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ESTIMATION OF RATE CONSTANTS IN THE MICHAELIS-MENTEN MODEL

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Nonlinear models leading to Michaelis - Menten scheme result from the study compartmental systems as well as in other contexts. Using difference equation approach, rate constants are estimated in some simple models. Simulation methods are used to derive the distributions of these estimates. It is shown that the assumptions of normality of errors leads to the normal distribution of the estimated rate constants.

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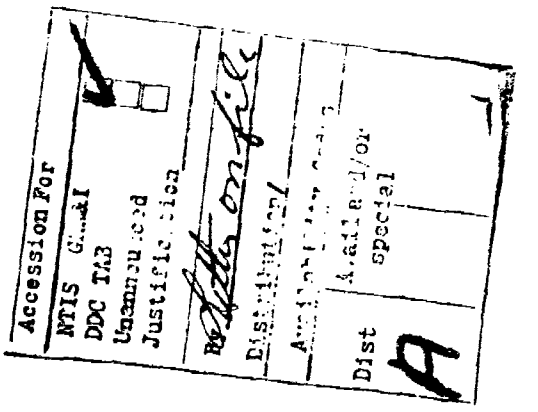

1. INTRODUCTION

Non-linear models arise in many practical applications. Models based on Michaelis-Menten differential equations are commonly used in biochemistry, biopharmaceutics, pharmacokinetics and other related disciplines. These differential equations result in general when compartmental models are applied. Linear differential equations usually suffice for the study of compartmental models with constant rates of exchange among compartments. However, there are many situations especially in the study of drug distribution where nonlinearity arises naturally. Several methods have been proposed in the literature to fit constants in such nonlinear models. In an earlier paper, Rustagi and Singh (1977) used difference equation approach to fit one- and two-compartment models under the assumptions of linear kinetics.

In this paper, the Michaelis-Menten elimination scheme is studied using the difference equation approach. The distribution of the estimates of the resulting rate constants is not easily obtainable in closed form. Using Monte-Carlo methods, the distribution of these constants is derived for the special case of normal errors. Similar methods can be utilized under different distributional assumptions for errors in the model.

Applications of Michaelis-Menten equations to pharmacokinetics has recently been made by Wagner (1973) and Sedman and Wagner (1974).

They obtained estimates of the rate constants by numerical methods but did not provide the distribution of these estimates. It is well known that it is not easy to obtain the distribution of estimates which are obtained through numerical techniques. These distributions are, however, necessary for statistical analysis, for example, in obtaining the confidence interval estimates for the rate constants or in comparing two different rate constants.

2. MICHAELIS-MENTEN MODELS

In a study dealing with enzyme kinetics, Michaelis and Menten (1913) provided a model for the following one compartment system, given by Figure 2.1.

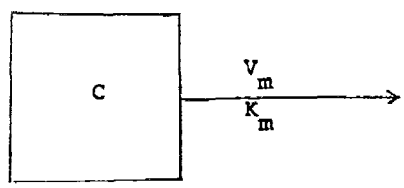

Figure 2.1

C is the concentration of certain substance in the compartment, K_m is the rate of output and V_m is the maximum rate of reaction. This system can be represented by the differential equation

$$\frac{dC}{dt} = -\frac{V_m C}{K_m + C} . \qquad (2.1)$$

Consider the difference equation analog of the equation (2.1) assuming that the process is observed at times $t_1, t_2, \ldots, t_n$. Using the

approximation for derivatives in the case of unequal time intervals, we have

$$\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = -\frac{V_m C(t_i)}{K_m + C(t_i)} . \tag{2.2}$$

Equation (2.2) is simplified in the following form.

$$K_m \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} + V_m C(t_i) + C(t_i)\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = 0 . \tag{2.3}$$

The model for estimating constants K_m and V_m is assumed to be

$$K_m \frac{C(t_{i+1})-C(t_i)}{t_{u+1}-t_i} + V_m C(t_i) + C(t_i)\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = u(t_i) . \tag{2.4}$$

We assume for simplicity that $u(t_i)$ are random errors with zero means and variances σ_i^2.

The weighted least-squares solution for K_m and V_m are obtained by minimizing the expression,

$$S(V_m,K_m) = \sum_{i=1}^{n-1} \frac{1}{\sigma_i}\left[K_m \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} + V_m C(t_i) + C(t_i)\frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i}\right]^2 , \tag{2.5}$$

where $\frac{1}{\sigma_i}$ is the weight for each data point. By taking the derivatives of equation (2.5) with respect to K_m and V_m, we get the normal equations given in (2.6) and (2.7) which provide the least-squares estimates for K_m and V_m.

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

$$+ \sum_{i=1}^{n-1} \frac{C(t_i)}{\sigma_i} \left(\frac{C(t_{i+1})-Ct_i)}{t_{i+1}-t_i} \right)^2 = 0 \qquad (2.6)$$

$$\hat{K}_m \sum_{i=1}^{n-1} \frac{C(t_i)}{\sigma_i} \cdot \frac{C(t_{i+1})-Ct_i)}{t_{i+1}-t_i} + \hat{V}_m \sum_{i=1}^{n-1} \frac{(C(t_i))^2}{\sigma_i}$$

$$+ \sum_{i=1}^{n-1} \frac{(C(t_i))^2}{\sigma_i} \cdot \frac{C(t_{i+1})-C(t_i)}{t_{i+1}-t_i} = 0 \qquad (2.7)$$

Example

Suppose the time-concentration data of alcohol elimination in human subject is given in Table 2.1. The data is fitted to (2.4) by using $\frac{1}{(C(t_i))^2}$ as the weights, we get K_m = 2.8021 mM, and V_m = 0.0882 mM/min. The ratio V_m/K_m is 0.0315 min^{-1}. This ratio is close to the theoretical approximate first-order rate constant for drug elimination which is known to be 0.0513 min^{-1}, Wagner (1971).

Table 2.1

Time (min)	5.0	48.0	78.0	105.0	135.0	163.0
Concen. (mM)	6.9	4.1	2.7	1.4	0.5	0.15

Note that the distribution of the estimates $\hat{K}_m$ and $\hat{V}_m$, which depend non-linearly on $C(t_i)$, are not easily obtainable for given n. Simulation methods are used later to obtain the properties of these estimates.

The characteristics of the two-compartment model with Michaelis-Menten kinetics have been studied by several authors, for example see Sedman and Wagner (1974). Suppose a drug is injected into a biological system intravenously. This system can be represented by a two-compartment open model where elimination occurs from the first compartment. A schematic diagram is given in Figure 2.2 using nonlinear kinetics.

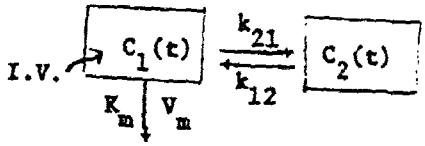

Figure 2.2

Here $C_1(t)$ and $C_2(t)$ are the concentrations in the two compartments, 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 concentrations results in the equations (2.8) and (2.9).

$$\frac{dC_1}{dt} = -(k_{21} + \frac{V_m}{K_m + C_1})C_1 + k_{12}C_2 \ , \tag{2.8}$$

$$\frac{dC_2}{dt} = k_{21}C_1 - k_{12}C_2 \ . \tag{2.9}$$

Suppose an experimenter can observe the concentration in only one compartment. From (2.8) we have

$$k_{12}C_2 = \frac{dC_1}{dt} + k_{21}C_1 + \frac{V_m}{K_m + C_1}C_1 \ . \tag{2.10}$$

Substituting (2.10) into (2.9), we get

$$\frac{dC_2}{dt} = -\frac{dC_1}{dt} - \frac{V_m}{K_m + C_1} . \tag{2.11}$$

Differentiating equation (2.8) once more, we have a second-order differential equation in terms of C_1,

$$\frac{d^2C_1}{dt^2}(K_m+C_1)^2 + [(K_m+C_1)^2(k_{21}-k_{12}) - V_mK_m]\frac{dC_1}{dt} + k_{12}V_m(K_m+C_1)C_1 = 0 . \tag{2.12}$$

Suppose now that the concentration in a given compartment is observed at equal intervals of time. For notational convenience we drop the subscript in C_1 and replace the derivative $\frac{dC}{dt}$ by $C(t+1) - C(t)$ and $\frac{d^2C}{dt^2}$ by $C(t+2) - 2(t+1) + C(t)$ to obtain the difference equation analog to equation (2.12). We have

$$[C(t+2) - 2C(t+1) + C(t)][K_m + C(t)]^2 + [C(t+1) - C(t)]\{[K_m + C(t)]^2 (k_{21}-k_{12}) - V_mK_m\} + C(t)[K_m + C(t)]k_{12}V_m = 0 . \tag{2.13}$$

The model for estimating the rate constants is

$$[C(t+2) - 2C(t+1) + C(t)][K_m + C(t)]^2 + [C(t+1) - C(t)]\{[K_m + C(t)]^2 (k_{21}-k_{12}) - V_mK_m\} + C(t)[K_m + C(t)]k_{12}V_m = u(t) \tag{2.14}$$

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

Notice that the equation (2.14) is a polynomial with degree three in parameters K_m, V_m, k_{12}, and k_{21} and results in a complicated

estimation procedure. With certain additional assumptions, such as those of some parameters known, nonlinear least-squares method may be utilized. The uniqueness of the resulting estimates is still questionable.

3. ESTIMATION OF RATE CONSTANTS

Several methods of estimation of rate constants in pharmacokinetics models are in common use. Numerical procedures leading to techniques using quasilinearization are due to Bellman and Kalaba (1965). Also computer programs such as NONLIN, by Metzler (1969) which is based on the modification of Gauss-Newton procedure are commonly used. We use the difference-equation approach discussed earlier for obtaining the estimates.

Using Runge-Kutta method, which is a part of NONLIN program, data are generated from the Michaelis-Menten model (2.1) with a given set of values of constants K_m and V_m. A sample of 25 observations is generated and each value is increased by a standard normal random variate. The estimates of V_m and K_m are then made using the difference equations model of the Michaelis-Menten equations as in equation (2.4). The resulting estimates are given in Table I for one hundred such samples.

The frequency distributions of $\hat{K}_m$ and $\hat{V}_m$ are given in Tables II and III. Means and standard deviations for $\hat{K}_m$ and $\hat{V}_m$ are

$$\bar{K}_m = 0.056 \qquad \bar{V}_m = 0.242$$

$$s_k = 0.0452 \qquad s_v = 0.0495$$

To test the hypotheses that the sample of one hundred $\hat{K}_m$ and $\hat{V}_m$ arise from normal distributions, classical chi-squared test of goodness-of-fit and Lilliefors test are used, Conover (1971). The chi-squared values for the samples $\hat{K}_m$ and $\hat{V}_m$ are

$$\chi^2_{K_m} = 6.454 \text{ with 5 degrees of freedom,}$$

$$\chi^2_{V_m} = 2.516 \text{ with 4 degrees of freedom,}$$

which are not significant at 0.05 level.

For Lilliefors non-parametric test, the test statistic used is

$$T = \sup_x |F(x) - S(x)| ,$$

where F(x) is the standard normal distribution function and S(x) is the empirical distribution function. The values have been standardized using the means and standard deviations obtained above. Absolute values of (F(x)-S(x)) for both samples are recorded in Table I. The maximum value for $\hat{K}_m$ occurs at x = 0.23, where S(x) equals 0.53, F(x) equals 0.59, and T is 0.06. The maximum value for $\hat{V}_m$ occurs at x = -0.56, where S(x) equals 0.35, F(x) is 0.29 so that T 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 exceeds its 0.80 quantile. The critical region is obtained from tables in Conover (1971). The hypotheses are accepted, and therefore we conclude that the estimates $\hat{K}_m$ and $\hat{V}_m$ are normally distributed.

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TABLE I

K_m	$(K_m-.056)/.045$	S(x)-F(x)	V_m	$(V_m-.242)/.0495$	S(x)-F(x)
0.0	-1.24	.02	0.0415	-4.05	.01
0.0	-1.24	.02	0.1407	-2.05	.002
0.0	-1.24	.02	0.1508	-1.84	.002
0.0	-1.24	.02	0.1567	-1.72	.002
0.0	-1.24	.02	0.1611	-1.63	.001
0.0	-1.24	.02	0.1665	-1.53	.003
0.0	-1.24	.02	0.1797	-1.26	.03
0.0	-1.24	.02	0.1829	-1.19	.04
0.0005	-1.23	.01	0.1864	-1.12	.04
0.0027	-1.18	.01	0.1875	-1.10	.04
0.0045	-1.14	.01	0.1882	-1.09	.03
0.0055	-1.12	.01	0.1883	-1.08	.02
0.0058	-1.11	.003	0.1892	-1.07	.01
0.0078	-1.07	.002	0.1895	-1.06	.004
0.0100	-1.02	.01	0.1915	-1.02	.003
0.010	-1.02	.01	0.1938	-0.97	.006
0.0114	-0.99	.01	0.1945	-0.96	.002
0.0123	-0.97	.02	0.2020	-0.81	.03
0.0204	-0.79	.02	0.2037	-0.77	.02
0.0206	-0.78	.01	0.2039	-0.77	.02
0.0221	-0.75	.006	0.2044	-0.76	.01
0.0221	-0.75	.006	0.2056	-0.74	.009
0.0225	-0.74	.01	0.2061	-0.73	.003
0.0243	-0.70	.002	0.2072	-0.70	.002
0.0262	-0.66	.004	0.2095	-0.66	.005
0.0295	-0.59	.01	0.2101	-0.64	.01
0.0299	-0.58	.01	0.2102	-0.64	.01
0.0305	-0.56	.01	0.2107	-0.63	.03
0.0309	-0.56	.01	0.2108	-0.63	.03
0.0318	-0.54	.01	0.2130	-0.59	.04
0.0325	-0.52	.01	0.2130	-0.59	.04
0.0332	-0.50	.02	0.2130	-0.59	.04
0.0344	-0.48	.02	0.2131	-0.58	.06
0.0357	-0.45	.02	0.2135	-0.58	.06
0.0362	-0.44	.02	0.2144	-0.56	.06
0.0377	-0.40	.03	0.2162	-0.52	.05
0.0379	-0.40	.03	0.2205	-0.43	.04
0.0394	-0.37	.03	0.2210	-0.42	.04
0.0426	-0.30	.01	0.2217	-0.41	.05
0.0429	-0.29	.03	0.2232	-0.38	.05
0.0429	-0.29	.03	0.2264	-0.32	.04
0.0439	-0.27	.03	0.2329	-0.18	.01
0.0449	-0.25	.03	0.2330	-0.18	.01
0.0497	-0.14	.004	0.2330	-0.18	.01
0.0499	-0.13	.03	0.2350	-0.14	.006
0.0501	-0.13	.03	0.2354	-0.13	.01
0.0503	-0.13	.03	0.2360	-0.12	.040
0.0531	-0.06	.01	0.2360	-0.12	.04

TABLE I (continued)

K_m	$(K_m-.056)/.045$	$S(x)-F(x)$	V_m	$(V_m-.242)/.0495$	$S(x)-F(x)$
0.0558	-0.00	.01	0.2360	-0.12	.04
0.0567	0.02	.008	0.2375	-0.09	.04
0.0583	0.05	.009	0.2378	-0.08	.04
0.0610	0.11	.02	0.2415	-0.01	.02
0.0662	0.23	.06	0.2435	0.03	.02
0.0667	0.24	.02	0.2440	0.04	.03
0.0668	0.24	.02	0.2445	0.05	.03
0.0668	0.24	.02	0.2450	0.06	.04
0.0670	0.24	.02	0.2487	0.14	.02
0.0674	0.25	.02	0.2539	0.24	.01
0.0678	0.26	.01	0.2573	0.31	.03
0.0689	0.29	.01	0.2585	0.33	.03
0.0699	0.31	.01	0.2595	0.35	.03
0.0740	0.40	.03	0.2601	0.37	.02
0.0758	0.44	.04	0.2625	0.41	.03
0.0764	0.45	.03	0.2673	0.51	.05
0.0769	0.46	.02	0.2675	0.52	.05
0.0805	0.54	.03	0.2707	0.58	.06
0.0806	0.54	.03	0.2710	0.59	.05
0.0824	0.58	.03	0.2741	0.65	.06
0.0827	0.59	.03	0.2747	0.66	.05
0.0841	0.62	.03	0.2759	0.68	.050
0.0844	0.63	.02	0.2761	0.69	.05
0.0853	0.65	.02	0.2774	0.72	.04
0.0880	0.71	.03	0.2784	0.74	.03
0.0885	0.72	.02	0.2788	0.74	.03
0.0895	0.74	.02	0.2809	0.79	.02
0.0906	0.77	.01	0.2810	0.79	.02
0.0906	0.77	.01	0.2812	0.79	.02
0.0909	0.77	.01	0.2818	0.80	.008
0.0925	0.81	.001	0.2824	0.82	.004
0.0946	0.85	.002	0.2852	0.87	.008
0.0952	0.87	.01	0.2873	0.92	.01
0.0984	0.94	.006	0.2884	0.94	.006
0.0994	0.96	.001	0.2892	0.95	.01
0.0998	0.97	.01	0.2892	0.95	.01
0.1001	0.98	.02	0.2914	1.00	.009
0.1028	1.04	.01	0.2923	1.02	.01
0.1043	1.07	.02	0.2974	1.12	.003
0.1090	1.17	.01	0.3006	1.18	.001
0.1137	1.28	.01	0.3032	1.24	.003
0.1138	1.28	.01	0.3052	1.28	.001
0.1143	1.29	.01	0.3057	1.29	.02
0.1169	1.35	.01	0.3058	1.29	.01
0.1178	1.37	.02	0.3094	1.36	.02

TABLE I (continued)

K_m	$(K_m - .056)/.045$	S(x)-F(x)	V_m	$(V_m - .242)/.0495$	S(x)-F(x)
0.1201	1.42	.02	0.3114	1.40	.02
0.1206	1.43	.03	0.3161	1.50	.02
0.1222	1.46	.04	0.3233	1.64	.01
0.1232	1.49	.04	0.3248	1.67	.02
0.1303	1.64	.04	0.3250	1.68	.03
0.1311	1.66	.04	0.3283	1.74	.04
0.1322	1.69	.05	0.3333	1.84	.04

TABLE II

FREQUENCY DISTRIBUTION OF $\hat{K}_m$

$\hat{K}_m$	Frequencies	Relative Frequencies	Cumulative Relative Frequencies
0	8	0.08	0.08
0.00-0.02	10	0.10	0.18
0.02-0.04	20	0.20	0.38
0.04-0.06	13	0.13	0.51
0.06-0.08	14	0.14	0.65
0.08-0.10	19	0.19	0.84
0.10-0.12	9	0.09	0.93
0.12-0.14	7	0.07	1.0
Total	100	1.0	

TABLE III

FREQUENCY DISTRIBUTION OF $\hat{V}_m$

$\hat{K}_m$	Frequencies	Relative Frequencies	Cumulative Relative Frequencies
0.00–0.17	6	0.06	0.06
0.17–0.20	11	0.11	0.17
0.20–0.23	24	0.24	0.41
0.23–0.26	20	0.20	0.61
0.26–0.29	23	0.23	0.84
0.29–0.32	11	0.11	0.95
0.32–0.35	5	0.05	1.0
Total	100	1.0	

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