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STUDY OF MICHAELIS-MENTEN EQUATION
WITH APPLICATIONS IN PHARMACOKINETICS.

The Ohio State University,
Ph.D., 1977
Biostatistics

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STUDY OF MICHAELIS-MENTEN EQUATION WITH APPLICATIONS IN PHARMACOKINETICS

DISSERTATION
Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
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The Ohio State University
1977

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ACKNOWLEDGMENT

I wish to thank my adviser, Dr. J. S. Rustagi, for his inspirational guidance and patience throughout the preparations of this dissertation. For their helpful suggestions and interest, I also wish to thank Dr. J.D. Powers and Dr. T.E. Obremski. I am grateful to Dr. R.A. Yeary for the data he provides.

I include special thanks to my parents and my husband for the encouragement they give me during my graduate studies.
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CHAPTER 1

Introduction

Compartmental models have been used for a long time in chemical kinetics, electrical engineering, economics, study of radioactivity and many other areas. Since the introduction of the use of radioactive tracer material, application of compartmental analysis in biological systems have developed extensively. Pharmacokinetics is one of the areas where compartmental models have been used for the study of the distribution and metabolism of drugs.

Pharmacokinetics is the application of kinetics to drugs. The goal of the study is to understand the speed of drugs being absorbed, the rate changes of drugs from one action site to others, and how fast the drug components are being metabolized. It also studies the rates at which the drug are metabolized and excreted and various factors which affect these parameters. Biological system consists of many components called compartments. These compartments may not coincide with the body organs or tissues. In some real applications, such as in biomedical experiments, how the compartments connect with each other, is assumed beforehand; in some others, it is just hypothesized. The rates at which the drugs or their metabolites are transported from one
compartment to another are referred to as the transfer rates. These are the pharmacokinetic parameters of the compartmental models.

The concepts of pharmacokinetics and compartmental analysis were first mentioned by Teorell (1937). He emphasized that the compartmental analysis be used in pharmacokinetics to derive mathematical relations so that the kinetics of distribution of substances in the body may be described. Since then, thousands of the papers have appeared in pharmacokinetics. A comprehensive discussion and references of this subject can be found in a book by Wagner (1971).

In classical compartmental analysis, we use ordinary differential equations to explain the pharmacokinetic behavior of drugs. Those models generally use the assumption that the transfer rates from one compartment to the other are proportional to the concentrations and result in linear differential equations. By using the Laplace transformation technique, most of the linear systems can be solved. In fitting these models to data, usual non-linear least-squares method is used. When the transfer rates are functions of the concentrations of at least one compartment and depend on some other parameters of the system, we have a non-linear compartmental system. A comprehensive survey of compartmental analysis is given by Jacquez (1972).

In this dissertation, pharmacokinetic applications of a non-linear system is studied. Essentially it is the study
of dose-dependent pharmacokinetics. There are many different situation in which non-linear drug kinetics occur. It is almost impossible to have uniform theory of non-linear dose-concentration relationship. Also analytical solutions of differential equations are hard to obtain. Our purpose is to estimate the constants of the system, and therefore we concentrate our attention on the original differential equations.

Many researchers in pharmacokinetics have proposed non-linear models for various drugs. Wanger (1971) presents a new generalized non-linear pharmacokinetic model. Several examples of the application of this non-linear model have been given. (1972). Krüger-Thiemer and Levine (1968) proposed non-linear models for systems with special characteristics, including saturability, substrate depletion and reversibility of chemical reactions. The non-linear models that this dissertation deals with are those associated with Michaelis-Menten kinetics.

The Michaelis-Menten equation is widely used in enzyme kinetics in biochemistry. Lunquist and Wolthers (1958) have derived a model with Michaelis-Menten kinetics for the metabolism of alcohol in pharmacokinetics. Wagner and Patel (1972) used alcohol to perform several experiments on a single subject. They utilized the statistical method to study the variations in absorption and elimination rates of alcohol. The useful properties of the Michaelis-Menten equation in pharmacokinetics have been analyzed by Wagner (1973).
He also pointed out the distortion in parameter values when data arising from Michaelis-Menten kinetics are evaluated by linear pharmacokinetic model. Sedman and Wagner (1974) stressed the importance of the use of appropriate pharmacokinetic models to analyze the Michaelis-Menten constants. The two-compartment model with Michaelis-Menten elimination process was first mentioned by them. Since the non-linear models with Michaelis-Menten process are increasingly applied to the study of pharmacokinetics, we devote most of this dissertation to problems with Michaelis-Menten kinetics.

The general linear compartmental models can be described by a system of differential equations. The solutions of these differential equations are linear combinations of exponentials. For estimating the parameters in these models, obtained as solutions of differential equations, the non-linear least-squares method with iterative procedure is used. The good initial estimates are necessary for the iterative process to get the least-squares estimates. It is known that for the two-compartment model with Michaelis-Menten elimination process, the iterative methods do not provide reasonable estimates see Rustagi and Singh (1977). Bellman and Kalaba (1965) suggested quasilinearization method, which also uses iterative process, for estimating the parameters of compartmental models. Rustagi and Singh (1977) have recently proposed difference equation method to estimate the constants in pharmacokinetic models. In many cases, it can be shown that the difference equation method gives improved estimates of the parameters
of the model. The difference equation method is used here for estimating the constants of one- and two-compartment models with Michaelis-Menten kinetics. A brief discussion and comparison of other methods are also given.

It is well known that numerical procedures do not provide an easy way to obtain the properties of the estimates. We find the probability distributions of estimates obtained from difference equation by using the computer simulation method. The difference equation used for linear compartmental models and their statistical analysis are discussed in great detail by Singh (1975). For the non-linear model with Michaelis-Menten process, it is shown here that the estimates have normal distributions.

A general review of compartmental analysis is given in Chapter 2. Methods for estimating the parameters of the models and the associated problems are included. The application of Michaelis-Menten equation to the study of pharmacokinetics is the topic of Chapter 3. An outline of mathematical study of the equation is also given. In Chapter 4, difference equation analogues of one- and two-compartment models with Michaelis-Menten kinetics are presented. The non-linear least-squares method and quasilineraization are discussed. Chapter 5 describes the computer simulation method and results. Several related discussions about the Michaelis-Menten equation can be found in Chapter 6.
CHAPTER 2
Compartmental Analysis

2.1 Introduction and definitions

A compartment can be defined as a group of materials or substances carrying certain characteristics such as chemical specie, biological entity etc., and occupying a given volume. Different compartments may be in the same place or may be separated by a permeable boundary. Several compartments connect together with the exchange of material from one to the other form a compartmental system. There are many types of compartmental systems, depending on how the compartments join together. If the system has exchange with enviroment, we call it an open system, otherwise it is a closed system. The compartmental models are the mathematical expressions to describe the systems. The theory and study of such models is called compartmental analysis.

The compartmental models are found very useful in certain branches of biological and physiological sciences. Jacquez (1972) and Wagner (1971) have given an exhaustive references. The experimenters in these areas use compartmental analysis to quantatify their experimental findings and get a plausible description of the system which is under study. Although in some applications such as pharmacokinetics, the compartmental models are only utilized as a reference aid, they may incorporate with
other information and give the experimenters more confidence about the analysis.

2.2 Classifications

We use boxes to represent the compartments. The substances associated with the compartments may be the amounts or the concentrations. In any case, we use the letter $C_i$ to stand for the quantity of substance in the $i$th compartment. Arrows tell the directions of the flow of the substance. Each arrow is labelled with a transfer rate $k_{ij}$ (from compartment $j$ to compartment $i$), that is the fraction of the amount in the compartment from which the arrow originates transferred per unit time, as shown in Figure 2.1

![Figure 2.1](image)

The following classification of compartmental systems was made by Jacquez (1972).

(a) System consisting of isolated subsystems

This type of compartmental systems is very easily recognized by looking at the connectivity diagram given in Figure 2.2, consisting of five compartments. For such a system, we can actually consider it as two sets of independent subsystems.
(b) Separable subsystems

This kind of systems may be divided into a hierarchy of subsystems \((1), (2), (3), \ldots, (k)\) such that \((1)\) receives no input from \((2), (3), \ldots, (k)\) and \((2)\) takes no input from \((3), (4), \ldots, (k)\). But may receive from \((1)\) etc. A diagram of six compartments is shown below in Figure 2.3.

For such a system, we perform the analysis for subsystem \((1)\), then take that as input for subsystem, do the analysis, etc.

(c) Trapping subsystems

A trap is a subsystem which receives input from the remainder of the system but no transfers out of the system. Sometimes the whole system is a trap, that means there is
no excretion in the system. If a system is a trap and no input from environment, then it is a closed system by definition. Figure 2.4 is a trap with three compartments. The compartments 3, 4, and 5 in Figure 2.2 also form a trap.

\[ \rightarrow \begin{array}{c} 1 \end{array} \leftarrow \begin{array}{c} 2 \end{array} \rightarrow \begin{array}{c} 3 \end{array} \]

\text{Figure 2.4}

(d) The catenary system

A system in which the compartments are arranged in a linear order and the exchanges of materials are made through the use of adjacent compartments is called a catenary system. Usually the input may only be into first compartment and excretion only from the last, as shown in Figure 2.5. The system is called "linear multicompartmental system" by Benet (1972) and Shah (1976).

\[ \rightarrow \begin{array}{c} 1 \end{array} \leftarrow \begin{array}{c} 2 \end{array} \rightarrow \cdots \cdots \cdots \leftarrow \begin{array}{c} n \end{array} \rightarrow \]

\text{Figure 2.5}

(e) The mammillary system with excretion from the central compartment

If the compartmental system has one central compartment, and all the others interact only with the central one, then we have a mammillary system. This type of model has been proved very useful in pharmacokinetics where the plasma often acts as the central compartment. Figure 2.6 shows the general mammillary system. Benet (1972) has made a thorough study of the mammillary system with excretion from any
compartments. Because of the importance of this system in our study, we will discuss its properties later.

Figure 2.6

(f) The extended mammillary system

This system is shown in figure 2.7, when the number of compartments, \( n \), is odd. It requires that the same number of compartments be associated with the central compartment. It is encountered often in biological systems. For example, the central compartment might be the plasma, the even-numbered compartments, such as 2, 4, ..., \( n-1 \), represent interstitial space and the odd-numbered for intracellular space in the organs.

Figure 2.7

2.3 Compartmental models

There are three areas of main interest in compartment analysis. First is to develop a plausible model for the existing system. This depends heavily on the understanding and knowledge of the field from which the problem comes. Sufficient background of the particular field should
provide reasonable justification about the use of the compartmental model and the associated parameters, in order to make them meaningful for the real system. The second problem is the development of analytical theory for the system given a compartmental model. It can usually be done with mathematical methods such as numerical approximations etc., if not always the easiest. The third one is the so-called inverse problem, which involves system identification and parameter estimation. This also is the problem which attracts mathematicians and statisticians. We consider the following mathematical models of various compartmental systems.

2.3.1 Linear compartmental models

The basic law governing the changes in a compartmental system is usually differential equations, which show the rate changes of materials in the compartments with respect to time. If the equations are linear in the fractional transfer rates, which are the parameters of the system, we have linear compartmental models. Suppose we have n compartments interconnecting with each other, there is exchange of flows between any two of them. Figure 2.8 shows any ith compartment:

![Figure 2.8]
The fractional transfer rate from the compartment i to the environment is $k_{oi}$, the transfer rate of material from compartment i to j is $k_{ji}$, and from compartment j to i is $k_{ij}$. By definition, $k_{ij}$'s are all positive. Then the differential equation for the ith compartment is:

$$\frac{dC_i}{dt} = -k_{oi}C_i - \sum_{i\neq j} k_{ji}C_i + \sum_{i\neq j} k_{ij}C_j.$$ (2.1)

Let $k_{ii} = k_{oi} + \sum_{i\neq j} k_{ji}$. Thus $k_{ii} \geq \sum_{i\neq j} k_{ji}$ (2.2)

The equality sign holds only if there no excretion from the compartment. Substituting (2.2) into (2.1), we have

$$\frac{dC_i}{dt} = -k_{ii}C_i + \sum_{i\neq j} k_{ij}C_j.$$ (2.3)

If we have n compartments, then the differential equations can be written in matrix form:

$$\frac{d\mathbf{C}}{dt} = \mathbf{K}\mathbf{C}.$$ (2.4)

where

$$\mathbf{K} = \begin{bmatrix}
-k_{11} & k_{12} & \cdots & k_{1n} \\
k_{21} & -k_{22} & & \\
& \ddots & \ddots & \\
k_{n1} & \cdots & -k_{nn}
\end{bmatrix}$$

and

$$\mathbf{C} = \begin{bmatrix}
c_1 \\
c_2 \\
\vdots \\
c_n
\end{bmatrix}.$$
Some of the $k_{ij}$'s may be zeros depending on the compartmental system. And because of the definition of the $k_{ij}$'s, $K$ is a non-singular matrix, as long as the system is open system.

The solutions of the system equations (2.4) is:

$$C = A V,$$

where

$$V' = \left(e^{\lambda_1 t}, e^{\lambda_2 t}, \ldots, e^{\lambda_n t}\right)$$

with $\lambda_1, \lambda_2, \ldots, \lambda_n$ are eigenvalues of matrix $K$, $A$ is arbitrary constant matrix, so that the solutions of the linear compartmental models are the sum of exponentials. The nature of these solutions is clearly determined by the nature of the matrix $K$. We will point out several major properties of $K$, the detailed discussion is in the paper by Hearon (1963).

We note that all $k_{ij}$ are non-negative and thus the signs of $k_{ij}$ and $k_{ji}$ are always the same, $K$ is a sign-symmetric matrix. In addition, we have shown in equation (2.2) that $k_{ii} \geq \sum k_{ji}$, a matrix satisfying this condition for all $i$ is said to be diagonally dominant. This condition assures us that the real part of all the root of $|K - \lambda I|$, i.e. the eigenvalues of $K$ will be non-positive and that there can be no pure imaginary roots. This property is required in most applications, for it guarantees the absence of any solutions which grow without bound. If $k_{ij} = k_{ji}$, then we have a symmetric matrix, all the roots must be real and non-negative. As a matter of fact, in applications, the roots of $K$ are all real even when $K$ is not symmetric.
2.3.2 Non-linear models

There is no unique definition about the non-linear compartmental models. Wager in his book (1971) has given a detailed discussion of the non-linear models, especially for applications in pharmacokinetics. He categorized all those compartmental models whose solutions cannot be written as the sum of exponentials, as the non-linear models. Jaquez (1972) defined the non-linear compartmental models as those systems which have at least one of the fractional transfer rates being a function of the quantity of at least one compartment and some other parameters, i.e., $k_{ji} = k_{ji}(C, K)$. Shah (1976) defines any compartmental system which has a multiple output or which is not arranged in a series to be a non-linear compartmental model. We follow the viewpoint that the non-linear compartmental models are those which are described by differential equations non-linear in parameters, or non-linear in quantities of substances of compartments.

There is little one can say about the nature of general solutions of non-linear models in general. Each system is an individual case. We illustrate this by the following two examples:

**EXAMPLE 1:**

Suppose we have a compartmental system as in Figure 2.9,
In this case, the transfer rate is not a constant. The differential equation for $C_1$ is
\[
\frac{dC_1}{dt} = -k_{21}C_1^2 + k_{12}C_2. \tag{2.6}
\]
The model is non-linear in concentration $C_1$.

**EXAMPLE 2:**

The following example is similar to the one given by Krüger-thiemer et al. (1968). Suppose we have four compartments arranged as in Figure 2.10,

![Figure 2.10](image)

The fractional transfer rate from compartment 1 to 2 is determined by two constants $K_1$ and $K_2$, then the differential equation for $C_1$ is:
\[
\frac{dC_1}{dt} = -k_{31}C_1 - \frac{K_1C_1}{K_2 + C_1}. \tag{2.7}
\]

### 2.3.3 Stochastic models

If the fractional transfer rates from one compartment
to another are not constants, but rather, random variables, then the compartmental systems are stochastic. The importance of the study of the stochastic compartmental models have been emphasized by many authors, see Rustagi (1964, 1965). We will only sketch some highlights of the studies.

Bartholomay (1958) was an early investigator of the stochastic assumptions, and solved the one-compartment model. Cornfield et al. (1960) concluded that the stochastic models should be studied for systems which have been approximately studied by deterministic models. Uppuluri, Fedor and Shenton (1967) considered stochastic one-compartment models for multiple dosing and random decay for radioactive material. Matis and Hartley (1971) considered the probability distributions of $p$ compartments for a finite tracer population and developed estimation procedures. They use the theories of Markov process and derived the solutions of the model similar to that of illness-death process.

In pharmacokinetics, the stochastic compartmental models mainly deal with the uncertainties in transfer rates. Only the linear stochastic compartmental models are considered here, in which the general model can be written as in vector-matrix form:

$$\frac{d\mathbf{C}}{dt} = \mathbf{A} \mathbf{C}(t), \quad \mathbf{C}'(t) = (C_1(t), C_2(t), ..., C_n(t)),$$

(2.8)
satisfying the initial condition

\[ \mathbf{c}(0) = \begin{bmatrix} c_1(0) \\ 0 \\ 0 \\ \vdots \\ 0 \end{bmatrix} \] (2.9)

with probability one.

The elements of the transfer rates matrix $\mathbf{K}$ are assumed to be random variables and satisfy with probability one the conditions,

\[ k_{ij} > 0 \quad i, j = 1, 2, \ldots, n \quad i \neq j \]

\[ \sum_{j} k_{ij} < 0 \quad j = 1, 2, \ldots, n \] (2.10)

to assure the physiological significance.

Soong and Dowdee (1974) showed that, if the underlying model is actually stochastic, and the deterministic model is incorrectly used, serious mistakes will result. Chuang and Lloyd (1974) used the statistical simulation of the time-concentration curves to identify the multicompartment models from a stochastic point of view.

2.4 Inverse Problem

Analytical solutions for the completely specified compartmental models are found by pure mathematical techniques. Often they can be solved by approximation methods. Numerical solutions to different types of differential equations should be feasible in most cases with the help of
computer. The inverse problem which involves parameter estimation and system identification is of practical importance. The difficulties in the solutions arise from the fact that in most experiments with n compartments, not everyone is accessible. The incomplete data makes the statistical estimations even harder. We mention a few methods to estimate the parameters of compartmental models. Two of these methods can be applied to both linear and non-linear compartmental models. We will show briefly the difficulties in estimating the parameters.

2.4.1 Parameter estimation

(a) Curve peeling method

For the linear compartmental models, the solutions are sums of exponentials. To illustrate this method, we consider the simple case for the two-compartment model.

\[ C = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t}, \]

\[ = A_2 e^{-\lambda_2 t} \left[ 1 + \frac{A_1}{A_2} e^{-(\lambda_1 - \lambda_2) t} \right] \]  \hspace{1cm} (2.11)

where \( A_1, A_2, \lambda_1, \lambda_2 \) are constants.

If \( \lambda_1 \) is sufficiently large than \( \lambda_2 \), then for \( t \) large, we have,

\[ C \approx A_2 e^{-\lambda_2 t} \]  \hspace{1cm} (2.12)

or

\[ \ln C \approx \ln A_2 - \lambda_2 t. \]  \hspace{1cm} (2.13)

Suppose the data are given in terms of concentrations \( C(t_1) \),
\(C(t_2), \ldots, C(t_n)\) at times \(t_1, t_2, \ldots, t_n\) respectively. A plot of the fitted model (2.13) for \(t\) large should give slope \(\hat{\lambda}_2\) and intercept \(\ln \hat{A}_2\). If we plot equation (2.13) on the semilog paper, we get \(\hat{\lambda}_2\) and \(\hat{A}_2\) directly. After determining \(\hat{\lambda}_2\) and \(\hat{A}_2\), subtract \(A^2 e^{-\lambda_2 t}\) from \(C(t_1)\) at all data points, obtain \(y(t_i)\) with,
\[
y = \hat{A}_1 e^{-\hat{\lambda}_1 t}\]
so that
\[
\ln y = \ln \hat{A}_1 - \hat{\lambda}_1 t. \tag{2.14}
\]
We get \(\hat{\lambda}_1\) and \(\hat{A}_1\). If we have more than two terms in the model (2.11), the process is repeated as before.

This method only works when we have less than three exponential terms, otherwise it is not accurate. Also it does not take into account the random errors inherited from data. Nevertheless, this method is easy and widely used. At least it can provide initial estimates for some other more complicated iterative methods.

(b) Least-squares method

One of the oldest technique in estimating parameters of a mathematical model is the least-squares method. For the linear compartmental models, we have,
\[
C = \sum_{i=1}^{n} A_i e^{-\lambda_i t}. \tag{2.15}
\]
Assume we have a set of \(m\) data points \(\{(t_j, C_j), j=1, 2, \ldots, m\}\) from one compartment. Suppose there are experimental errors in \(C_j\), but that errors in \(t_j\) are negligible. Then the measure-
ment $C_j$ at time $t_j$ is given by,

$$C_j = \sum_{i=1}^{n} A_i e^{-\lambda_i t_j} + e_j.$$  

In general, $e_j$, the errors, are assumed to have mean zero and variance $\sigma^2$ and are uncorrelated with each other. That is, $E(e_j) = 0$, $V(e_j) = \sigma^2$, $E(e_i e_j) = 0$ for $i \neq j$.

The least-squares method provides estimates of $A_i$'s and $\lambda_i$'s so as to minimize,

$$SS(\lambda, A) = \sum_{j=1}^{m} \frac{1}{\sigma^2_j} (C_j - \sum_{i=1}^{n} A_i e^{-\lambda_i t_j})^2 \quad (2.16)$$

Note that equation (2.15) is not a linear function in $\lambda_i$'s. There are many unusual properties associated with the non-linear least-squares estimation. We mention a few of them. Further details and references can be found in Metzler(1969).

Normal equations of non-linear least-squares are not linear equations and do not provide unique solutions. The difficulty is seen by considering $SS(\lambda, A)$ as a surface in $(2n+1)$ dimensional space of $\lambda_1, \lambda_2, \ldots, \lambda_n, A_1, A_2, \ldots, A_n$ and $SS(\lambda, A)$. In the linear case, the $SS(\lambda, A)$ is a quadratic surface and the minimum is unique. But in the non-linear case, $SS(\lambda, A)$ is not a quadratic surface and may have ridges and valleys, there may be several minima. Sometimes when numerical procedures are used, the convergence may be slow and impossible. In such cases, a good choice of initial estimates of the parameters is very important.

Cornfield et al. (1960) have also discussed the difficulties of non-linear least-squares, especially in case of
the sum of exponential models.

(c) Difference equation method

In biological compartmental systems, usually the experimenter can only observe one compartment at various times. The compartmental equations given by (2.4) are a system of \( n \) linear first order differential equations. In general we can reduce them to an \( n \)th order differential equation with constant coefficients. Thus the differential equation for any \( i \)th compartment can be written as follows,

\[
\frac{d^n C_i}{dt^n} + \sum_{p=q}^n \lambda_p \frac{d^{n-1} C_i}{dt^{n-1}} + \sum_{p<q} \lambda_p \lambda_q \frac{d^{n-2} C_i}{dt^{n-2}} + \ldots \nonumber
\]

\[
+ (\lambda_1 \lambda_2 \ldots \lambda_n) \frac{dC_i}{dt} = 0. \quad (2.17)
\]

The particular solution for the \( n \)th order differential equation is,

\[
C_i = \sum_{j=1}^n A_{ij} e^{\lambda_j t}, \quad i=1,2,\ldots,n. \quad (2.18)
\]

In section 2.4.2, we will show that there are \( (2n-1) \) equations available to solve \( n^2 \) unknowns, for the general compartmental model if the \( i \)th compartment is observed. Hence the estimates are not unique. For the purpose of estimating the \( \lambda_j \)'s most efficiently, the following approach can be used under the assumption of equal time interval.

Equation (2.17) is approximated by the \( n \)th order difference equation. For the notational convenience, we drop the subscript \( i \), the \( n \)th order difference equation is given by,
\[ \Delta^n C(t) + \left( \sum_{i=1}^{k} \lambda_p \right) \Delta^{n-1} C(t) + \left( \sum_{p,q} \lambda_p \lambda_q \right) \Delta^{n-2} C(t) + \ldots + (\lambda_1 \lambda_2 \ldots \lambda_n) C(t) = 0, \]  
\[ (2.19) \]

where \( \Delta C(t) = C(t+1) - C(t) \), and

\[ \frac{d^k C(t)}{dt^k} \] is replaced by \( \Delta^k C(t) \),

\[ \Delta^k C(t) = \Delta^{k-1} C(t+1) - \Delta^{k-1} C(t) \quad k=1,2,\ldots,n \]

\[ \Delta^0 = 1. \]  
\[ (2.20) \]

The statistical model for equation (2.19) is the following,

\[ \Delta^n C(t) + \left( \sum_{i=1}^{k} \lambda_p \right) \Delta^{n-1} C(t) + \left( \sum_{p,q} \lambda_p \lambda_q \right) \Delta^{n-2} C(t) + \ldots + (\lambda_1 \lambda_2 \ldots \lambda_n) C(t) = u(t+n), \]  
\[ (2.21) \]

\( u(t+n) \) is the error component. Because the same compartment is observed at different times, the error components for different times are correlated.

Substituting (2.20) into (2.21), we obtain,

\[ C(t+n) + b_1 C(t-1+n) + b_2 C(t-2+n) + \ldots + b_n C(t) = u(t+n), \]  
\[ (2.22) \]

where \( b_i, i=1,2,\ldots,n \) are linear combinations of \( \left( \sum_{p=1}^{k} \lambda_p \right), \left( \sum_{p,q} \lambda_p \lambda_q \right), \ldots, (\lambda_1 \lambda_2 \ldots \lambda_n) \), given by the following system of equations:

\[
\begin{bmatrix}
1 & 0 & \ldots & 0 & 0 \\
-(n) & 1 & \ldots & 0 & 0 \\
(n) & -(n-1) & \ldots & 0 & 0 \\
\vdots & \ddots & \ddots & \ddots & \ddots \\
(-1)^{n-2}(n) & \ldots & (n-3)(n) & \ldots & 1 & 0 \\
(-1)^{n-1}(n) & \ldots & (n-2)(n) & \ldots & -2 & 1 \\
(-1)^n & \ldots & 1 & -1 & \ldots & 0 \\
\end{bmatrix}
\begin{bmatrix}
1 \\
\Lambda_1 \\
\Lambda_2 \\
\Lambda_n \\
\end{bmatrix}
= \begin{bmatrix}
b_1 \\
b_2 \\
b_{n-2} \\
b_{n-1} \\
b_n \\
\end{bmatrix}
\]  
\[ (2.23) \]
where \( \Lambda_1 = \sum_1^\Lambda p_1 p_2 \ldots p_n \), \( l=1,2,\ldots,n \) \hspace{1cm} (2.24)

the summation is over \( (\binom{n}{1}) \) different combination.

In matrix form, the equation (2.21) can be written as,

\[
\mathbf{M} \Lambda = \mathbf{B}
\]

with

\[
\mathbf{\Lambda} = \begin{bmatrix} 1 & \Lambda_1 & \Lambda_2 & \ldots & \Lambda_n \end{bmatrix}
\]

\[
\mathbf{B} = \begin{bmatrix} 1 & b_1 & b_2 & \ldots & b_n \end{bmatrix}
\]

and

\[
\mathbf{M} = [m_{ij}] (n+1)(n+1)
\]

\[
(m_{ij}) = \begin{cases} 
0 & \text{if } j > i \\
1 & \text{if } j = i \\
(-1)^{i-j}(n+1-j) & \text{if } j < i.
\end{cases}
\] \hspace{1cm} (2.26)

Notice that

\[ 1 + \sum_{i=1}^\Lambda b_i = \Lambda_1 \Lambda_2 \ldots \Lambda_n. \]

Hartley (1948) used the difference equation method to estimate the parameters in one-compartment model. He called the method "internal least squares". Shah (1973) suggested similar concepts to get the preliminary estimates for the two-exponential models. Singh (1975), Rustagi and Singh (1977) generalized the difference equation method to n-compartment models. They discussed some of the statistical properties of the difference equation method.

This method will be utilized in the study of proposed pharmacokinetic models with the help of Michaelis-Menten equation to discussed in Chapter 3.
2.4.2 Problems in estimating parameters of compartmental models

One of the important reasons which cause difficulties in estimation of parameters of compartmental models is the availability of incomplete data sets. It has already been pointed out that data may be available only from one compartment in a system with several compartments. The following theorem illustrates the problem.

Theorem: In a general linear compartmental system with more than one compartment, if a substance is introduced in the first compartment, and only that compartment is observed all the times, then the first-order transfer rates are not uniquely estimable.

Proof: In general, there are \( n^2 \) parameters in the matrix \( K \) that are to be estimated.

Substituting (2.5) into (2.4), we get,

\[
\frac{dC}{dt} = \frac{d}{dt}AY = A \frac{dY}{dt} = A \left( e^{-\lambda_1 t}, e^{-\lambda_2 t}, \ldots, e^{-\lambda_n t} \right)
\]

\[
= AA^\top = KAV,
\]

where

\[
A = \begin{bmatrix} \lambda_1 & 0 & \cdots & 0 \\ 0 & \lambda_2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \lambda_n \end{bmatrix}
\]

So that \( AA^\top = KAV \). It implies that the columns of \( A \) are eigenvectors of \( K \).

If the eigenvalues of \( K \) are not all distinct, we may
have powers of \( t \) appearing in certain columns. However, in compartmental models, we assume implicitly that there is no power of \( t \) appearing in the solutions.

In the case that \( \zeta \) is completely determined, i.e., we have data from every compartment, then \( A \) and \( \Lambda \) are known, \( K \) may be calculated from equation (2.28),

\[
K = AA^{-1}. \tag{2.28}
\]

Since only the first compartment is observed, with initial condition,

\[
\zeta(0) = \Lambda y(0), \tag{2.29}
\]

we have knowledge of \((2n-1)\) parameters, \( n \) eigenvalues \( \lambda_i 's \) and \( a_{1j}, j=1,2,\ldots,n \), only \((n-1)\) are independent because of equation (2.29).

We see that if only one compartment is observed, there are \((2n-1)\) equations for \( n^2 \) unknowns, and hence it is impossible to estimate all the \( k_{ij} 's \) unless \( n=1 \). In that case, \( n^2=2n-1 \).

There is no definite answer for estimating the parameters uniquely in compartmental analysis. It is generally recommended that the experimenter put some side conditions for the incomplete data. One of the relations between \( K \) and the parameters obtained from the observed data is seen by multiplying both sides of equation (2.29) by \( A^{-1} \), we get,

\[
A^{-1} \zeta(0) = y(0). \tag{2.30}
\]
Multiplying both sides of equation (2.28) by \( Y(0) \) and utilizing equation (2.30), we get,

\[
K_{\mathbf{2}}(0) = \mathbf{A}_2 \mathbf{A}_1^{-1} \mathbf{y}(0) = \mathbf{A}_1 Y(0), \quad (2.31)
\]

(2.31) provides a set of \( n \) equations, and if one compartment is observed, one row of \( \mathbf{A} \) is known. Hence only one equation is available to estimate \( k_{ij} \) from the data. In any case, equation (2.31) are the additional constraints, and they cannot apply to all schemes of compartmental models.

The following example is a two-compartment model with elimination from the first compartment. This model is widely used for dealing with pharmacokinetics data. The example demonstrate that the equation (2.31) may be utilized as constraint to give a set of unique estimates.

**EXAMPLE:** The system is given by Figure 2.11,

![Figure 2.11](image)

In this case, we have,

\[
K = \begin{pmatrix}
-(k_{01} + k_{21}) & k_{12} \\
k_{21} & -k_{12}
\end{pmatrix}
\quad (2.32)
\]

The initial condition is,

\[
\mathbf{z}(0) = \begin{bmatrix} C_1(0) \\ 0 \end{bmatrix} = \begin{bmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{bmatrix} \begin{bmatrix} e^{\lambda_1 t} \\ e^{\lambda_2 t} \end{bmatrix} = \begin{bmatrix} A_{11} + A_{12} \\ A_{21} + A_{22} \end{bmatrix}
\]

So that \( C_1(0) = A_{11} + A_{12} \). \quad (2.33)
Suppose first compartment is observed, then we have,
\[ C_1 = A_{11}e^{\lambda_1 t} + A_{12}e^{\lambda_2 t} \quad \text{with } \lambda_2 < \lambda_1 < 0 \] (2.34)
The eigenvalues of equation (2.32) can be written as the following expressions,
\[ -\lambda_1 - \lambda_2 = k_{01} + k_{21} + k_{12} \] (2.35)
\[ \lambda_1 \lambda_2 = k_{01} k_{12} \] (2.36)
and from equation (2.31), we have,
\[ -(k_{01} + k_{21}) C_1(0) = \lambda_1 A_{11} + \lambda_2 A_{12} \] (2.37)
Since \( A_{11} + A_{12} = C_1(0) \), \( -(k_{01} + k_{21}) \) is uniquely determined.
Combined with equations (2.35) and (2.36), we will have unique solutions for \( k_{01}, k_{21}, \) and \( k_{12} \). These results have been used in pharmacokinetics literature extensively in the past. (Cramer et al. 1974)

Shah (1976) used a similar model except that he assumed the possibility of elimination occurring from both compartments. He showed that there are no unique estimates for the \( k_{ij} \)'s if we only have data from first compartment.
CHAPTER 3
Applications of Michealis-Menten Equation to Pharmacokinetics

3.1 Introduction
Two assumptions are usually made in the classical one- and two-compartment models. Firstly, it is assumed that the body tissues can accept an infinite amount of drug, and secondly that the elimination of the drug from body follows the first-order kinetics. However, it has been shown that for many drugs, the above assumptions are not adequate to describe the processes. Levy et al. (1968) pointed out that the break-down of salicylic acid (aspirin) in human adults is according to zero-order kinetics if the drug concentration is above certain level. Similar behavior applies to benzoid acid, one of the food perservatives, for references, see Bray, Thorpe and White (1952). Widmark (1932) revealed the zero-order decay of ethanol (alcohol) concentration in blood. Lundquist and Wolthers (1958) provided a model with Michaelis-Menten kinetics for drug metabolism.

3.2 Michaelis-Menten kinetics
Michaelis-Menten kinetics deal with enzyme-catalyzed reactions. A compound acted upon by an enzyme is called
the substrate. In a paper published in 1913, L. Michaelis and M. Menten hypothesized that the mechanism of enzyme-catalyzed reaction could be described by the following chemical equations.

$$[E] + [C] \xrightarrow{k_1}{k_2} [EC] \xrightarrow{k_3} [P] + [E]$$ (3.1)

where $[E]$ is the enzymes; $[C]$, the substrate; $[EC]$, enzyme-substrate complex; $[P]$, product of reaction; and $k_i$'s are the first-order rate constants associated with each step of the reactions. The equation (3.1) describes the process that substrate goes through a transition state $[EC]$ to form the final product $[P]$. The rate of decrease in $[C]$ equals to the rate of increase in $[P]$, that is,

$$-\frac{d[C]}{dt} = \frac{d[P]}{dt}.$$ (3.2)

Since the publication of Michaelis and Menten, there has been an extensive study of the Michaelis-Menten equation. We follow the development described by Briggs-Haldane (1930). We assume that the amount of enzyme is fixed.

The rate of formation of $[EC]$ from $[E]$ and $[C]$ at any time is given by the follows,

$$\frac{d[EC]}{dt} = k_1([E] - [EC])[C],$$ (3.3)

where $([E] - [EC])$ is the uncombined enzyme. Similarly, the rate of break-down of $[EC]$ is given by,
\[- \frac{d[\text{EC}]}{dt} = k_2[\text{EC}] + k_3[\text{EC}] = (k_2 + k_3)[\text{EC}]. \] (3.4)

When the formation rate equals the break-down rate, a steady-state is reached, \([\text{EC}]\) remains constant. We have,

\[k_1([E] - [\text{EC}])[C] = (k_2 + k_3)[\text{EC}]\]

then

\[
\frac{([E] - [\text{EC}])[C]}{[\text{EC}]} = \frac{k_2 + k_3}{k_1} = K_m
\] (3.5)

The combined constant, \(K_m\), is called Michaelis-Menten constant. Solving \([\text{EC}]\) in equation (3.5), we have,

\[ [\text{EC}] = \frac{[E][C]}{K_m + [C]} \] (3.6)

From equation (3.1) and (3.2), we can see,

\[- \frac{d[C]}{dt} = \frac{d[P]}{dt} = k_3[\text{EC}] = k_3 \frac{[E][C]}{K_m + [C]} \] (3.7)

When the substrate concentration is so high that all the enzymes in the system are present as the \([\text{EC}]\) complex, we reach the maximum reaction rate \(V_m\), for which we can write,

\[ V_m = k_3[E] \] (3.8)

Substituting (3.8) into (3.7), we obtain,

\[- \frac{d[C]}{dt} = \frac{V_m[C]}{K_m + [C]} \] (3.9)

Equation (3.9) is known as the Michaelis-Menten equation. It depends on the constants, \(K_m\) and \(V_m\), and the reaction
shows the phenomenon of saturation of substrate.

Bartholomay (1964) derived the same equation from the deterministic mathematical model. He used the following set of ordinary differential equations from equation (3.1),

$$\frac{d[C]}{dt} = -k_1([E] - [EC])[C] + k_2[EC]$$  \hspace{1cm} (3.10)

$$\frac{d[E]}{dt} = -k_1([E] - [EC])[C] + (k_2+k_3)[EC]$$  \hspace{1cm} (3.11)

$$\frac{d[EC]}{dt} = k_1([E] - [EC])[C] - (k_2+k_3)[EC]$$  \hspace{1cm} (3.12)

$$\frac{d[P]}{dt} = k_3[EC].$$  \hspace{1cm} (3.13)

Using steady-state conditions, that is, either $\frac{d[E]}{dt} = 0$ or $\frac{d[EC]}{dt} = 0$, we get the same result as equation (3.7), followed by equations (3.8) and (3.9). A stochastic approach to the kinetics of enzyme behaviours was also given by Bartholomay and is one of the few papers dealing with the stochastic models for chemical kinetics.

The Michaelis-Menten equation derived above under the condition of steady state is only an approximation to the general process. Because enzyme kinetics plays an important role in biochemistry, several authors made attempts to study the general Michaelis-Menten process, see for references, Wong (1965), Heiveken et al. (1967). Otten (1974) provides a more complete descriptions of the equation
in biochemistry.

From equation (3.1), the rates of change with respect to time for enzyme \([E]\) and substrate \([C]\), we get,

\[
\frac{d[C]}{dt} = -k_1([E] - [EC])[C] + k_2[EC]
\]

(3.14)

\[
\frac{d[E]}{dt} = -k_1([E] - [EC])[C] + (k_2+k_3)[EC].
\]

(3.15)

The steady-state of the process implies \(\frac{d[E]}{dt}=0\), we have,

\[
k_1([E] - [EC])[C] = (k_2+k_3)[EC].
\]

(3.16)

Equation (3.16) is exactly the same as equation (3.5) and will lead to the Michaelis-Menten equation (3.9). However, before the process reaches the steady state, it should go through transient phase. That is, \(\frac{d[E]}{dt}=0\). Otten solved the differential equation (3.15) in great detail under different conditions. The solutions of the differential equations are not the purpose of our study, we will not discuss them here.

3.3 Michaelis-Menten kinetics in pharmacokinetics

In pharmacokinetics, lots of drugs have the protein-binding trait. That is, before the drug is eliminated from the body, its components may react with enzymes to form metabolites, such as vitamin C, riboflavin, 5-fluouricil. The compartmental models no longer deal with first-order
differential equations linear in parameters. There are no general models one can follow, each situation is considered for each drug individually. The analyses of these models are far more complicated than the linear compartmental models, Wagner (1974). We give a few examples here.

Krüger-Thiemer and Levine (1968) are among the first few authors to provide an extensive review of the pharmacokinetic models. They proposed eight different models according to the chemical properties, such as reversibility, saturability and substrate depletion, and metabolism and excretion of the drugs. They also provided very thorough investigation of drug transformation in the body. One of the eight models deals with the Michaelis-Menten process. It can be briefly described by Figure 3.1.

\[
\begin{array}{c}
\text{C}_1 \\
\downarrow p \\
\text{C}_2
\end{array} \xrightarrow{K_m, V_m} \begin{array}{c}
\text{M}_1 \\
\downarrow q \\
\text{M}_2
\end{array}
\]

Figure 3.1

\(\text{C}_1\) is the drug concentration in compartment one, it may either go through Michaelis-Menten process to form a metabolite \(\text{M}_1\), or just go to another compartment \(\text{C}_2\) by a first-order transfer rate \(p\), in the meantime, \(\text{M}_1\) can go to other compartment \(\text{M}_2\) by a different first-order transfer rate \(q\).
The general mathematical expressions for this scheme are given below,

\[
\frac{dC_1}{dt} = -pC_1 - \frac{V_mC_1}{K_m + C_1} \tag{3.17}
\]

\[
\frac{dC_2}{dt} = pC_1 \tag{3.18}
\]

\[
\frac{dM_1}{dt} = \frac{V_mC_1}{K_m + C_1} - qM_1 \tag{3.19}
\]

\[
\frac{dM_2}{dt} = qM_1. \tag{3.20}
\]

Krüger-Thiemer et al. gave the analytical solutions for the differential equations, and simplified this model under different assumptions. They also suggested that these types of models be fitted by analog computer. However, they did not mention a particular method to fit the models except using analog computer to simulate some graphs from the models.

Wagner is one of the pioneers in the study of Michaelis-Menten equation and its applications to pharmacokinetics. Wagner and Petel (1972) used ethanol as the experimental drug. The experiment required that the same subject take five different amounts of ethanol. Concentration data was fitted to the on-compartment model with the following Michaelis-Menten scheme,

\[
\frac{dC}{dt} = -\frac{V_mC}{K_m + C} \tag{3.21}
\]

The authors used differential and integrated forms of Michaelis-
Menten equation to fit the same data. This is the simplest model with Michaelis-Menten process. It shows the importance of the applications of Michaelis-Menten kinetics to the pharmacokinetics. Besides, Wagner and Petel seem to be the first authors using statistical methods to analyze pharmacokinetic data.

3.4 Properties of the compartmental models with Michaelis-Menten kinetics

The properties of Michaelis-Menten equation used in pharmacokinetics and its integrated solutions was studied by Wagner (1973). Using computer simulation technique, he showed that if the time-concentration data follow the one-compartment model with Michaelis-Menten kinetics, the graph should look like a "hockey-stick", as shown in Figure 3.2. That is, in the beginning, the concentration of drug in the compartment drops rapidly, the time-concentration relation in that period is almost linear. Wagner calls it pseudo-linear phase, because the slope is a hybrid parameter, not a rate-changing constant, then after the saturation of the drug, the concentration decreases exponentially. Several simulated data sets were used to find bias and error, if the constants in Michaelis-Menten model are approximated by first-order transfer rates.

Two-compartment model with Michaelis-Menten elimination has been discussed by Sedman and Wagner (1974). The scheme is given by Figure 3.3,
Figure 3.2
Simulated C,t data which obey equation (3.21) parameter values employed are $V_m=0.22$, $K_m=0.1$ and $C_0=0.2, 0.5, 1.0, 1.15, 2.0$ and $3.0$. Portions above the arrows indicate "pseudo-linear" phase.

Figure 3.3
The mathematical model for the rates of change of concentration $C_1$ and $C_2$ is,

$$\frac{dC_1}{dt} = -(k_{21} + \frac{V_m}{K_m C_1})C_1 + k_{12}C_2 \quad (3.22)$$

$$\frac{dC_2}{dt} = k_{21}C_1 - k_{12}C_2 \quad (3.23)$$

where $k_{12}$, $k_{21}$ are first-order transfer rates, $V_m$ is the maximum velocity of the reaction and $K_m$ is the Michaelis-Menten constant.

The difficulties of fitting these differential equations occur on account of the intricacy of all the constants and the dependence on the concentrations of each compartment as we will see in chapter 4. Sedman et al. pointed out the importance of a correct compartmental model, and the method of distinguishing the one-compartment from multi-compartment models by looking at the graphs of time-concentration data.
CHAPTER 4

Statistical Studies of Models With Michaelis-Menten Kinetics

4.1 Introduction

In Chapter 2, we have presented the general concepts of difference equation method. It can be applied to the estimation of the parameters of compartmental models. Difference equation methods were used to get either the exact unique estimates or the preliminary estimates for other methods. Most of these studies are concentrated on the linear differential equations, which are linear in parameters. We will use difference equation method for fitting the one- and two-compartment models with Michaelis-Menten elimination process. In section 4.4, some other commonly used methods will be mentioned for estimating the parameters in the non-linear compartmental models.

4.2 Difference equations for one-compartment model

As pointed out in chapter 3, the differential equation describing the one-compartment model with Michaelis-Menten kinetics has been studied extensively in biochemistry. In pharmacokinetic applications, apparently Wagner (1973) is one of the few to study the properties of this model in detail. We will use difference equation method for this
The scheme for the one-compartment model with Michaelis-Menten elimination process is given by Figure 4.1,

\[
\begin{array}{c}
C \\
\rightarrow \\
\leftarrow
\end{array}
\quad \frac{K_m, V_m}{m}
\]

Figure 4.1

where \( C \) is the concentration of the compartment, \( K_m \) and \( V_m \) have the same meaning as in chapter 3. The mathematical model is given as,

\[
\frac{dC}{dt} = - \frac{V_mC}{K_m + C} \quad (4.1)
\]

Suppose the process is observed at times \( t_1, t_2, \ldots, t_n \). Then using difference equation analog of (4.1) for unequal time intervals, we have,

\[
\frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} = - \frac{V_mC(t_i)}{K_m + C(t_i)} \quad (4.2)
\]

Simplifying equation (4.2), we get,

\[
\frac{K_m}{t_{i+1} - t_i} \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} + V_mC(t_i) + C(t_i) \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} = 0. \quad (4.3)
\]

The statistical model for estimating constants \( K_m \) and \( V_m \) is,

\[
\frac{K_m}{t_{i+1} - t_i} \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} + V_mC(t_i) + C(t_i) \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} = u(t_i) \quad (4.4)
\]
where \( u(t_i) \) are random errors with zero mean and variance \( \sigma_i^2 \). The weighted least-squares solution for \( K_m \) and \( V_m \) are obtained by minimizing the following expression,

\[
\sum_{i=1}^{n-1} \frac{1}{\sigma_i} \left[ K_m \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} + V_m C(t_i) + C(t_i) \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} \right]^2
\]

(4.5)

the \( \frac{1}{\sigma_i} \) is the weight for each data point, that is, we put more weight on the observation with higher variation. By taking the derivatives of equation (4.5) with respect to \( K_m \) and \( V_m \), we get the following normal equations. The normal equations provide the unique least-squares estimates for \( K_m \) and \( V_m \),

\[
\hat{K}_m \sum_{i=1}^{n-1} \frac{1}{\sigma_i} \left( \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} \right)^2 + \hat{V}_m \sum_{i=1}^{n-1} \frac{1}{\sigma_i} \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} \\
+ \sum_{i=1}^{n-1} \frac{C(t_i)}{\sigma_i} \left( \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} \right)^2 = 0 \quad (4.6)
\]

\[
\hat{K}_m \sum_{i=1}^{n-1} \frac{C(t_i)}{\sigma_i} \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} + \hat{V}_m \sum_{i=1}^{n-1} \frac{C(t_i)}{\sigma_i} \\
+ \sum_{i=1}^{n-1} \frac{C(t_i)^2}{\sigma_i} \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} = 0 \quad (4.7)
\]

Table I is the time-concentration data of alcohol

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>5.0</th>
<th>48.0</th>
<th>78.0</th>
<th>105.0</th>
<th>135.0</th>
<th>163.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concent. (mM)</td>
<td>6.9</td>
<td>4.1</td>
<td>2.7</td>
<td>1.4</td>
<td>0.5</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Figure 4.2 Semilogarithmic plot of alcohol elimination from a human subject
elimination in human subject. Figure 4.2 is the semilog plot of the data. The data is fitted to (4.4) by using \( \frac{1}{C(t_i)} \) as the weight, we get \( K_m = 2.8021 \text{ mM} \), and \( V_m = 0.0882 \text{ mM/min} \). The ratio \( \frac{V_m}{K_m} \) is 0.0315 min\(^{-1}\) which is close to the theoretical approximate first-order rate constant for drug elimination 0.0513 min\(^{-1}\).

Note that the distribution of the estimates \( \hat{K}_m \) and \( \hat{V}_m \), depending non-linearly on \( C(t_i) \), are not easily available for given \( n \). In chapter 5, computer simulation methods are used to obtain the properties of the estimates given by equations (4.6) and (4.7).

4.3 Difference equations for two-compartment model

The characteristics of the two-compartment with Michaelis-Menten kinetics in pharmacokinetics have been discussed in detail by Sedman et al. (1974). Suppose a drug whose components follow the Michaelis-Menten elimination process is injected into a biological system intravenously, then the two-compartment open model with elimination occurring from the central compartment is shown schematically in Figure 4.3,

\[
\begin{array}{c}
\text{I.V.} \\
C_1(t) \xleftarrow{k_{21}} \xrightarrow{k_{12}} C_2(t) \\
K_m \downarrow V_m
\end{array}
\]

Figure 4.3
where $C_1(t)$ and $C_2(t)$ are the concentrations in compartments one and two, $k_{12}$ and $k_{21}$ are the first-order rate constants, and $K_m$, $V_m$ have the same meaning as before. The mathematical model for the rates of change of concentrations $C_1(t)$ and $C_2(t)$ is given by the following equations,

$$\frac{dC_1}{dt} = -(k_{21} + \frac{V_m}{K_m + C_1})C_1 + k_{12}C_2, \quad (4.8)$$

$$\frac{dC_2}{dt} = k_{21}C_1 - k_{12}C_2. \quad (4.9)$$

Suppose only one compartment is observed, the difference equation analog can be obtained by eliminating equation (4.9) as follows.

From equation (4.8), we have,

$$k_{12}C_2 = \frac{dC_1}{dt} + k_{21}C_1 + \frac{V_m}{K_m + C_1}C_1. \quad (4.10)$$

Substituting (4.10) into (4.9), we obtain,

$$\frac{dC_2}{dt} = -\frac{dC_1}{dt} - \frac{V_m}{K_m + C_1}. \quad (4.11)$$

Differentiating equation (4.8) once more, and substituting (4.11) for $\frac{dC_2}{dt}$, we have a second-order differential equation in terms of $C_1$ given by the following,

$$\frac{d^2C_1}{dt^2}(K_m + C_1)^2 + [(K_m + C_1)^2(k_{21} - k_{12}) - V_m K_m]\frac{dC_1}{dt} + k_{12}V_m(K_m + C_1)C_1 = 0. \quad (4.12)$$

Replacing $\frac{dC_1}{dt}$ by $C(t+1) - C(t)$
and \( \frac{d^2C}{dt^2} \) by \( C(t+2)-2C(t+1)+C(t) \),

where we assume equal time intervals, the difference equation corresponding to differential equation (4.12) is

\[
(C(t+2)-2C(t+1)+C(t))(K_m+C(t))^2+(C(t+1)-C(t))(K_m+C(t))^2(k_{21} - k_{12})-V_mK_m + C(t)(K_m+C(t))k_{12}V_m = 0. \quad (4.13)
\]

The statistical model for equation (4.13) is given by equation (4.14),

\[
(C(t+2)-2C(t+1)+C(t))(K_m+C(t))^2+(C(t+1)-C(t))(K_m+C(t))^2(k_{21} - k_{12})-V_mK_m + C(t)(K_m+C(t))k_{12}V_m = u(t) \quad (4.14)
\]

where \( u(t) \) is the error term with zero mean and appropriate variance.

It can be seen that the above equation (4.14) is a polynomial with degree three in parameters \( K_m, V_m, k_{12}, \) and \( k_{21} \). The estimation procedure is not an easy one. With certain assumptions, such as those of one or two parameters known, non-linear least-squares method may be used. In any case, the estimates may not be unique.

4.4 Other commonly used methods of estimation

In recent years, the digital electronic computers have been used to analyze the pharmacokinetic data. Equations derived from the models are generally fitted to the experimental blood or urinary excretion time-concentration data. There are several methods and computer programs available, such as NONLIN Program, Metzler (1969), quasilinearization,
Bellman and Kalaba (1965), and method of residuals, Powers (1976). The first two methods have been developed and used not only in the linear compartmental models, but also in the non-linear compartmental models with Michaelis-Menten kinetics by authors in pharmacokinetic and biological areas. We briefly describe the methods in the following sections.

4.4.1 NONLIN Program

This is a computer program developed by Metzler (1969). Non-linear least-squares method is the basic mathematical tool for this computer program. It has been proved very useful in the study of linear pharmacokinetic models. Wagner and Patel (1972) also utilized the program NONLIN in determining parameters of the Michaelis-Menten equation for one-compartment model. In the following, we provide a brief description of this algorithm.

Suppose we have a non-linear function,

\[ f(\theta_1, \theta_2, \ldots, \theta_k, x_1, x_2, \ldots, x_m) = f(\hat{\theta}, x), \quad (4.15) \]

where \( \theta \) is the k-dimensional vector of parameters and \( x \) is the vector of independent variables. It is desired to fit the function to a set of observations. Then we have the following,

\[ y_i = f(\hat{\theta}, x) + e_i, \quad i=1,2,\ldots,n \quad (4.16) \]

where \( y_i \) is the ith observation. It is assumed that the errors \( e_i \) are independent.
The purpose of non-linear least-squares is to choose to minimize $SS(\theta)$ where

$$SS(\theta) = \sum_{i=1}^{n} \left[ y_i - f(\theta, x) \right]^2 w_i. \quad (4.17)$$

$w_i$ is the weight for $i$th observation. It is known that the normal equations obtained from equation (4.17) are not linear in parameters $\theta$. Usually an iterative procedure is called for. The following Gauss-Newton linearization method is used.

Assume there is an initial estimate $\hat{\theta}^1$ of $\theta$ in the neighborhood of the least-squares estimates $\hat{\theta}$. Approximate $f(\theta, x)$ by the Taylor's expansion about the point $\hat{\theta}^1$, using only the first derivative term. This can be written as equation (4.18),

$$f(\theta, x) \approx f(\hat{\theta}^1, x) + \sum_{j=1}^{k} \frac{\partial f(\hat{\theta}^1, x)}{\partial \theta_j} [\theta_j - \hat{\theta}_j^1]. \quad (4.18)$$

In particular, the equation (4.16) may be written as follows,

$$y_i - f(\hat{\theta}^1, x) \approx \sum_{j=1}^{k} \frac{\partial f(\hat{\theta}^1, x)}{\partial \theta_j} [\hat{\theta}_j - \hat{\theta}_j^1] + u_i, \quad (4.19)$$

where $u_i$ are the new error terms. Equation (4.19) is a linear multiple regression equation with proper identification of dependent and independent variables. Let

$$\Delta \theta = \hat{\theta} - \theta^1 \quad (4.20)$$

$$a_{ij} = \sum_{\hat{q}=1}^{n} \frac{\partial f(\hat{\theta}^1, x_\hat{q})}{\partial \theta_j} \frac{\partial f(\hat{\theta}^1, x_\hat{q})}{\partial \theta_i} \quad (4.21)$$

$$b_i = \sum_{\hat{q}=1}^{n} \frac{f(\hat{\theta}^1, x_\hat{q})}{\partial \theta_i} [y_\hat{q} - f(\hat{\theta}^1, x_\hat{q})] w_\hat{q}. \quad (4.22)$$
Then the normal equations for the linear multiple regression equation (4.19) are the following,

$$A\Delta \theta = B$$

(4.23)

with $A = [a_{ij}]$ and $B = [b_j]$.

If $A$ is non-singular, and equation (4.18) is exact, solving for $\Delta \theta = A^{-1}B$, we have the least-squares solution $\hat{\theta} = \theta^1 + \Delta \theta$.

However (4.18) is not exact, $\theta^2 = \theta^1 + \Delta \theta$ is a second approximation to $\hat{\theta}$. This procedure continues until $SS(\hat{\theta}^p)$ is not much less than $SS(\hat{\theta}^{p-1})$, then the procedure has converged to the least-squares solution $\hat{\theta} = \hat{\theta}^p$.

The convergence of non-linear least-squares methods may be slow or may never be obtained. There are several modifications of the Gauss-Newton procedure. The one used in NONLIN program is suggested by Hartley(1961). After the modification, the convergence could be assured and usually hastened.

Most biological systems, or more specifically, the compartmental models, are described by the differential equations, such as,

$$\frac{df(\hat{\theta}, x)}{dx_i} = g_i(\hat{\theta}, x). \quad i=1,2,\ldots,m$$

These equations should be integrated numerically first in order to apply the least-squares method. One convenient feature of NONLIN is that the program has a built-in subroutine to supply the numerical method to integrate the
differential equations.

Boxenbaum et al. (1974) made a review of the statistical significance of the estimates obtained from the non-linear least-square methods. They especially emphasize the statistical aspects of pharmacokinetic analyses. They provide a general methodology, so as to form a preliminary model from the data, to develop a criterion of a good model and the importance of an appropriate analysis for the pharmacokinetic researchers. The NONLIN program was used by them to demonstrate these concepts.

4.4.2 Quasilinearization

The primary objective of the quasilinearization is to supply a uniform approach for the solutions of ordinary and partial differential equations subject to initial and boundary-value conditions. It connects with classical geometry and analysis, and does not have any simple pattern. The quasilinearization method has been developed by Bellman and Kalaba and a lucid exposition is given in their book, (1965). The method has been used in many applications, especially in physics, engineering, economics and biology. In pharmacokinetic context, the application of quasilinearization is given by Bellman, Jacquez, Kalaba and Schwimmer (1967) and Buell and Kalaba (1969).

Quasilinearization provides a method of successive approximation to solutions of differential equations.
Let $x(t)$ be an $N$-dimensional vector and be the solution of the equation,

$$\frac{dx}{dt} = f(x), \quad x_i(0) = \alpha_i, \quad i = 1, 2, \ldots, n \quad (4.24)$$

The first $n$ components of $x_i(0)$ are specified and remaining $N-n$ are free so as to minimize,

$$Q = \sum_{i=1}^{M} \left[ y_i - x(t_i) \right]^2 \quad M > N-n \quad (4.25)$$

$y_i$ is the observation at $t_i$. Integrating equation (4.24) numerically with a selected initial approximation to the missing initial conditions, and call this vector $x^0(t)$. Using Taylor's expansion, we get the first approximation of (4.24), given by,

$$\frac{dx^1}{dt} = f(x^0) + (x^1 - x^0) \frac{\partial f}{\partial x^0} \quad (4.26)$$

A particular solution on the interval $0 < t < t_n$ is $p(t)$, say, with some convenient initial conditions. For example,

$$p_i(0) = \begin{cases} \alpha_i & \text{i = 1, 2, \ldots, n} \\ 0 & \text{otherwise.} \end{cases} \quad (4.27)$$

The general solutions are obtained by producing numerically $N-n$ independent vector solutions of the homogeneous equation,

$$\frac{dv_j}{dt} = \frac{\partial f}{\partial x^0} v_j \quad j = n+1, n+2, \ldots, N \quad (4.28)$$

where $v_j$ is an $N$-dimensional vector. The initial conditions are chosen as the following,
\[ x_j(0) = \delta_{ij} \quad j=n+1,n+2,\ldots,N \]

where
\[ \delta_{ij} = \begin{cases} 1 & \text{if } i=j, \\ 0 & \text{otherwise.} \end{cases} \quad (4.29) \]

Then the solution of equation (4.26) is given by,
\[ \tilde{x}(t) = p(t) + \sum_{j=n}^{N} c_j v_j(t), \quad (4.30) \]
\[ c_{n+1}, c_{n+2}, \ldots, c_N \] are the constants to be determined. These constants are given by the solutions of the equations,
\[ \frac{\partial Q}{\partial c_j} = 0 \quad j=n+1,n+2,\ldots,N \quad (4.31) \]
where \( \tilde{x}=x^1 \) given by (4.30) in \( Q \). New approximation to the initial vector \( x(0) \) is given by,
\[ \tilde{x}(0) = \begin{pmatrix} x_1 \\ \vdots \\ x_{n+1} \\ \vdots \\ x_{n+2} \\ \vdots \\ x_N \end{pmatrix}. \quad (4.32) \]

The next iteration follows similarly to obtain an improved estimate. This method has been shown to be convergent usually after the third iteration, if convergent at all.

The inverse problems in compartmental analysis deal with parameter estimation rather than the solution of differential equations. When the quasilinearization technique is applied, the problem of estimating constants within the
equations is transformed into the one of estimating the initial conditions for the system of differential equations. For examples, see Bellman et al. (1967) and Buell et al. (1969).

4.5 Comments and Comparison

The purpose of this section is not to make judgements about the good and bad features of the above methods, or to set criteria for an ideal method of estimating parameters in pharmacokinetics. We are trying to compare the methods in order to suggest some objective guidelines.

All the methods use approximations in certain degree to the original models. Difference equation method approximates the differential equations by difference equations, while NONLIN program and quasilinearization use Taylor's expansion to approximate the equations. One advantage of the difference equation method is that, in most cases, it does not need initial estimates and iterative procedures. This is particularly clear in the linear compartmental models and even in the one-compartment model with Michaelis-Meten elimination process. A good initial estimate certainly will reduce the number of iterations, and reach the desired estimate faster. However, in cases where no previous data can be referred to, the initial estimates are obtained according to the experience of the experimenter. Besides, most iteration procedure needs large computer storage, it is not
feasible in some situations.

One important statistical aspect of the estimates is the distributions of the estimates. There is still no uniform argument about the distributions of estimates obtained from the non-linear least-squares methods. The users of quasilinearization have not mentioned about the distributions of their estimates. For the linear compartmental models, the difference equation method reduces the models to those of time-series. The distributions of the estimates have been discussed in detail by Anderson (1971). As for the non-linear compartmental models, we will present a simulation method to find the distributions of the estimates.
CHAPTER 5

Simulation Experiments for Statistical Analysis

5.1 Introduction

In most of the pharmacokinetic models, methods of estimation of rate constants or other parameters of interest are all approximate procedures. The properties of such estimates are extremely difficult to obtain except through simulation methods. In this chapter, simulation procedures are presented to obtain certain properties of these estimates. Empirical distribution functions of the rate constants are obtained based on one hundred samples of twenty-five observations generated for each experiment. Standard tests of goodness of fit and Lilliefors non-parametric test show that the distributions of rate constants of the one-compartment model with Michaelis-Menten process obtained by the method of difference equation are normal.

The computer system we use is the IBM 370. The proposed simulation procedure does not require comprehensive understanding of computer knowledge except the basic FORTRAN language. Because the model imposed is in differential equation form, certain numerical integration method should be used to get the one hundred samples. We will describe the whole procedure in detail in the following sections.
5.2 Statistical inferences about $K_m$ and $V_m$

It is of importance and interest to find the confidence intervals for $K_m$ and $V_m$. In order to reach the goal however, first of all, we have to know the distributions of $\hat{K}_m$ and $\hat{V}_m$. In the linear compartmental models, the properties of the estimates obtained from the difference equation approach have been studied in various publications. The distributions of the estimates are so complicated that we only can get the asymptotic distributions, see Anderson (1971). As for the non-linear pharmacokinetic models, solutions of $\hat{K}_m$ and $\hat{V}_m$ from equations (4.6) and (4.7) are the quotients of two polynomials, these make the search for the exact distributions of $\hat{K}_m$ and $\hat{V}_m$ impossible. Therefore, we use computer simulation method to find the distributions.

5.2.1 Generating data sets

In order to generate 100 samples of size 25 with the model \( \frac{dC}{dt} = \frac{V_mC}{K_m + C} \) for given values of $K_m$ and $V_m$, Runge-Kutta method is used to get the time-concentration data. The Runge-Kutta method is widely used in numerical analysis to approximate the solutions for a given differential equation. A brief description of Runge-Kutta method can be found in Appendix.

Equation (5.1), which is usually called Runge-Kutta fourth-order method, is the basic computer algorithm we use to get the samples from the model \( \frac{dC}{dt} = \frac{V_mC}{K_m + C} \).
Predictor-corrector method is also used to improve the value $C(t_{i+1})$.

$$C(t_{i+1}) = C(t_i) + \frac{1}{6}(k_0 + 2k_1 + 2k_2 + k_3),$$

$$k_0 = (\Delta t) f(t_i, C(t_i)), \quad k_1 = (\Delta t) f(t_i + \frac{\Delta t}{2}, C(t_i) + \frac{k_0}{2}), \quad k_2 = (\Delta t) f(t_i + \frac{\Delta t}{2}, C(t_i) + \frac{k_1}{2}), \quad k_3 = (\Delta t) f(t_i + 1, C(t_i) + k_2),$$

(5.1)

where $\Delta t = t_{i+1} - t_i$, $f$ stands for the function of $t$'s and $C$'s.

After obtaining the one hundred samples, random errors from standard normal distribution are added to each observation of the generated time-concentration data. The random numbers are from the generation routine RNOR which is drawn from the random number generator package SUPER-DUPER issued by McGill University School of Computer Science. Initialization routines used with this routine is RSTART, see Dudewicz (1976). The random numbers have normal distribution, with mean zero and variance one.

Since the data for our pharmacokinetic model for a single dose are monotonically decreasing, we, therefore, make the assumption that errors which are proportional to observations are also monotonically decreasing. For this reason, we assign the random error, $e(t_i)$, at time $t_i$ as follows,
where $r(t_i)$ is the random number generated for time $t_i$, and $a$ is the percentage of discrepancy of the experimental value from the real value. In a particular example, if we expect that the observed values are only, say, five percent away from the real values, then $a=0.05$. The random numbers we generate have standard normal distribution. However, one can use other distributions as uniform, gamma or any other probability distributions as long as the random number generator is available in the computer compiler. By observing equation (5.2), one sees that $e(t_i)$ have finite means and variances.

5.2.2 Fitting the data by difference equation method

We use difference equation approach of chapter 2 and fit the generated time-concentration data sets to the original model equation (4.1). Because the random errors we added in equation (5.2) are linear functions of each observation, the ideal weight for using the weighted least squares should be proportional to the observations. We, therefore, choose $\left(\frac{1}{C(t_i)}\right)^2$ as the weight. Under the assumption of unequal time intervals, equation (4.5) is,

\[
\begin{align*}
\text{minimize} \sum_{i=1}^{n-1} \frac{1}{K_m \cdot V_m} \left[ \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} + V_mC(t_i) \right] \left[ \frac{C(t_{i+1}) - C(t_i)}{t_{i+1} - t_i} + C(t_i) \right]^2
\end{align*}
\]
The usual linear least-squares method can, then, be applied to get the estimates \( \hat{K}_m \) and \( \hat{V}_m \). These estimates are unique. We use FORTRAN language to evaluate the 100 \( \hat{V}_m \)'s and \( \hat{K}_m \)'s.

5.2.3 Results

We follow the above procedures and get 100 \( \hat{K}_m \)'s and \( \hat{V}_m \)'s. Their values are given in Table II. \( \hat{K}_m \) is the estimate of Michaelis-Menten constant which is a hybrid parameter as given in equation (3.5). \( \hat{V}_m \) is that of maximum velocity, both values should be positive. Hence those negative values are replaced by zeros. Means and standard deviations for 100 \( \hat{K}_m \)'s and \( \hat{V}_m \)'s are given below,

\[
\begin{align*}
\overline{K}_m &= 0.056 \\
\overline{V}_m &= 0.242 \\
s_K &= 0.0452 \\
s_V &= 0.0495.
\end{align*}
\]

We want to test the hypotheses that these one hundred \( \hat{K}_m \)'s and \( \hat{V}_m \)'s are from normal distributions with unknown means and variances. Classical chi-square goodness of fit test and Lilliefors non-parametric test are used. The frequency distributions of \( \hat{K}_m \) and \( \hat{V}_m \) are given in Table III and IV. They seem to be close to the normal distributions. The chi-square values for the samples \( \hat{K}_m \) and \( \hat{V}_m \) are,

\[
\chi^2_k = 6.454 \text{ with } 5 \text{ degrees of freedom},
\]
\[ \chi^2 = 2.516 \] with 4 degrees of freedom, which are not significant at the level of 0.05.

It is known that the chi-square goodness of fit test depends on the number of classes and the boundaries of each class one chooses. For this reason, we also use Lilliefors non-parametric test, the test statistic is,

\[ T_2 = \text{Sup}_x |F(x) - S(x)|, \quad (5.4) \]

where \( F(x) \) is the standard normal distribution function. \( S(x) \) is the empirical distribution function obtained from the normalized sample. For the sample of \( K_m \), the \( x \) is defined as,

\[ x = \frac{k_m - 0.056}{0.0452}, \quad (5.5) \]

and for the sample of \( V_m \), we have,

\[ x = \frac{v_m - 0.242}{0.0495}. \quad (5.6) \]

We compute the 100 absolute values of \( (F(x) - S(x)) \) for both samples, as recorded in the columns 3 and 6 of Table II. The maximum value for \( K_m \) occurs at \( x=0.23 \), where \( S(x) \) equals 0.53, \( F(x) \) equals 0.59, and \( T_2 \) is 0.06. The maximum value for \( V_m \) occurs at \( x=-0.56 \), where \( S(x) \) equals 0.35, \( F(x) \) is 0.29, and \( T_2 \) equals 0.06. The maximum value 0.06 also occurs at other points, but at no point does the absolute difference of \( S(x) \) and \( F(x) \) exceed 0.06.

The Lilliefors test calls for rejection of our hypotheses at \( \alpha=0.20 \) if \( T_2 \) exceeds its 0.80 quantile, which is
given as,

\[ w_{.80} = \frac{0.736}{\sqrt{n}} = \frac{0.736}{\sqrt{100}} = 0.0736. \]

The critical region is obtained from the book by Conover (1971). Because \( T_2 \) for both samples equals 0.06, and is less than 0.0736, the hypotheses are accepted. From the Lilliefors test, even at the level of 0.20, we cannot reject the hypotheses. It is reasonable to conclude that in our case, the samples of \( \hat{k}_m \) and \( \hat{V}_m \) are from normal distributions.
<table>
<thead>
<tr>
<th>KM</th>
<th>(KM-0.056)/0.045</th>
<th>S(X)-F(X)</th>
<th>VM</th>
<th>(VM-0.242)/0.0495</th>
<th>S(X)-F(X)</th>
</tr>
</thead>
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<tr>
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<td>-1.24</td>
<td>.02</td>
<td>0.0415</td>
<td>-4.05</td>
<td>.01</td>
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### Table 5.2

Frequency Distribution of $\hat{K}_m$

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Table 5.3
Frequency Distribution of $\hat{V}_m$

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Statistical methods have been used for estimating Michaelis-Menten constants $K_m$ and $V_m$, not only in pharmacokinetic models, but also in biochemical data. Recent references include Sattelmeyer and Lerner (1975), Sakoda and Hiromi (1976), Newman, Atkins and Nimmo (1974) and Porter and Trager (1977). Atkins and Nimmo (1975) have given a comparison of seven methods of fitting the Michaelis-Menten equation. In most of this literature, the equation,

$$v = \frac{V_m[S]}{K_m + [S]}$$

(6.1)

where $[S]$ is the concentration of substrate, $v$ is the velocity of the chemical reaction, is fitted when observations on $v$ and $[S]$ are given. Various transformations are used to utilize results of linear regression theory and to provide outlier resistant procedure, even non-parametric procedures have been proposed. The pharmacokinetic studies do not lead to measurements of $v$. Here we have actually the times of taking concentration data. Therefore their methods do not have straightforward applications to pharmacokinetic models. However, all these show the necessity and importance of statistical analysis in chemical and pharmacological studies.
Equations derived from the pharmacokinetic models are generally in differential equation forms. Mathematically speaking, these are equations for continuous observations. But the experimental data can only be taken at discrete points. In this sense, difference equations are sometimes more appropriate to describe the models. In many cases, especially those of linear compartmental models, the integrated solutions are usually used instead of the original differential equations. There are constants introduced in the solutions of the differential equations by the initial conditions. They do not appear in the original models. Hence if the integrated solution forms are used, more constants need to be estimated than if the original differential equations are used. The properties of the random errors inherited from the observations in the differential equation models are different from those in the integrated solution forms. Besides, the models become more complicated, and for the non-linear compartmental systems, the integrated solutions are hard to obtain. Many times only approximate forms are used. For these reasons, it is important to fit the original differential equation models. The difference equation method proposed in this dissertation will reduce the drawbacks resulting in fitting the integrated solutions. We do not imply that difference equation method is perfect and that it will give more accurate estimates. We emphasize that our method is simpler and, in some cases, more feasible.
The computer simulation procedure presented in chapter 5 can be applied to situations where the distributions of estimates are impossible to obtain. The statistical analysis of the pharmacokinetic models will be greatly facilitated by knowing the distributions of the estimates. Such results are needed for finding confidence interval for the parameters or for testing hypotheses about the parameters. They may help in finding the minimum number of compartments needed to fit the data. Through the simulation techniques, more appropriate distributions of the estimates can be used instead of the asymptotic or approximate ones. The model we have used is a simple one, but the technique can be practically applied for any models.

More work is still needed for the pharmacokinetic models involving Michaelis-Menten kinetics. As a matter of fact, there is still much to be done in developing the theory of non-linear compartmental models. More systems are found to be non-linear than linear. Deviation from linearity may be due to the absorption process, drug distribution, tissue binding of the drug, metabolic and excretory processes. Non-linear compartmental models involving Michaelis-Menten process are few of the models used in pharmacological studies. One-compartment model with Michaelis-Menten process has been examined in this dissertation. As for the two-compartment model, because of the complexity of the model itself, the problem of finding estimates of the parameters still needs to be solved.
One interesting aspect about the Michaelis-Menten equation is the mathematical solution of the equation (6.2),

\[ \frac{dC}{dt} = -\frac{V_mC}{K_m + C}. \]  

Solving equation (6.2), we get the following equation by integration,

\[ K_m(ln C) + C = -V_m t + A, \]  

where A is an arbitrary constant. With the initial condition \( C(0) = C_0 \) at time \( t=0 \), one may write equation (6.3) as,

\[ C_0 - C + K_m \ln \frac{C_0}{C} - V_m t = 0 \]  

(6.4)

The statistical model for equation (6.4) can be postulated as follows,

\[ (C_0 - C(t)) + K_m \ln \frac{C_0}{C(t)} - V_m t = u(t) \]  

(6.5)

where \( u(t) \) are the random errors with certain assumptions.

Note that if \( C_0 \) is a given quantity, equation (6.5) is linear in parameters \( K_m \) and \( V_m \). Ordinary least-squares method can be applied directly to equation (6.5) for estimating \( K_m \) and \( V_m \). The distributions of estimates \( \hat{K}_m \) and \( \hat{V}_m \), however, do not seem to be easily obtainable.

Further understanding about the pharmacokinetic models with multiple doses and oral intakes of drugs is still needed. The development of experimental designs and the determination of optimal sample size for non-linear regression designs are important areas of further investigations. A stochastic
model related to the Michaelis-Menten equation would be of great value to the experimenters.
APPENDIX

Runge-Kutta Method

The method is used to get the numerical solutions for given differential equations. It can be described as follows.

Suppose the initial-value problem of the first-order differential equation (A.1),

\[
\frac{dy}{dx} = y' = f(x,y) \quad \text{for } x \in (a,b)
\]

with \( y(a) = \alpha \)

If the interval \((a, b)\) is divided into \(n\) equal parts, each of length \(\Delta x\), and set \(a=x_0<x_1<x_2<...<x_n=b\). Next approximate \(y'\) at each point by the forward-difference approximation,

\[
y' \approx \frac{y_{i+1}-y_i}{\Delta x} = f(x_i, y_i) \quad \text{(A.2)}
\]

We consider the more general formula of equation (A.2) as follows,

\[
\frac{y_{i+1}-y_i}{\Delta x} = \beta_0 f(x_i, y_i) + \beta_1 f(x_i + \Delta x, y_i + \int \Delta x) \quad \text{(A.3)}
\]

where \(\beta_1, \beta_0\), and are parameters to be determined.

Equation (A.3) can also be written as,

\[
y_{i+1} = y_i + \beta_0 (\Delta x) f(x_i, y_i) + \beta_1 (\Delta x) f(x_i + \Delta x, y_i + \int \Delta x)
\]

The Taylor's expansion of \(f\) is given below,
\begin{align*}
    f(x_i+y\Delta x, y_i+\Delta y) &= f(x_i, y_i) + \gamma(\Delta x) \frac{\partial f(x_i, y_i)}{\partial x} + \delta(\Delta x) \frac{\partial f(x_i, y_i)}{\partial x} \\
    &+ \frac{1}{2} \left[ \gamma^2(\Delta x)^2 \frac{\partial^2 f(x_i, y_i)}{\partial x \partial x} + 2\gamma \delta(\Delta x)^2 \frac{\partial^2 f(x_i, y_i)}{\partial x \partial y} \\
    &+ \delta^2(\Delta x)^2 \frac{\partial^2 f(x_i, y_i)}{\partial y \partial y} \right] + O((\Delta x)^3) \quad (A.4)
\end{align*}

Substituting equation (A.4) into (A.3) and recombining terms,

\begin{align*}
    y_{i+1} &= y_i + \Delta x \left[ (\beta_0 + \beta_1 f(x_i, y_i) \right] + \frac{(\Delta x)^2}{2} \left[ 2\beta_1 \gamma \frac{\partial f(x_i, y_i)}{\partial x} \\
    &+ 2\beta_1 \delta \frac{\partial f(x_i, y_i)}{\partial y} \right] + \frac{(\Delta x)^3}{6} \left[ 3\beta_1 \gamma^2 \frac{\partial^2 f(x_i, y_i)}{\partial x \partial x} + 6\beta_1 \gamma \delta \frac{\partial^2 f(x_i, y_i)}{\partial x \partial y} \\
    &+ 3\beta_1 \delta^2 \frac{\partial^2 f(x_i, y_i)}{\partial y \partial y} \right] + \beta_0 O((\Delta x)^4) \quad (A.5)
\end{align*}

Suppose also that the exact solution of equation (A.1) can be written as Taylor's series,

\begin{align*}
    y_{i+1} &= y_i + (\Delta x) y_i' + \frac{(\Delta x)^2}{2} y_i'' + \frac{(\Delta x)^3}{6} y_i''' + \ldots \quad (A.6)
\end{align*}

Because \( y' = f(x, y) \), we have,

\begin{align*}
    y'' &= \frac{\partial f}{\partial x} + (\frac{\partial f}{\partial y}) y' \quad (A.7)
\end{align*}

\( y''' = \frac{\partial^2 f}{\partial x \partial x} + 2f \frac{\partial^2 f}{\partial x \partial y} + f^2 \frac{\partial^2 f}{\partial y \partial y} + (\frac{\partial f}{\partial x}) (\frac{\partial f}{\partial y}) + f(\frac{\partial^2 f}{\partial y})^2 \quad (A.8)

Substituting (A.7) and (A.8) into (A.6), yields,
There are different ways that we can choose the parameters \( \beta_0, \beta_1, \gamma, \delta \) in (A.5) to approximate (A.9). For example, we may construct formulas for which (A.5) agrees with (A.9) through at least the \((\Delta x)^2\) terms, wetting the corresponding coefficients, we have,

\[
\beta_0 + \beta_1 = 1
\]
\[
2\beta_1 \gamma = 1 \quad (A.10)
\]
\[
2\beta_1 \delta = f(x_i, y_i)
\]

The system has infinite solutions, the most convenient one of which is,

\[
\beta_0 = \beta_1 = \frac{1}{2}, \quad \gamma = 1, \quad \delta = f(x_i, y_i) \quad (A.11)
\]

Substituting these parameters in (A.5), we get,

\[
y_{i+1} = y_i + \frac{\Delta x}{2} f(x_i, y_i) + \frac{\Delta x}{2} (f(x_{i+1}, y_i) + (\Delta x) f(x_i, y_i))
\]

or

\[
y_{i+1} = y_i = \frac{1}{2} (k_0 + k_1) \quad (A.12)
\]

where

\[
k_0 = (\Delta x) f(x_i, y_i)
\]
\[
k_1 = (\Delta x) f(x_{i+1}, y_{i+k_0})
\]
If we approximate $(A.9)$ through $(\Delta x)^4$, it was shown by Kutta in 1901 that,

$$y_{i+1} = y_i + \frac{1}{6}(k_0 + 2k_1 + 2k_2 + k_3) \quad (A.12)$$

where

$$k_0 = (\Delta x)f(x_i, y_i)$$

$$k_1 = (\Delta x)f(x_i + \frac{\Delta x}{2}, y_i + \frac{k_0}{2})$$

$$k_2 = (\Delta x)f(x_i + \frac{\Delta x}{2}, y_i + \frac{k_1}{2})$$

$$k_3 = (\Delta x)f(x_{i+1}, y_{i+}k_2)$$

This is the formula we use to generate our data sets in chapter 5.
LIST OF REFERENCES


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