \to \infty,$$  

where $a$ is the radius of the catheter and $C_0$ is the concentration at the catheter tip. The solution (13) of equation 1 using the boundary and initial conditions of equations 2 is:

$$C(r,t) = \frac{aC_0}{2r} \left\{ \exp \left( -(r-a) \sqrt{\frac{k}{D}} \right) \cdot \text{erfc} \left( \frac{r-a}{2\sqrt{D \cdot t}} - \sqrt{k \cdot t} \right) \right\}$$

11
The methods for solution of the diffusion equation are provided below. Assuming steady-state and applying the boundary conditions from equations 2, equation 1 can be solved using a series technique as is shown below. Alternatively, the steady-state solution can also be found from equation 3 by letting time go to infinity:

\[
C(r) = C_0 \frac{a}{r} \exp\left(-a \sqrt{\frac{k}{D}} \left(\frac{r}{a} - 1\right)\right). \tag{4}
\]

However, this would require knowing the full solution to begin with. The series solution technique for the steady-state case and the methods to find the general solution of the diffusion equation are complex and are not easily found in the literature. They are provided here to show their correctness and detailed structure.

The Brownian diffusion coefficient for dexamethasone in water was estimated from the Stokes-Einstein equation:

\[
D = \frac{R \cdot T}{6\pi \cdot \mu \cdot r_s \cdot N_A}. \tag{5}
\]

\(r_s = 0.657 \text{ M}^{1/3} \times 10^{-10} \text{ m}\) and is the equivalent spherical solute radius; \(M\) is the molecular weight of dexamethasone (392.46 MW); \(R\) is the ideal gas constant 8.314 J K\(^{-1}\) mol\(^{-1}\); \(T\) is temperature; \(\mu\) is the dynamic viscosity; and \(N_A\) is Avogadro’s number.

The calculated diffusion constant of dexamethasone in water at 37 °C is \(D = 6.82 \times 10^{-10}\) m\(^2\)/s. The Stokes-Einstein equation under predicts the actual diffusion coefficient for small solutes of molecular weight less than several hundred, and over predicts it for large solutes of molecular weight greater than several thousand \((14)\). Measured concentration profiles for [\(^3\)H]-dexamethasone at \(t = 6\) hours, \(t = 24\) hours, and \(t = 60\) hours were
compared to the transient equation 3. Typical concentration profiles predicted by equation 3 for two values of $\varphi$ for various times are shown in Figure 2, a and b. The dimensionless parameter $\varphi$, where $\varphi = a \frac{k}{\sqrt{D}}$, is analogous to the Thiele modulus obtained in analysis of heterogeneous catalysis (4) and is a predictor of the extent of drug penetration from the catheter tip.

The radius of the catheter was approximated at 0.6 mm. Values for $D$ and $k$ were found in the following manner. First, initial estimates for $D$ and $k$ were found. For the initial estimate of $D$, the diffusion constant for dexamethasone in water was used, $D=6.82 \times 10^{-10}$ m$^2$/s. This $D$ was used in the steady-state solution of the diffusion equation (equation 4) to find an initial estimate for $k$, which was found by using the Marquardt-Levenberg technique (15) with two independent variables, $k$ and $C_0$, to minimize the residual of the sum-squared-error between the predicted and experimental concentrations. Note that the steady-state solutions are generally expected to be close to the profiles for the 6, 24, and 60 hour data. (Figure 3.) Second, these initial estimates for the $k$ and $D$ values were then used as the starting points for the Marquardt-Levenberg algorithm using the transient equation (equation 3) with the $k$, $D$, and $C_0$ being the three independent variables over which the residual of the sum squared error between the predicted and experimental concentrations was to be minimized. The initial value for $C_0$ was always the maximum concentration in the measured data set. The Marquardt-Levenberg algorithm efficiently searched over the $k$, $D$, and $C_0$ space to find the point which best fits the data (16). This technique was repeated to find $k$ and $D$ for 6, 24, and 60 hours. For each of these times, the calculations were repeated for the autoradiographic scans at various angles. The Marquardt-Levenberg algorithm was written in MATLAB.
Figure 3. Theoretical Concentration versus Distance Profiles for Implantation. Concentration versus distance profiles can be obtained by solving the transient diffusion and elimination equation (Equation 3) for various times until steady-state is reached. Panels a and b demonstrate the dependence of the penetration depth with the modulus $a \cdot \sqrt{k/D}$. Panel a (modulus 0.2) has a larger penetration depth than panel b (modulus 1).
Mathematical Formulation, Injection

In order to investigate the diffusion coefficient for the injection experiments, the model required four specific assumptions. The model assumed (1) that the diffusing substance is deposited within a sphere of radius \( a \) at \( t=0 \), (2) isotropic and homogeneous diffusion transport of drug through the subcutaneous tissue, (3) negligible fluid convection, and (4) negligible elimination. Assuming that the elimination is negligible is justified as tissue samples were obtained from a sacrificed rat. Note that the absence of blood flow eliminates most clearance mechanisms normally present in vivo (8). Hence, the governing equation for diffusion of a drug in the subcutaneous tissue is

\[
\frac{\partial C}{\partial t} = D \left( \frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right). \tag{6}
\]

The following initial conditions also come from the assumptions:

\begin{align*}
C &= 0, t = 0, r \geq a, \\
C &= C_0, t = 0, r < a, \\
C &= 0, t > 0, r \to \infty.
\end{align*} \tag{7}

The analytic solution for equation 1 using the above initial and boundary conditions is (13,17):

\[
C(r,t) = \frac{C_0}{2} \left\{ \text{erf} \left( \frac{r + a}{2\sqrt{D \cdot t}} \right) - \text{erf} \left( \frac{r - a}{2\sqrt{D \cdot t}} \right) - \\
\frac{2\sqrt{D \cdot t}}{r \cdot \pi^{1/2}} \left[ \exp\left( - (r - a)^2 / (4 \cdot D \cdot t) \right) - \exp\left( - (r + a)^2 / (4 \cdot D \cdot t) \right) \right] \right\}, \tag{8}
\]

where \( a \) is the radius of the sphere. If \( r \) is very much greater than \( a \), then expression 8 becomes (13):
\[
C = \frac{m}{8 \cdot (\pi \cdot D \cdot t)^{3/2}} \cdot \exp\left(-\frac{r^2}{4 \cdot D \cdot t}\right) \cdot \left\{1 + \left(\frac{r^2}{D \cdot t} - 6\right) \cdot \frac{a^2}{40 \cdot D \cdot t}\right\}, \quad (9)
\]

where \( m = VCo = 4/3 \pi a^3 Co \) and \( V \) is the injected volume. If the radius of the sphere tends to zero, \( a \to 0 \), with \( m \) remaining constant \( (13) \),

\[
C = \frac{m}{8 \cdot (\pi \cdot D \cdot t)^{3/2}} \cdot \exp\left(-\frac{r^2}{4 \cdot D \cdot t}\right), \quad (10)
\]
or

\[
\frac{C(r, t)}{C_0} = \frac{a^3}{6 \cdot \pi^{1/2} \cdot (D \cdot t)^{3/2}} \cdot \exp\left(-\frac{r^2}{4 \cdot D \cdot t}\right), \quad (11)
\]

Equation 11 has the same solution as that for the instantaneous point source in 3D \( (18) \). However, Nicholson \( (19) \) suggests that, at measurement locations sufficiently far from the source, equation 10 (or equation 11) will provide a useful approximation. Moreover, Thorne et al. \( (8) \) suggest that when the injection time is very brief compared to the time of the subsequent diffusion measurements, the concentration can be described by equation 11 (or equation 10). Typical concentration profiles for \([^3H]\)-dexamethasone predicted by equation 11 at \( t=2.5 \) minutes, \( t=5 \) minutes, \( t=10 \) minutes, and \( t=20 \) minutes are shown in Figure 4. The radius of the injected spherical volume was 2.1 mm. For equation 11 to be a useful approximation, data away from the source were used \( (19) \). For the 20 minute experiment, a portion of the concentration profile from the tail end was used in the mathematical model. This portion ranged from the tail-end to a position 3 mm toward the source from the first-zero event value. The tail-end was defined as the furthest position on the curve from the source. The first-zero event value was defined as the position where the events first decrease to a zero value. For the 2.5 minute
experiment, a first-zero event value could not be used as a reference point. Instead, a location on the profile where the profile “bends” from a steep curve to a plateau region was used as a reference. This bend was defined to occur at a position where the number of events was 100. The number of events at the bend is dependent on the detection time in the Micro-Imager. If the detection time is increased, more events are detected. However, the detection time was the same for all profiles in the 2.5 min experiment.

Therefore, the portion of the concentration profile used in the mathematical model was from the tail-end to a distance 0.7 mm toward the source after the bend. The reason that the first-zero event value could not be used as a reference point is discussed further below. The value for \( D \) was found in an iterative manner. First, an initial estimate for \( D \) was needed. The diffusion constant for dexamethasone in water was used, \( D=6.82\times10^{-10} \) m\(^2\)/s. This initial estimate for \( D \) was then used as the starting points for the Marquardt-Levenberg algorithm (15) with the \( D \) and \( C_0 \) being the two independent variables over which the residual of the sum-squared-error between the predicted and experimental concentrations was to be minimized. The initial value for \( C_0 \) was always the maximum concentration in the measured data set. The initial values for \( D \) and \( C_0 \) should be physiologically reasonable to avoid final estimates that are based on meaningless local minima in the sum-squared-error function. While sensitivity to the initial guess was not specifically investigated, careful thought was given as to where to start the searching in the \( D \) and \( C_0 \) space. Quick convergence, along with physiologically reasonable results using the Marquardt algorithm, led us to believe that our strategy was generally sound.

The Marquardt-Levenberg algorithm efficiently searched over the \( D \) and \( C_0 \) space to find the point which best fits the data (16). This technique was repeated to find \( D \) at 2.5 and
20 minutes for each scan. The Marquardt-Levenberg algorithm was again written in MATLAB.
Figure 4. Theoretical Concentration versus Distance Profiles for Injection. Concentration profiles for diffusion when a concentrated bolus of solute is deposited within a small region are shown. The curves shown result from equation 5 with $D = 4.11 \times 10^{-10} \text{ m}^2/\text{s}$, $a=2.1 \text{ mm}$, and $t=2.5 \text{ minutes}$, 5 minutes, 10 minutes, and 20 minutes.
Figure 5. Example Measured Injection Profiles. Typical number of events versus distance profiles obtained using the Micro-Imager at (a) 2.5 and (b) 20 minutes after injection are shown. Data from only one scan are shown. The ordinate represents the location of the center of injection. For (a) all data shown was used for diffusion coefficient estimation.
Steady-State Solution With Elimination, Implantation

The diffusion partial differential equation with spherical symmetry that is needed as the starting expression for this work is:

\[ 0 = D \left( \frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right) - k C. \quad (12) \]

Now divide through by \( D \) and set \( k / D \) to \( k_1 \).

\[ 0 = \left( \frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right) - k_1 C. \quad (13) \]

Next, for clarity we shift to the following notational form which is the same equation but closer to classical notation used for this category of problem. Let \( C \) be replaced by \( y \) and the independent variable \( r \) by \( x \). We then get:

\[ x^2 \cdot y'' + 2 \cdot x \cdot y' - x^2 \cdot k_1 \cdot y = 0. \quad (14) \]

This differential equation can be solved using a power series. By taking successive derivatives of the general series form assumed as the solution we get:

\[ y = \sum_{n=0}^{\infty} a_n x^{r+n} = a_0 x^r + \sum_{n=1}^{\infty} a_n x^{r+n}, \quad (15) \]

\[ y' = \sum_{n=0}^{\infty} (r + n) a_n x^{r+n-1} = r a_0 x^{r-1} + \sum_{n=1}^{\infty} (r + n) a_n x^{r+n-1}, \quad (16) \]

\[ y'' = \sum_{n=0}^{\infty} (r + n)(r + n - 1) a_n x^{r+n-2} = r \cdot (r - 1) \cdot a_0 \cdot x^{r-2} + \sum_{n=1}^{\infty} (r + n)(r + n - 1) a_n x^{r+n-2}. \quad (17) \]
Now substitute these derivatives into the differential equation.

\[ r \cdot (r - 1) a_0 x^r + \sum_{n=1}^{\infty} (r + n)(r + n - 1) a_n x^{r+n} \]

\[ + 2 \cdot r \cdot a_0 \cdot x^r + 2 \sum_{n=1}^{\infty} (r + n) a_n x^{r+n} \]

\[ - a_0 x^{r+2} k_1 - k_1 \sum_{n=1}^{\infty} a_n x^{r+n+2} = 0. \]  
(18)

Combining terms and simplifying, a sequence of straightforward steps can be taken:

\[
\left[ r (r - 1) + 2r \right] \cdot a_0 x^r \\
+ \sum_{n=1}^{\infty} \left[ (r + n)(r + n - 1) + 2(r + n) \right] \cdot a_n x^{r+n} \\
- k_1 \sum_{n=0}^{\infty} a_n x^{r+n+2} = 0. \]  
(19)

\[ r (r + 1) a_0 x^r \\
+ \sum_{n=1}^{\infty} (r + n)(r + n + 1) a_n x^{r+n} \\
- k_1 \sum_{n=0}^{\infty} a_n x^{r+n+2} = 0. \]  
(20)

\[ r (r + 1) a_0 x^r + \sum_{n=1}^{\infty} (r + n)(r + n + 1) a_n x^{r+n} \\
- k_1 \sum_{n=2}^{\infty} a_{n-2} x^{r+n} = 0. \]  
(21)

\[ r (r + 1) a_0 x^r + (r + 1)(r + 2) a_1 x^{r+1} \]

\[ + \sum_{n=2}^{\infty} [(r+n)(r+n+1)a_n - k_1 a_{n-2}] \cdot x^{r+n} = 0. \]  
(22)

22
At this point there are many different paths that can be taken to provide the two independent solutions needed for our second order situation. For our case the best and most general choice will be $r = -1$ and $a_0, a_1 \neq 0$.

We now need to develop a recurrence relation for $n \geq 2$. Therefore, if both $a_0$ and $a_1$ are taken as nonzero values, for $n \geq 2$ the recurrence relationship that must always be true is:

$$a_n = \frac{k_1 a_{n-2}}{(n)(n-1)}.$$  \hspace{1cm} (23)

Specifically, for example, this would mean:

$$a_2 = \frac{k_1 a_0}{(2)(1)}, \hspace{1cm} (24)$$

$$a_3 = \frac{k_1 a_1}{(3)(2)}, \hspace{1cm} (25)$$

$$a_4 = \frac{k_1^2 a_0}{4!}, \hspace{1cm} (26)$$

$$a_5 = \frac{k_1^2 a_1}{5!}, \hspace{1cm} (27)$$

$$a_6 = \frac{k_1^3 a_0}{6!}, \hspace{1cm} (28)$$

$$a_7 = \frac{k_1^3 a_1}{7!}, \hspace{1cm} (29)$$

$$a_8 = \frac{k_1^4 a_0}{8!}, \hspace{1cm} (30)$$

and $a_9 = \frac{k_1^4 a_1}{9!}$.  \hspace{1cm} (31)
For the even series, in general for \( n = 1, 2, 3, \ldots \) we get \( a_{2n} = \frac{k_1^n a_0}{(2n)!} \).  \( \text{(32)} \)

For the odd series, in general for \( n = 1, 2, 3, \ldots \) we get \( a_{2n+1} = \frac{k_1^n a_1}{(2n+1)!} \).  \( \text{(33)} \)

Going back to the original series form with \( r = -1 \) we get:

\[
y = \sum_{n=0}^{\infty} a_n x^{r+n} = \frac{a_0}{x} + a_1 + a_2 x^1 + a_3 x^2 + a_4 x^3 + a_5 x^4 + a_6 x^5 + \ldots \quad \text{or} \quad \text{(34)}
\]

\[
y = \frac{a_0}{x} + a_1 + \frac{k_1 a_0 x}{2!} + \frac{k_1 a_1 x^2}{3!} + \frac{k_1^2 a_0 x^3}{4!} + \frac{k_1^2 a_1 x^4}{5!} + \frac{k_1^3 a_0 x^5}{6!} + \ldots \quad \text{(35)}
\]

If we now let \( a_0 = 1 \) and \( a_1 = \sqrt{k_1} \) this general solution becomes:

\[
y = \frac{1}{x} + \sqrt{k_1} + \frac{k_1 x}{2!} + \frac{k_1 \sqrt{k_1} x^2}{3!} + \frac{k_1^2 x^3}{4!} + \frac{k_1^2 \sqrt{k_1} x^4}{5!} + \frac{k_1^3 x^5}{6!} + \ldots \quad \text{(36)}
\]

This can now obviously be written as:

\[
y = \frac{\exp(\sqrt{k_1} x)}{x}. \quad \text{(37)}
\]

Similarly, if we chose \( a_0 = 1 \) and \( a_1 = -\sqrt{k_1} \) the solution becomes:

\[
y = \frac{\exp(-\sqrt{k_1} x)}{x}. \quad \text{(38)}
\]

Returning to our original notation, these 2 independent solutions can be combined to form the full solution:

\[
C(r) = A \frac{\exp\left(\frac{k}{\sqrt{D}}\right)}{r} + B \frac{\exp\left(-\frac{k}{\sqrt{D}}\right)}{r}. \quad \text{(39)}
\]
Next, the boundary conditions $C(a) = C_0$ and $C(\infty) = 0$ must be met. Therefore, the steady-state solution is:

$$C(r) = C_0 \frac{a}{r} \exp \left( -a \sqrt{\frac{k}{D}} \left( \frac{r}{a} - 1 \right) \right) = C_0 \frac{a}{r} \exp \left( - \phi \left( \frac{r}{a} - 1 \right) \right). \quad (40)$$

This is the first important equation that we will need to model the diffusion behavior in some of our experimental situations. This equation will be particularly useful in finding a first estimate of $k$, as described earlier. This equation will also be useful in deriving the more sophisticated transient cases as will now be shown.

**General Solution, Implantation**

We will now take recourse to the Laplace method to derive a solution for the full diffusion differential equation. The ultimate solution will involve the elimination term, but first we must go back to the solution for the diffusion equation without elimination. Laplace transform methods will be used to derive the full solution. However, if the solution for the diffusion equation without elimination is understood, it will make finding the full solution easier. Taking the Laplace transform of Equation 1 with a zero initial condition can quickly identify the Laplace transform for the diffusion equation without elimination. It must simply be the steady-state solution with $k$ replaced by $s$:

$$\mathcal{C}(s, r) = \frac{C_0 a}{r} e^{-s \sqrt{\frac{r-a}{D}}} \quad (41).$$

When returning to the time domain this formula will handle the $C(0, r) = 0$ initial condition. Next consider some of the simple properties of the Laplace Transform:
\[ F(s - a) \leftrightarrow \exp(at) \cdot f(t), \]

where

\[ F(s) \leftrightarrow f(t). \]

However, the differential equation we really want to solve is:

\[ \frac{\partial C}{\partial t} = D \cdot \left( \frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C}{\partial r} \right) - k \cdot C. \tag{42} \]

By going through a similar argument to that just given for the no elimination case we can make progress toward the desired full solution. Taking the Laplace transform of both these equations with the zero initial conditions (which is our case) we get:

\[ s\bar{C} = D \cdot \left( \frac{\partial^2 \bar{C}}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial \bar{C}}{\partial r} \right) - k \cdot \bar{C}. \tag{43} \]

The bar over the concentration term is the notation for the Laplace transform.

Now rearranging we can say:

\[ 0 = D \cdot \left( \frac{\partial^2 \bar{C}}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial \bar{C}}{\partial r} \right) - (k + s) \cdot \bar{C}. \tag{44} \]

This means the transform we want to work with is:

\[ \bar{C}(s, r) = \frac{C_0 a}{r} e^{-\sqrt{\frac{s + k}{D}}(r - a)}. \tag{45} \]

Next, the boundary condition \( C(a, t) = C_0 \) for \( t>0 \) must be taken into account. This can be done by multiplying by a \( (1/s) \) factor. The need for doing this is clear since there can only be a solution for time greater than zero. This requirement comes from the basic properties of the Laplace transform as can be verified by examining any Laplace transform table. Therefore, the transform we want to invert is:
\[ \overline{C}(s,r) = \frac{C_0a}{r \cdot s} e^{-\sqrt{s + k}/D (r-a)} \]  

(46)

Note that we can expect to get the final and full solution to the diffusion equation because all the initial and boundary conditions will be met. However, an algebraic manipulation is needed before we will find the proper expressions in Laplace transform tables. This algebraic manipulation is:

\[
\overline{C}(s,r) = \frac{C_0a}{r \cdot s} e^{-\sqrt{s + k}/D (r-a)} = \frac{C_0a}{r} \cdot \frac{1}{s} \cdot \frac{2\sqrt{s + k}}{2\sqrt{s + k}} \cdot e^{-\sqrt{s + k}/D (r-a)} \\
= \frac{C_0a}{2r} \cdot \frac{2\sqrt{s + k}}{s} \cdot \frac{1}{\sqrt{s + k}} \cdot e^{-\sqrt{s + k}/D (r-a)} \\
= \frac{C_0a}{2r} \left( \frac{1}{\sqrt{s + k} - \sqrt{k}} + \frac{1}{\sqrt{s + k} + \sqrt{k}} \right) \cdot \frac{1}{\sqrt{s + k}} \cdot e^{-\sqrt{s + k}/D (r-a)} \\
= \frac{C_0a}{2r} \left( \frac{1}{\sqrt{s + k} - \sqrt{k}} \cdot \frac{1}{\sqrt{s + k}} \cdot e^{\sqrt{s + k}/D (r-a)} + \frac{1}{\sqrt{s + k} + \sqrt{k}} \cdot \frac{1}{\sqrt{s + k}} \cdot e^{-\sqrt{s + k}/D (r-a)} \right). 
\]

(47)

(48)

(49)

The two terms in the previous expression are commonly available in Laplace transform tables (20). This will allow us to return to the time domain. The needed transform pairs are:

\[
\frac{1}{\sqrt{s + c_2} + \sqrt{c_2}} \cdot \frac{1}{\sqrt{s + c_2}} \cdot e^{-c_1 \sqrt{s + c_2}} \Leftrightarrow \exp \left( \frac{c_1}{\sqrt{c_2}} \right) \cdot \text{erfc} \left( \sqrt{c_2} \cdot t + \frac{c_1}{2\sqrt{t}} \right), \]

\[
\frac{1}{\sqrt{s + c_2} - \sqrt{c_2}} \cdot \frac{1}{\sqrt{s + c_2}} \cdot e^{-c_1 \sqrt{s + c_2}} \Leftrightarrow \exp \left( -\frac{c_1}{\sqrt{c_2}} \right) \cdot \text{erfc} \left( -\sqrt{c_2} \cdot t + \frac{c_1}{2\sqrt{t}} \right). 
\]
Remember that because of the exponential terms, we are making a shift in the complex plane. That is, when coming back to the time domain there must be a factor of $\exp(-c_2 \cdot t)$ if the $s$ is shifted by $+c_2$. This is why the $\exp(c_2 \cdot t)$ term drops out of the transform pair found in the tables.

$$\frac{1}{\sqrt{s} + \sqrt{c_2}} \cdot \frac{1}{\sqrt{s}} e^{-c_1 \sqrt{s}} \Leftrightarrow \exp\left(c_1 \sqrt{c_2}\right) \cdot \exp\left(c_2 t\right) \cdot \text{erfc}\left(\sqrt{c_2} \cdot t + \frac{c_1}{2 \sqrt{t}}\right). \quad (52)$$

The $\exp(c_2 t)$ term drops out due to the $s + c_2$ terms.

To get back to the time domain just let $c_1 = \frac{r-a}{\sqrt{D}}$ and $\sqrt{c_2} = \pm \sqrt{k}$.

Note that we have assumed $k>0$ in this development.

So finally we get:

$$C(r,t) = \frac{aC_0}{2r} \left\{ \exp\left(-(r-a) \sqrt{\frac{k}{D}}\right) \cdot \text{erfc}\left(\frac{r-a}{2\sqrt{D} \cdot t} - \sqrt{k} \cdot t\right) + \exp\left((r-a) \sqrt{\frac{k}{D}}\right) \cdot \text{erfc}\left(\frac{r-a}{2\sqrt{D} \cdot t} + \sqrt{k} \cdot t\right) \right\}. \quad (53)$$

**General Solution, Injection**

Using the assumptions described earlier for the injection studies, the governing equation for diffusion of a drug in the subcutaneous tissue is:

$$\frac{\partial C}{\partial t} = D \cdot \left( \frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C}{\partial r} \right). \quad (54)$$

The following initial conditions also come from the assumptions:

$$C = 0, t = 0, r \geq a,$$
$$C = C_0, t = 0, r < a,$$
$$C = 0, t > 0, r \to \infty. \quad (55)$$
A transformation of the diffusion equation can be used to simplify the problem by making a simple variable change.

\[ C(t, r) = u(t, r) / r. \]

\[
\frac{\partial C}{\partial r} = -\frac{u}{r^2} + u' \tag{56}
\]

\[
\frac{\partial^2 C}{\partial r^2} = \frac{2u}{r^3} - \frac{2u'}{r^2} + u'' \tag{57}
\]

By substitution, this reduces the problem to the following simpler case:

\[
\frac{\partial u}{\partial t} = D \left( \frac{\partial^2 u}{\partial r^2} \right). \tag{57}
\]

The initial conditions change to:

\[
u = 0, t = 0, |r| \geq a,
\]

\[
u = C_0 r, t = 0, |r| < a, \tag{58}
\]

\[
u = 0, t > 0, |r| \to \infty.
\]

The point source solution to equation 54 is easily found and verified by substitution. This solution is:

\[
u_p(t, r) = \frac{1}{2(\pi D t)^{1/2}} e^{-r^2/(4Dt)}. \tag{59}
\]

If equation 59 is a solution, then the following equation must also be true for some general function \( f(r') \), which can also be quickly verified by substitution:

\[
u(t, r) = \frac{1}{2(\pi D t)^{1/2}} \int_{-\infty}^{\infty} f(r') e^{-(r-r')^2/(4Dt)} dr'. \tag{60}
\]

In our particular case the function we want to chose is \( f(r') = C_0 \cdot \delta(r') \). \( C_0 \) will be ignored for now. Now, let \( C(t, r) = C_0 v(t, r) = C_0 u(t, r)/r \). Using the initial conditions (58), we get:
Next, it is just a matter of simplifying the mathematic expressions and getting them into a more tractable form. First, just split the integral:

\[
\int_{-a}^{a} r'^{2} e^{-(r' r' + r'' r'') / (4 D t)} dr' = \int_{0}^{a} r'^{2} e^{-(r' r' + r'' r'') / (4 D t)} dr' + \int_{0}^{0} r'^{2} e^{-(r' r' + r'' r'') / (4 D t)} dr'.
\]  

(62)

Now just substitute \( -r'' \) for \( r' \) in the second integral, changing the integral and its limits appropriately one gets:

\[
= \int_{0}^{a} r'^{2} e^{-(r' + r'') r' / (4 D t)} dr' + \int_{a}^{a} r''^{2} e^{-(r' + r'') r'' / (4 D t)} dr''.
\]

(63)

Reversing the limits of the second integral, we get:

\[
= \int_{0}^{a} r'^{2} e^{-(r' + r'') r' / (4 D t)} dr' - \int_{0}^{a} r''^{2} e^{-(r' + r'') r'' / (4 D t)} dr''.
\]

(64)

Then reducing these integrals into one, we get:

\[
= \int_{0}^{a} r'^{2} \left( e^{-(r' r' + r'' r'') / (4 D t)} - e^{-(r' + r'') r'/ (4 D t)} \right) dr'.
\]

(65)

Returning to the full expression for the solution, but still disregarding \( C_0 \), we get:

\[
v(t, r) = \frac{1}{2r(\pi \cdot D \cdot t)^{1/2}} \int_{0}^{a} r'^{2} \left( e^{-(r' r' + r'' r'') / (4 D t)} - e^{-(r' + r'') r'/ (4 D t)} \right) dr'.
\]

(66)

Doing the indicated integration is straightforward. By simple change of variables the first part of the integral can be easily evaluated. Letting \( r' = r - r'' \), where \( r \) is taken as a
constant, changing integration limits appropriately, and splitting the integral into two parts, gives the following sequence of equalities:

\[
\int_0^a r \cdot \left( e^{-(r-r')^2/(4Dt)} \right) \, dr' = \int_{r}^{r-a} (r' - r) \cdot \left( e^{-(r'^2)/(4Dt)} \right) \cdot (-dr'). \quad (67)
\]

\[
= \int_{r-a}^{r} (r - r'n) \cdot \left( e^{-(r'^2)/(4Dt)} \right) \cdot dr'. \quad (68)
\]

\[
= \int_{r-a}^{r} r \cdot \left( e^{-(r'^2)/(4Dt)} \right) \cdot dr' - \int_{r-a}^{r} r'n \cdot \left( e^{-(r'^2)/(4Dt)} \right) \cdot dr'. \quad (69)
\]

Next, the precise definitions of the error function needs to be understood and adjusted for these integrals. The error function is:

\[
erf(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-z^2} \, d\xi. \quad (70)
\]

Now making the variable change:

\[
\xi = \frac{r'}{\sqrt{4 \cdot D \cdot t}}, \quad (71)
\]

we get:

\[
erf(x) = \frac{2}{\sqrt{\pi}} \int_0^{x\sqrt{4Dt}} e^{-\left(\frac{r'^2}{4Dt}\right)} \frac{dr'}{\sqrt{4Dt}}. \quad (72)
\]

With further simplification the following equality results.

\[
erf\left(\frac{r}{\sqrt{4Dt}}\right) = \frac{1}{\sqrt{\pi \cdot D \cdot t}} \int_0^r e^{-\left(\frac{r'^2}{4Dt}\right)} \, dr'. \quad (73)
\]

Using equation 69, further progress can now be made with equation 66.
\[
\int_{r}^{r-a} r \cdot \left( e^{-r^2/(4Dt)} \right) \, dr - \int_{r}^{r-a} r'' \cdot \left( e^{-r^2/(4Dt)} \right) \, dr''
\]
\[
= r \int_{r}^{r-a} e^{-r^2/(4Dt)} \, dr'' - \int_{r}^{r-a} r'' \cdot \left( e^{-r^2/(4Dt)} \right) \, dr'' .
\] (74)

Now, just doing the integration and using the specified limits the resulting expression is:
\[
= r \cdot \sqrt{\pi} \cdot D \cdot t \cdot \text{erf} \left( \frac{r}{\sqrt{4Dt}} \right) - r \cdot \sqrt{\pi} \cdot D \cdot t \cdot \text{erf} \left( \frac{r-a}{\sqrt{4Dt}} \right)
\]
\[
+ \frac{4 \cdot D \cdot t}{2} \left( e^{-\frac{r^2}{4Dt}} - e^{-\frac{(r-a)^2}{4Dt}} \right) .
\] (75)

By symmetry a further integral result can be immediately written:
\[
\int_{0}^{a} r' \left( e^{-r'^2/(4Dt)} \right) \, dr'
\]
\[
= r \cdot \sqrt{\pi} \cdot D \cdot t \cdot \text{erf} \left( \frac{r}{\sqrt{4Dt}} \right) - r \cdot \sqrt{\pi} \cdot D \cdot t \cdot \text{erf} \left( \frac{r+a}{\sqrt{4Dt}} \right)
\]
\[
+ \frac{4 \cdot D \cdot t}{2} \left( e^{-\frac{r^2}{4Dt}} - e^{-\frac{(r+a)^2}{4Dt}} \right) .
\] (76)

Equations 74 and 76, when put into equation 66, provide the solution needed for the injection studies. This final result is:
\[
\nu(r,t) = \frac{C_0}{2} \left\{ \text{erf} \left( \frac{r+a}{2\sqrt{D} \cdot t} \right) - \text{erf} \left( \frac{r-a}{2\sqrt{D} \cdot t} \right) - \frac{2\sqrt{D} \cdot t}{r \cdot \pi^{1/2}} \left[ \exp \left( -(r-a)^2 / (4 \cdot D \cdot t) \right) - \exp \left( -(r+a)^2 / (4 \cdot D \cdot t) \right) \right] \right\} .
\] (77)
where $C_0$ is the concentration of the drug in subcutaneous tissue, $D$ is the diffusion coefficient of the drug in subcutaneous tissue, $r$ is the radial distance from the center of the injection, and $t$ is time. The initial concentration is $C_0$ in the sphere $0 \leq r < a$ and zero for $r > a$. Additionally, a boundary condition is $C(+\infty,t) = 0$. The analytic solution for equation 54 using the above initial and boundary conditions is (13,17):

$$C(r,t) = \frac{C_0}{2} \left[ \text{erf} \left( \frac{r + a}{2\sqrt{D \cdot t}} \right) - \text{erf} \left( \frac{r - a}{2\sqrt{D \cdot t}} \right) - \frac{2\sqrt{D \cdot t}}{r \cdot \pi^{1/2}} \exp \left( - \frac{(r - a)^2}{4 \cdot D \cdot t} \right) - \exp \left( - \frac{(r + a)^2}{4 \cdot D \cdot t} \right) \right],$$

where $a$ is the radius of the sphere. This completes the needed derivations and mathematical background for this research.

Marquardt Curve Fitting

Equipped with the solutions for the steady-state and general solutions to the diffusion equation, curve fitting techniques can be employed to do the estimation of the $D$ and $k$ values. The Marquardt technique offers a way to do the needed curve fitting (13, 27). The Marquardt method is based on two principles. It combines a gradient search with a Gauss-Newton technique. This method balances these two principles to provide a stable yet efficient search in the solution space for a multivariable nonlinear modeling formula. In this particular case, the desire is to find estimates of the diffusion coefficient, the elimination constant, and the maximum count value. In essence, the desire is to find the best values for these parameters to fit the measured data. The Marquardt method minimizes a sum-squared-error expression to get the best estimate for the desired parameters. A brief description of the formulation of the Marquardt Method is given in
Appendix I. An example of the actual Marquardt program in MATLAB is provided in Appendix II. To start the Marquardt algorithm, a beginning point in the solution space must be specified. The choice of the starting point for the least squares optimization process is crucial for stability and quick convergence. How the initial point is found for the various cases related to implantation and injection have already been described.
Results

Radiolabeled dexamethasone spread through the subcutaneous tissue after implantation of the osmotic pump (Figure 1). The local concentration of drug within the tissue was quantified from the autoradiographic images using the Beta Vision+ software. The Beta Vision+ software was used to construct the number of events as a function of distance profiles. An event is a radioactivity decay event (12). The number of events was greatest at the tip of the catheter. A high number of events on the autoradiographic image represent a high drug concentration. The number of events at the tip of the catheter can be calibrated to the known concentration in the pump. Hence, the local concentration of the drug in the subcutaneous tissue surrounding the catheter can be estimated by comparing the local number of events to the number of events at the catheter tip. In general, at distances more than a few millimeters from the catheter tip, the radioactivity was not significantly different from background. Figure 1 is representative of the autoradiographic images obtained using the Micro-Imager after implantation of the osmotic pumps for 6, 24, or 60 hours. Concentration profiles obtained from the autoradiographic images of the subcutaneous tissue surrounding the catheter tip were examined and compared to the mathematical model of diffusion and first-order elimination to find the best estimates for $D$ and $k$. Figures 6 to 11 are representative of the measured profiles with their final curve fit. The best estimates obtained for $D$ and $k$ are given in Table 1. A single-factor analysis of variance (ANOVA) indicated (26) that there was no significant difference between the 6 hour and 24 hour data for $k$ ($p > 0.05$)
or for $D$ ($p > 0.05$). There was not enough data at 60 hours for comparison. The average, based on the 6, 24, and 60 hour data, for the diffusion coefficient is $D = 4.11 \pm 1.77 \times 10^{-10}$ m$^2$/s and for the elimination constant is $k = 3.65 \pm 2.24 \times 10^{-5}$ s$^{-1}$. To quantify differences in drug penetration with time after release from the osmotic pump, the best fit concentration profiles were used to find the distance where the local concentration drops to 10% of its maximum value. For the 6 hour case, the majority of the drug was confined to a region within $2.22 \pm 0.42$ mm from the tip of the catheter. For the 24 and 60 hour cases, the majority of the drug was confined to a region within $2.70 \pm 0.38$ mm and $1.80$ mm from the tip of the catheter, respectively. (Table 2.) The penetration distance of $[^3]$H]-dexamethasone increased from 6 to 24 hours but decreased from 24 to 60 hours.

Radiolabeled dexamethasone spread through the subcutaneous tissue after injection. Figure 2 is representative of the autoradiographic images obtained using the Micro-Imager after injection of a radiolabeled drug. The local concentration of drug within the tissue was again quantified from the autoradiographic images using the Beta Vision+ software. Figure 5 is representative of the number of events versus distance profiles obtained from the autoradiographic images for the injection experiments. The number of events was greatest at the center of the injection. The number of events can be calibrated to concentration to obtain concentration versus distance profiles. For the 20 min case, the concentration profile from the tail-end to 3 mm toward the source from the first-zero event value was compared to the mathematical model of diffusion to find the best estimate for $D$. For the 2.5 min case, the concentration profile from 0.7 mm toward the source from the bend to the tail-end was compared to the mathematical model of
diffusion to find the best estimate for $D$. The best estimates obtained for $D$ are given in Table 5.
Figure 6. Implantation Profile with Curve Fit, 24 Hours, 45 Degrees. A concentration versus distance example profile obtained by solving the transient diffusion and elimination equation (equation 3) is shown. This curve fit is for the data obtained from the 24 hour case at an angle of 45 degrees. Note that there were points that went into the curve fit in producing this graph that fell below the zero line due to background adjustments that are not shown.
Figure 7. Implantation Profile with Curve Fit, 24 Hours, 0 Degrees. A concentration versus distance example profile obtained by solving the transient diffusion and elimination equation (equation 3) is shown. This curve fit is for the data obtained from the 24 hour case at an angle of 0 degrees. Note that there were points that went into the curve fit in producing this graph that fell below the zero line due to background adjustments that are not shown.
Figure 8. Implantation Profile with Curve Fit, 60 Hours, 195 Degrees. A concentration versus distance example profile obtained by solving the transient diffusion and elimination equation (equation 3) is shown. This curve fit is for the data obtained from the 60 hour case at an angle of 195 degrees. Note that there were points that went into the curve fit in producing this graph that fell below the zero line due to background adjustments that are not shown.
Figure 9. Implantation Profile with Curve Fit, 6 Hours, 60 Degrees. A concentration versus distance example profile obtained by solving the transient diffusion and elimination equation (equation 3) is shown. This curve fit is for the data obtained from the 6 hour case at an angle of 60 degrees. Note that there were points that went into the curve fit in producing this graph that fell below the zero line due to background adjustments that are not shown.
Figure 10. Implantation Profile with Curve Fit, 6 Hours, 75 Degrees. A concentration versus distance example profile obtained by solving the transient diffusion and elimination equation (equation 3) is shown. This curve fit is for the data obtained from the 6 hour case at an angle of 75 degrees. Note that there were points that went into the curve fit in producing this graph that fell below the zero line due to background adjustments that are not shown.
Figure 11. Implantation Profile with Curve Fit, 6 Hours, 30 Degrees. A concentration versus distance example profile obtained by solving the transient diffusion and elimination equation (equation 3) is shown. This curve fit is for the data obtained from the 6 hour case at an angle of 30 degrees. Note that there were points that went into the curve fit in producing this graph that fell below the zero line due to background adjustments that are not shown.
Figure 12. Injection Profile with Curve Fit. Concentration profiles near the tail end at (a) 2.5 and (b) 20 min after injection are shown. Data from only one scan per time period are shown. Combining data from all other scans would make the figure unreadable. The solid lines show the diffusion model in which $D$ and $C_0$ were varied to minimize the residual of the sum-squared-error between the predicted and experimental values.
Table 1. Resultant Estimated $D$ and $k$ Values Using Implantation. The diffusion coefficient and elimination constant were determined by fitting a model of diffusion and elimination to the concentration profiles measured near the tip of a catheter attached to an osmotic pump. For the 60 hour data, only was very thin, allowing measurement without boundary effects only in one case. The outliers and obviously questionable numeric values were excluded to arrive at these statistical results.
Table 2. Penetration Distance of Radioactivity from the Tip of the Catheter. The penetration distance is the distance where the local concentration drops to 10% of the concentration at the catheter tip. This radial distance was found using the best fit curve through the data and corresponds to the location where $C/Co = 0.1$. The dimensionless parameter, $\varphi = a\sqrt[2]{\frac{k}{D}}$, determines the extent of drug penetration and was found using the corresponding $k$ and $D$ values in Table 1.
<table>
<thead>
<tr>
<th>Medium</th>
<th>D [m$^2$/s]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>6.82 x10$^{-10}$</td>
<td>Stokes-Einstein equation</td>
</tr>
<tr>
<td>Subcutaneous tissue</td>
<td>4.11 ± 1.77x10$^{-10}$</td>
<td>This study</td>
</tr>
<tr>
<td>Brain</td>
<td>2.0 x10$^{-10}$</td>
<td>Saltzman and Radomsky, 1991 (21)</td>
</tr>
<tr>
<td>Cellulose acetate membrane</td>
<td>3.15 x10$^{-10}$</td>
<td>Barry and Brace, 1977 (22)</td>
</tr>
</tbody>
</table>

Table 3. Diffusion Coefficients for Dexamethasone in Various Media. The diffusion coefficient of dexamethasone in subcutaneous tissue was compared to the diffusion coefficient for dexamethasone in other media from the literature.
Table 4. Elimination Constants of Various Agents in Subcutaneous Tissue. The elimination constant of dexamethasone in subcutaneous tissue was compared to the elimination constant of other agents in subcutaneous tissue from the literature. a rat serum albumin. b vascular endothelial growth factor.

<table>
<thead>
<tr>
<th>Agent</th>
<th>$k$ [1/s]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>$6.42 \pm 1.19 \times 10^{-5}$</td>
<td>Kim and Burgess, 2002) (24)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>$3.65 \pm 2.24 \times 10^{-5}$</td>
<td>This study</td>
</tr>
<tr>
<td>VEGF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>$3.50 \pm 1.03 \times 10^{-5}$</td>
<td>Kim and Burgess, 2002) (24)</td>
</tr>
</tbody>
</table>
Table 5. Estimated $D$ from Injection Experiments. The diffusion coefficient was determined by fitting a model of diffusion to the concentration profiles from the tail-end of the profiles for injection analysis.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>$D \times 10^{-10}$ (m$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>$2.69 \pm 1.08$</td>
</tr>
<tr>
<td>20</td>
<td>$4.01 \pm 2.01$</td>
</tr>
</tbody>
</table>
Discussion

The purpose of this research was to investigate the capabilities of using the solutions to the diffusion equation with spherical symmetry along with a nonlinear curve fitting technique to estimate the diffusion coefficient and the elimination constant for dexamethasone in subcutaneous tissue. While the estimates were in the expected range and the general technique did work, there is much than can be done to improve the overall estimation calculations. One of the shortcomings was that some data had to be considered outliers and eliminated for doing the final estimation (Table 1). Specifically, the data at some angles was such that the tails were clearly not representative of a diffusion phenomenon. One explanation for this discrepancy is that, in this analysis, convection was not taken into account. Due to channeling by anatomical structures convection could have been significant in some directions. Future research could investigate the importance of convection to get a better result in the modeling of the subcutaneous mass transfer. A second weakness in what has been done here is that only the tails of the concentration profiles were used to get the estimates of the diffusion coefficients and elimination constants. This was appropriate because of the focus on diffusion and elimination. However, an improvement in this technique could be made if different criteria were used to decide which data represented the diffusion process. Here the maximum looking back from the tail was used as the cutoff point for curve fitting. Other criteria might be established for achieving better results. For example, a curvature criterion could be used to establish which data on the profile tails should be used in curve
fitting. Clearly, much research could be done to investigate how to better estimate the diffusion and elimination properties.

Another area that could be investigated is the control of the Marquardt algorithm. While the Marquardt algorithm worked quite well, there are some controlling parameters that could be investigated to improve the results. Specifically, the initial lambda setting could be investigated in detail to make sure it is being set appropriately. There were instances of curve fitting for which the initial lambda was changed so that the initial first step would not be so large as to put the calculation into a mode which provided non-physiological results. While, in this work, the Marquardt algorithm generally provided physiological results, it may be possible to discover strategies for picking lambda which are more methodical than what was done here. Similarly, better starting points for the Marquardt search may be able to be found with more work. The strategies used for picking the initial lambda and starting point were carefully considered, but no investigation was actually done to see if other strategies might be more appropriate as this issue was beyond the scope of this research.

The diffusion coefficient, \(D\), of dexamethasone in subcutaneous tissue at 6 and 24 hours after implantation was \(3.63 \pm 1.06 \times 10^{-10} \text{ m}^2/\text{s}\) and \(4.92 \pm 1.97 \times 10^{-10} \text{ m}^2/\text{s}\), respectively. The 60 hour data suggests a \(D\) of \(1.73 \times 10^{-10} \text{ m}^2/\text{s}\). There was no significant difference between the 6 and 24 hour data for \(D\) \((p > 0.05)\). A comparison with the 60 hour data was not made as the sample size was too small. Even though the concentration profile at 6 hours has not yet reached steady-state (Figure 3a), the value found for \(D\) should not be different from that found for the 24 hour case, which is very close to steady-state. (Note that at 6 hours \(\varphi = 0.22\) (Table 2), and Figure 3a shows concentration
profiles for $\varphi = 0.2$ for various times.) The concentration profile at 60 hours has reached steady-state (Figure 1a). $D$ should have similar values at 6, 24, and 60 hours because the best estimate for $D$ and $k$ for all cases was achieved using the transient diffusion and elimination equation (equation 3). Since the transient equation takes time into account, be it for a short time period or for a long time period, the $D$ and $k$ values for the same agent in the same tissue should be the same. $D$ and $k$ are assumed to be constants. As time becomes large, the transient equation (equation 3) reduces to the steady-state equation (equation 4). Hence, the average diffusion coefficient $D = 4.11 \pm 1.77 \times 10^{-10}$ m$^2$/s, based on the 6, 24, and 60 hours data, results in a reasonable value for dexamethasone in subcutaneous tissue. The diffusion coefficient of dexamethasone in subcutaneous tissue is slightly less than in water but slightly greater than in brain tissue (Table 3). Our diffusion coefficient for dexamethasone in rat subcutaneous tissue is slightly greater than the diffusion coefficient of sodium fluorescein (MW 376) in rat subcutaneous tissue $D = 2.35 \pm 0.24 \times 10^{-10}$ m$^2$/s (23). Sodium fluorescein has a molecular weight similar to that of dexamethasone (MW 392). The elimination constant, $k$, at 6 and 24 hours was $4.80 \pm 2.56 \times 10^{-5}$ s$^{-1}$ and $2.52 \pm 1.65 \times 10^{-5}$ s$^{-1}$, respectively. The 60 h data suggests a $k$ of $4.70 \times 10^{-5}$ s$^{-1}$. There was no significant difference between the 6 and 24 hour data for $k$ ($p > 0.05$). A comparison with the 60 hour data was not made, as the sample size was too small. The average, based on the 6, 24, and 60 hour data, for the elimination constant is $k = 3.65 \pm 2.24 \times 10^{-5}$ s$^{-1}$. This value is quite reasonable despite the fact that the 6 hour case has not yet reached steady-state for the reasons given in the paragraph above. Table 4 shows values for $k$ of other agents in subcutaneous tissue. Our elimination constant for dexamethasone in rat subcutaneous tissue is slightly greater than that of dexamethasone.
in rat brain $k = 1.19 \times 10^{-5} \text{ s}^{-1}$ (25). Although only two rats were used for each time point, variation was not observed between the two rats as they were the same age, sex, size, and strain and were all from the same vendor. A detailed study would be useful to demonstrate that the age, sex, size, strain and vendor have no significant effect on the values of $D$ and $k$.

When a substance is injected into tissue in a period that is effectively instantaneous, it may exhibit two distinct behaviors: (1) form a fluid-filled cavity or (2) infiltrate the extracellular space of the tissue (19). The subsequent diffusion from each case can be described by its own set of expressions (19). In this study, we have assumed that the substance does not form a cavity but infiltrates the extracellular space and then diffuses away. Hence, the appropriate solutions and approximations have been used for this case. The approximations to the case where substance infiltrates the extracellular space lead to equation 11. The two criteria for equation 11 to provide a useful approximation are that the measurement locations be sufficiently far from the source (19) and that the injection time is very brief compared to the time of the subsequent diffusion measurements (8). To comply with these criteria, the data near the tail-end of the concentration profiles were used as described below. The measurement distance was kept as small as possible while large enough to provide meaningful data. To investigate criterion 2, two diffusion times were chosen, $t=2.5$ minutes and $t=20$ minutes. For this study, radiolabeled dexamethasone was introduced into the subcutaneous tissue by injection. The highest concentrations of the agent were assumed to be at the location of the center of the injection. This assumption is supported by our theoretical curves (Figure 4). The local distribution of the agent in the subcutaneous tissue surrounding the
center of injection was measured (Figure 5). The local distribution of the agent at the tail-end of the distribution was compared to the mathematical model of diffusion. For the 20 minute case, the mathematical model was compared to the local distribution from the tail-end to a distance 3 mm toward the center of the injection from the first-zero event value. For the 2.5 minute case, the mathematical model was compared to the local distribution from the tail-end to a distance 0.7 mm toward the center of the injection from a bend. The concentration profile bends from a steep curve to a plateau region. The bend was defined to be the position where the number of events had a value of 100 (Figure 5a). The plateau region was defined as having a relatively flat profile where the events values were between 0 and 100. The position of the first zero event value could not be used as a reference as the plateau region varied greatly in length. Hence, it would not be possible to set a specified measurement distance from the first zero event value. A plateau region was not seen with the 20 minute data. The distribution of the agent within the subcutaneous tissue near the tail-end of the concentration profile was consistent with the mathematical model of diffusion (Figure 12). The mathematical model was compared to the experimental data in order to obtain values for the diffusion coefficient, $D$, at 2.5 and 20 minutes after injection. The diffusion coefficient, $D$, of dexamethasone in subcutaneous tissue slices at 2.5 and 20 minutes after injection was $2.69 \pm 1.08 \times 10^{-10}$ and $4.01 \pm 2.01 \times 10^{-10}$ m$^2$/s, respectively. As mentioned above, there were two criteria for equation 5 to provide a useful approximation. Also, to comply with the criteria, the data near the tail-end of the concentration profiles were used. However for a few of the concentration profiles for the $t=2.5$ minute case, using data 0.7 mm toward the source from the bend meant using all the data as the profile was very steep (Figure 12a). Hence,
at $t=2.5$ minutes the criteria could not be complied with. For the 20 minute case, there was an offset ranging from 0.77 to 2.25 mm from the center of the injection (Figure 12b). Although this offset is not large, it may be sufficient enough to comply with criterion 1. In addition, data in Nicholson (19) showed that the accuracy of equation 5, at measurement distances near the source, increases with time. Further, the criteria required that the injection time be very brief compared to the time of the subsequent diffusion measurements. The approximate duration of the injection was 1 second. Hence, the injection of 0.04 mL of substance was very brief. The two diffusion times were $t=2.5$ minutes and $t=20$ minutes. The $t=2.5$ minute concentration profile had a plateau region that was not seen in the $t=20$ minute concentration profile (Figure 12). It could be that, at $t=2.5$ min, the injected substance both formed a fluid filled cavity and infiltrated the extracellular space to some degree, producing the plateau region. If this were the case, then equation 5 would not be the appropriate expression. This is a phenomenon that needs to be investigated further and is beyond the scope of this present study. The concentration profile at $t=20$ minute is similar in shape to the theoretical curves realized by using equation 11 (Figure 4), whereas the concentration profile for $t=2.5$ minutes is not similar due to the plateau region. Hence, we assume that for $t=2.5$ minutes that the diffusion time was not long enough and that equation 11 does not provide a useful approximation. A diffusion time of $t=20$ minutes probably provides an adequate diffusion time, and, hence, equation 11 does provide a useful approximation in this case. Therefore, the best estimate for the diffusion coefficient of dexamethasone in subcutaneous tissue slices based on the $t=20$ minute data is $D=4.01 \pm 2.01 \times 10^{-10}$ m$^2$/s. This compares with $D=4.11 \pm 1.77 \times 10^{-10}$ m$^2$/s for the implantation case. These values
for $D$ are very similar, suggesting that equation 11 provides an adequate approximation as long as the two criteria are met. Our mathematical model assumed that the diffusing substance was deposited within a sphere at $t=0$. Figure 2a shows that the shape of the injection at 2.5 minutes is relatively spherical. Hence, the assumption that the injected volume at $t=0$ was spherical is acceptable. The mathematical model also assumed isotropic diffusional transport of drug through the subcutaneous tissue. Figure 2b shows the diffusion of the drug at $t=20$ minutes, and diffusion is relatively spherical away from the site of injection. Hence, our assumption of isotropic diffusional transport is reasonable. However, the actual shape is not perfectly spherical, and this resulted in a relatively large standard deviation. The elimination constant was assumed to be negligible as the injections were made in either harvested subcutaneous tissue or in a sacrificed rat so that the normal clearance processes that depend on circulation of blood were eliminated (8). It is recommended that for future diffusion experiments with other substances in other tissue that (1) the injection volume be small, i.e., 0.04 mL or less; and (2) higher radioactivity be used, i.e., 0.65 $\mu$Ci or higher. The experimental duration time can be estimated a priori by (1) finding a diffusion coefficient for a substance similar to the one of interest in the tissue, (2) making theoretical curves like these shown in figure 4, and (3) choosing a curve that shows a large diffusion distance away from the source (e.g., the 20 minute curve in Figure 5 shows a large diffusion distance away from the source). In conclusion, equation 11 provides an adequate approximation for measurement locations sufficiently far from the source and for diffusion times much longer than the injection time (8, 19). The main advantages of this injection technique to determine an approximation for the diffusion coefficient are that it is a relatively simple
technique and can be applied to any radiolabeled substance of interest injected into any tissue of interest.
References


Appendix A: The Levenberg-Marquardt Algorithm

The Levenberg-Marquardt method of optimization is a minimum sum squared error numerical technique for fitting a nonlinear expression to a set of data. The optimization can be easily extended to several variables. This makes it an ideal technique for the problem addressed in this research (27). The solution of the diffusion equation contains a set of parameters, C₀, D, and k, over which one wishes to search for the best possible values so that the experimental measurements are adequately summarized.

More generally, we have a function $F$ which relates a single independent variable to a single dependent variable. $y_i = F(x_i)$. The independent and dependent variables form an ordered pair $(x_i, y_i)$. The measured data that we work with is a set, $m$, of these ordered pairs. $F$ contains a set of parameters, $\beta_i$, that must be found to best represent the data with the function. The goal becomes finding the best set $\{\beta_i\}$ that minimizes the sum squared error between the function and the raw data:

$$S.S.E. = \sum_i (y_i - \hat{y}_i)^2 = E' \cdot E, \quad (79)$$

where $E_i = y_i - \hat{y}_i$, $E = \begin{bmatrix} E_1 \\ E_2 \\ \vdots \\ E_i \\ \vdots \end{bmatrix}$ and $\hat{y}_i = F(\beta_0, \beta_1, \beta_2, x_i)$. The first strategy that the Marquardt technique uses is to find the gradient of the sum-squared-error with respect to
the function parameters:

\[
\frac{1}{2} \frac{\partial (E' \cdot E)}{\partial \beta} = -X' \cdot Y + X' \cdot F(\beta) = -X' \cdot E. \quad (80)
\]

Note that \(X\) is an \(m\) by \(n\) matrix containing the partial derivatives of \(F\) with respect to the parameters, \(X = \frac{\partial F}{\partial \beta}\), and \(E\) is an \(n\) by 1 matrix containing the error at each data point in the set.

The gradient is used to help determine the direction to move in the \(\beta_i\) space to achieve the smallest sum-squared-error:

\[
\beta_{i+1} = \beta_i + k \cdot X' \cdot E. \quad (81)
\]

The variable \(k\) controls how far to move in the direction opposite to the gradient in updating the parameter values. The disadvantage of the gradient method is that while it can tell the best direction to move for finding a solution, it does not specify how far to move. The Marquardt algorithm rectifies this problem by using a Gauss-Newton technique.

The Gauss technique assumes that the function of interest can be expanded in a Taylor series around the present location in the \(\beta_i\)-space. Only the linear terms are kept in this approach:

\[
F(\beta) = F(\beta_o) + X \cdot (\beta - \beta_o) + \cdots \quad (82)
\]

Now, by assuming there is an exact \(\beta_i\) location that will make the above equation exact or, in other words, when the error is at zero, the following algebra leads to an update formula.

\[
X' \cdot [F(\beta_o) + X \cdot (\beta - \beta_o)] = X' \cdot Y. \quad (83)
\]
\[(X' \cdot X) \cdot (\beta - \beta_0) = X' \cdot Y - X' \cdot F(\beta_0). \quad (84)\]

So that finally, the Gauss method gives an update formula:

\[\beta_{i+1} = \beta_i + (X' \cdot X)^{-1} \cdot X' \cdot E. \quad (85)\]

Note that this update formula tells us everything we need to get to a better solution in the \(\beta_i\)-space. However, as opposed to the steepest decent gradient method it requires a matrix inverse calculation which can be unstable under certain conditions.

The Marquardt algorithm combines the philosophy of these two approaches to provide a stable yet complete way to move to a better position in the solution space. Marquardt uses the following update formula:

\[\beta_{i+1} = \beta_i + (X' \cdot X + \lambda I)^{-1} \cdot X' \cdot E. \quad (86)\]

This formula balances the gradient-steepest-decent and the Gauss approaches by essentially forcing the two together by use of the \(\lambda\) scaling parameter. Note that if \(\lambda\) goes to zero the formula becomes the Gauss expression and if \(\lambda\) becomes large the formula goes to the steepest decent expression with very small movement. So by controlling \(\lambda\) we can keep the inverse calculation stable and yet still move in a good direction even when the inverse is not stable.

The key then becomes picking a good starting point in the solution space. Additionally, developing a good strategy for updating \(\lambda\) is important. The Marquardt algorithm handles this issue by increasing the \(\lambda\) if the error gets larger on that particular iteration of the updating loop and decreasing \(\lambda\) if the error got better. In this way, the distance moved gets more aggressive as the error gets smaller but uses the Gauss concept to know precisely how far to move. This technique is then able to provide a stable, efficient, easily programmable, easily understood, and mathematically elegant approach.
for finding the optimal solution for a nonlinear function containing unknown parameters.

(Figure 13.)
Figure 13. Flow Chart Showing the Marquardt Algorithm. This diagram is a detailed flowchart for the Marquardt Algorithm used for the curve fitting.
Appendix B: The Marquardt Program

% This program will fit a curve to autoradiographic data to determine diffusion and elimination constants.
%
% The initializations.
%
clear all;
format short e;
format compact;
warning off all
%
% Get or generate the data needed.
% The number of data points should be specified as n.
% This data should have the following form for each sample row:
% 1. First Column; distance from source sample.
% 2. Second Column; time of the sample.
% 3. Third Column; concentration value of the sample.
%
% R is the distance from the source.
%
% k is the elimination constant.
%
% D is the diffusion constant.
%
% C is concentration level. C0 is the source concentration.
%
% Thin line 45 degrees background already subtracted.
%
a=0.0006; % a is the radius of the source.
%
InData=[
1.47336 40.692426
1.4944 32.927726
1.51545 35.045326
1.5365 35.045326
1.55755 23.751226
1.5786 29.398226
1.59964 29.398226
1.62069 42.104126
1.64174 26.574726
1.66279 26.574726
1.68384 23.751226
1.70488 23.751226
1.72593 21.633526
1.74698 21.633526
1.76803 23.045326
1.78908 28.692426
1.81012 28.692426
1.83117 26.574726
]
| 1.85222 | 20.927726 |
| 1.87327 | 23.751226 |
| 1.89432 | 23.751226 |
| 1.91536 | 14.574726 |
| 1.93641 | 17.398226 |
| 1.95746 | 17.398226 |
| 1.97851 | 14.574726 |
| 1.99955 | 23.045326 |
| 2.0206 | 23.045326 |
| 2.04165 | 17.398226 |
| 2.0627 | 23.045326 |
| 2.08375 | 13.868826 |
| 2.10479 | 13.868826 |
| 2.12584 | 6.104126 |
| 2.14689 | 12.457126 |
| 2.16794 | 12.457126 |
| 2.18899 | 10.339426 |
| 2.21003 | 8.927726 |
| 2.23108 | 19.515926 |
| 2.25213 | 19.515926 |
| 2.27318 | 18.810026 |
| 2.29423 | 24.457126 |
| 2.31527 | 24.457126 |
| 2.33632 | 28.692426 |
| 2.35737 | 12.457126 |
| 2.37842 | 21.633526 |
| 2.39947 | 21.633526 |
| 2.42051 | 19.515926 |
| 2.44156 | 20.221826 |
| 2.46261 | 20.221826 |
| 2.48366 | 13.868826 |
| 2.50471 | 11.751226 |
| 2.52575 | 3.280626 |
| 2.5468 | 3.280626 |
| 2.56785 | 15.986526 |
| 2.5889 | 7.515926 |
| 2.60995 | 7.515926 |
| 2.63099 | 21.633526 |
| 2.65204 | 13.868826 |
| 2.67309 | 13.868826 |
| 2.69414 | 11.045326 |
| 2.71518 | 12.457126 |
| 2.73623 | 5.398226 |
| 2.75728 | 5.398226 |
| 2.77833 | 11.045326 |
| 2.79938 | 6.810026 |
| 2.82042 | 6.810026 |
| 2.84147 | 12.457126 |
| 2.86252 | 10.339426 |
| 2.88357 | 8.927726 |
| 2.90462 | 8.927726 |
| 2.92566 | 16.692426 |
| 2.94671 | 11.045326 |
| 2.96776 | 11.045326 |
| 2.98881 | 3.280626 |
| 3.00986 | 1.163026 |
| 3.0309 | 9.633526 |
| 3.05195 | 9.633526 |
| 3.073 | 6.104126 |
| 3.09405 | 3.986526 |
| 3.1151 | 3.986526 |
| 3.13614 | 3.280626 |
\texttt{[n,m]=size(InData);}

\texttt{R=zeros(1,n);}
\texttt{C=zeros(1,n);}

\texttt{for i=1:n}
\texttt{
T(i)=24*60*60; \% T is the time.}
\texttt{R(i)=(InData(i,1)-InData(1,1))*0.001+a;}
\texttt{C(i)=InData(i,2);}
\texttt{\% if C(i)<0.0}
\texttt{\% \quad C(i)=0.0;}
\texttt{\% end}
\texttt{end}
\texttt{
\% The following creates the initial estimates for the optimization starting}
\texttt{\% point.}
\texttt{\%}
\texttt{estC0=max(C);}
\[ C_0 = \text{est}C_0; \]
\[ k = 4.5825 \times 10^{-5}; \]
\[ D = 6.82 \times 10^{-10}; \]
\[ D_{\text{old}} = 1.0 \times 10^{-10}; \]

\% \%

\texttt{figure(1);}
\texttt{plot(R-a,C,'r+');
grid on;
\texttt{title('Concentration vs. Distance from Source');
xlabel('Position (meters)');
ylabel('Concentration');
\%}
\%

\texttt{Creation of the necessary matrices for the Marquardt algorithm.}
\%
\texttt{X=zeros(n,3);
XT=zeros(3,n);
XTX=zeros(3,3);
INV_XTX=zeros(3,3);
DELTA=zeros(n,1);
ADJUST=zeros(3,1);
INIT=zeros(3,1);
NEW=zeros(3,1);
Cest=zeros(1,n);
LAMBDA=eye(3);
\%}
\%

\texttt{The following are the initial estimates with other needed settings.}
\%
\texttt{epsilon=0.0001; \% Small numbers needed for the curve fit.}
\texttt{lambda=100000000000000;}
\texttt{oldSSQE=0.0; \% Storage for the sum squared error and its update.}
\texttt{newSSQE=0.0;
INIT(1,1)=C_0;
INIT(2,1)=k;
INIT(3,1)=D;
\%}
\%

\texttt{Calculate the sum squared error of the concentration with the latest}
\%
\texttt{concentration model.}
\%
\texttt{iteration=0;
newSSQE=0.0;
for i=1:n
\texttt{Cest(i)=C_0*a/(2*R(i))*(exp(-(R(i)-a)*sqrt(k/D))*erfc((R(i)-a)/(2*sqrt(D*T(i)))-sqrt(k*T(i))) ...
+exp((R(i)-a)*sqrt(k/D))*erfc((R(i)-a)/(2*sqrt(D*T(i)))+sqrt(k*T(i))));
newSSQE = newSSQE + (Cest(i)-C(i))^2;}
\texttt{end}
\%

\texttt{Iterate until convergence, but loop at least 5 times.}
\texttt{while (abs(newSSQE-oldSSQE)/oldSSQE<epsilon)&(iteration<100)&(abs(D-Dold)/D<epsilon)
\texttt{newSSQE}
\texttt{oldSSQE=newSSQE;
Dold=D;
iteration=iteration+1}
\%

\texttt{Fill the X matrix and do the matrix calculations.}
\%
\texttt{The derivatives have been split up for efficiency and clarity.}
\%
\texttt{for i=1:n
fparm1=(R(i)-a)/(2*sqrt(D*T(i)))-sqrt(k*T(i));
fparm2=(R(i)-a)/(2*sqrt(D*T(i)))+sqrt(k*T(i));
fparm3=(R(i)-a)*sqrt(k/D);
fparm4=0.5*sqrt(T(i)/k);
fparm5=0.5*sqrt(k/(D^3));
fparm6=(R(i)-a)*0.5/sqrt(k*D);
fparm7=a/(2*R(i));
X(i,1)=fparm7*(exp(-fparm3)*erfc(fparm1) ...
+exp(fparm3)*erfc(fparm2));
X(i,2)=C0*fparm7*((-fparm6)*exp(-fparm3)*erfc(fparm1) ...
+(2.0/sqrt(pi)*exp(-(fparm1^2)))*(-fparm4)*exp(-fparm3) ...
+(fparm6)*exp(fparm3)*erfc(fparm2) ...
+(2.0/sqrt(pi)*exp(-(fparm2^2)))*fparm4*exp(fparm3));
X(i,3)=C0*fparm7*(((R(i)-a)*fparm5)*exp(-fparm3)*erfc(fparm1) ...
+(-2.0/sqrt(pi)*exp(-(fparm1^2)))*((R(i)-a)/(-4*sqrt(D^3*T(i))))*exp(-fparm3) ...
+(-2.0/sqrt(pi)*exp(-(fparm2^2)))*((R(i)-a)/(-4*sqrt(D^3*T(i))))*exp(fparm3));

DELTA(i,1)=C(i)-C0*fparm7*(exp(-fparm3)*erfc(fparm1) ...
+exp(fparm3)*erfc(fparm2));

end
%
% The essence of the Marquardt algorithm.
%
XT=X';
%
LAMBD(A(1,1)=lambda;
 LAMBD(A(2,2)=lambda;
 LAMBD(A(3,3)=lambda;
%
XTX = XT*X + LAMBD(A;
INV_XTX=inv(XTX);
%
ADJUST=INV_XTX*XT*DELTA
%
NEW=ADJUST+INIT
%
INIT=NEW;
%
C0=INIT(1,1);
k=INIT(2,1);
D=INIT(3,1);
%
newSSQE=0.0;
for i=1:n
 Cest(i)=C0*a/(2*R(i))*(exp(-(R(i)-a)*sqrt(k*D))*erfc((R(i)-a)/(2*sqrt(D*T(i))))-sqrt(k*T(i))) ...
 +exp((R(i)-a)*sqrt(k*D))*erfc((R(i)-a)/(2*sqrt(D*T(i))))+sqrt(k*T(i)));
 newSSQE = newSSQE + (Cest(i)-C(i))^2;
end
%
if oldSSQE>newSSQE
 lambda=lambda/2;
else
 lambda=lambda*2;
end
lambda
end
%
% Create the found curve for plotting with the data.
%
Rsg=zeros(1,300);
Cestg=zeros(1,300);
Resg=zeros(1,300);
kn=4.5825e-5
Dn=6.82e-10
k
D
\begin{verbatim}
for i=1:300
    Rsg(i)=(i-1)*0.000015+a;
    Cestg(i)=a/(2*Rsg(i))*(exp(-(Rsg(i)-a)*sqrt(kn/Dn))*erfc((Rsg(i)-a)/(2*sqrt(Dn*T(1)))-sqrt(kn*T(1)))) ...
           +exp((Rsg(i)-a)*sqrt(kn/Dn))*erfc((Rsg(i)-a)/(2*sqrt(Dn*T(1)))+sqrt(kn*T(1))));
    Restg(i)=a/(2*Rsg(i))*(exp(-(Rsg(i)-a)*sqrt(k/D))*erfc((Rsg(i)-a)/(2*sqrt(D*T(1)))-sqrt(k*T(1)))) ...
           +exp((Rsg(i)-a)*sqrt(k/D))*erfc((Rsg(i)-a)/(2*sqrt(D*T(1)))+sqrt(k*T(1))));
end
%
for i=1:300
    if Restg(i)<0.1
        r10=Rsg(i)-a
        break;
    end
end
C=C/C0;
%
figure(2);%
plot((R-a)*1000,C,'b*',(Rsg-a)*1000,Cestg,(Rsg-a)*1000,Restg);
grid on;
title('Concentration vs. Distance from Source, Thin-line 24Hr. 45 Degrees');
xlabel('Position (mm)');
ylabel('Concentration/Counts');
text(3.0,0.6,'D = ',num2str(D),'FontWeight','bold');
text(3.0,0.8,'k = ',num2str(k),'FontWeight','bold');
end
\end{verbatim}