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STATISTICAL PROPERTIES OF THE LEAST SQUARES ESTIMATOR OF MAXIMUM EXCRETED DRUG AMOUNT

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Красимира Проданова. СТАТИСТИЧЕСКИЕ СВОЙСТВА ОЦЕНКИ НАЙМЕНШИХ КВАДРАТОВ ДЛЯ МАКСИМАЛЬНОГО ЭКСКРЕТИРАНОГО КОЛИЧЕСТВА ЛЕКАРСТВА

Неточность оценки максимального экскретираного количества лекарства U^{∞} влечет за собой ошибки при определении некоторых фармакокинетических параметров лекарств. Обычно оценивание U^{∞} делается, используя наблюдаемые стойности для кумулятивной экскреции $U(t)$. В данной работе используется стохастическая модель для оценивания ошибки оценки U^{∞}. Распределение лекарства описывается n-частевой фармакокинетической модели ($n = 1, 2, \ldots$) с всасыванием. Преимущество модели в ее приложимости в случаях, когда отбор проб $U(t)$ не делан через равные промеждутки времени.

Krasimira Prodanova. STATISTICAL PROPERTIES OF THE LEAST SQUARES ESTIMATOR OF MAXIMUM EXCRETED DRUG AMOUNT

Inaccuracy in estimation of the maximum excreted drug amount (U^{∞}) is the main cause of errors in determination of some pharmacokinetic parameters of drugs. Usualy, one estimates U^{∞} using observed values of the cummulative excretion ($U(t)$). This paper uses a new stochastic model to obtain the least squares estimator of U^{∞}. The advantages of the model are that it is applicable for any drug, whose pharmacokinetics may be formalized with a linear n-compartment model ($n = 1, 2, 3, \ldots$) with or without phase of absorption and it may be used when the measurements for $U(t)$ are non-equidistant.

1. INTRODUCTION

The basic information concerning pharmacokinetics of a given drug is obtained by measurement the plasma concentrations in appropriate moments of time. The pharmacokinetics model best describing the drug behavior is determined on the ground of these data. According this model some basic pharmacokinetic parameters, such as rate constants of absorption, distribution, elimination, maximum plasma concentration, apparent volume of distribution, total clearance etc., are estimated. Additional information for the pharmacokinetic drug behavior is obtained by simultaneous measurement of both plasma and urinary concentrations. On the basis of the model and this information the kinetics of the urinary excretion is studied. This makes possible the determination of the rate constant of excretion, renal clearance, maximum cumulative amount of drug excreted in the urine, etc. On the other hand, the knowledge of the total and renal clearance of a drug makes possible the determination of the rate of urinary excretion and the extend of other extra renal mechanisms taking part in this elimination.

The measured data describing the kinetic of the excretion generally are presented as a relation between the velocity of the process of excretion $[dU(t)/dt]$ and the time t (a differential approach) or as a relation between the cumulative excreted drug amount $U(t)$ and the time t (an integral approach).

When employing the first approach, the instantaneous velocities are calculated using the ratio of eliminated drug amount ΔU and the corresponding interval of time Δt. However, the time intervals Δt, during which the urine tests are made, are usually of the same order as the drug elimination half-life (i.e. too big). This reduces essentially the reliability of the estimated pharmacokinetic constants.

The defects of the integral approach are due mainly to the lack of effective methods for determination the maximum drug amount excreted by the urine — $U^{\infty} = \lim_{t \to \infty} U(t)$, on which depends the accuracy of the estimated pharmacokinetic constants. Namely, the value U^{∞} could be found analytically by the methods of Guggenheim [1] or Mandelsdorff [2] if the intervals Δt are equal. However, usually it is not so. In [3] a comparatively simple method for estimation of U^{∞} is proposed, but it may be applied only for drugs, whose pharmacokinetics is described by linear one-compartment model without phase of absorption.

In the present paper, employing the integral approach, a method for estimation of U^{∞} is proposed. It offers the following advantages:

- Does not restrict the choice of the intervals Δt;
- Is applicable still for drugs, whose pharmacokinetics is described by a linear n-compartment ($n = 1, 2, \ldots$) model with phase of absorption;
- Provides the opportunity for estimation the statistic error of the estimated U^{∞}.

2. PROBLEM STATEMENT AND MATHEMATICAL MODEL

Let perform a single treatment of a given drug on an individual. In the moments of time $t_1, t_2, \ldots, t_m$ there are measured values of the plasma concentrations $y_1, y_2, \ldots, y_m$.

Let the drug distribution in the body is described by an n-compartment pharmacokinetic model. Then the drug concentration (plasma concentration) in the central compartment is given by the relation

$$C(t) = \sum_{i=1}^{n} C_i e^{-k_i t}, \tag{1}$$

where C_i and $k_i > 0$ are constants [1]. If there is a phase of absorption, then the time t_{lag}, the drug shows up, is the solution of the equation $C(t_{\text{lag}}) = 0$.

Let us have for the assumed model $y_i = C(t_i)$, $i = 1, 2, \ldots, m$.

The velocity of changing the cumulative drug amount excreted by the urine is given by the formula

$$\frac{dU(t)}{dt} = kVC(t), \tag{2}$$

where k and V are correspondingly the constants of excretion and the volume of the central compartment (the constant product kV is the so-called renal clearance).

After integration of (2) in the interval $[t_{\text{lag}}, t]$ and taking into account the initial condition $U(t_{\text{lag}}) = 0$ for the cumulative drug amount excreted till the moment t, we obtain

$$U(t) = kV \sum_{i=1}^{n} \frac{C_i}{k_i} \left[e^{-k_i t_{\text{lag}}} + e^{-k_i t} \right]. \tag{3}$$

According to [4] the maximum cumulative drug amount excreted in the urine, U^∞, is defined by the limit

$$U^\infty = \lim_{t \to \infty} U(t),$$

i.e.

$$U^\infty = kV \sum_{i=1}^{n} \frac{C_i}{k_i} e^{-k_i t_{\text{lag}}}. \tag{4}$$

Then (3) may be presented in the form

$$U(t) = U^\infty \left[1 - \frac{\sum_{i=1}^{n} (C_i/k_i) e^{-k_i t}}{\sum_{j=1}^{n} (C_j/k_j) e^{-k_j t_{\text{lag}}}} \right], \tag{5}$$

or

$$U(t) = U^\infty \varphi(t), \tag{6}$$

where $\varphi(t)$ denotes the expression in brackets from (5).

Let us assume that along with the plasma concentrations for the same individual are measured also the drug amounts U_i excreted till the moments of time τ_i $(i = 1, 2, \ldots, N)$. We suppose that

$$U(\tau_i) = U_i.$$

Now we shall estimate U^∞ employing U_i and using formula (6).

Let us consider the function

$$g(U^\infty) = \sum_{i=1}^{N} [U_i - U^\infty \varphi(t_i)]^2 . \tag{7}$$

We shall minimize the function $g(U^\infty)$. This function obtains its global minima when

$$U^\infty = \frac{\sum_{i=1}^{N} U_i \varphi(t_i)}{\sum_{j=1}^{N} [\varphi(t_j)]^2}. \tag{8}$$

Let us denote by $U^\top = (U_1, U_2, \ldots, U_N)$ ($\top$ — transpose of a matrix) the vector of experimental data for the cumulative excreted amount and by $W^\top = (W_1, W_2, \ldots, W_N)$ the vector with components

$$W_i = \frac{\varphi(t_i)}{\sum_{j=1}^{N} [\varphi(t_j)]^2}, \quad i = 1, 2, \ldots, N. \tag{9}$$

Then U^∞ may be presented in the form

$$\hat{U}^\infty = U^\top W. \tag{10}$$

In order to estimate the error of U^∞, we shall generalize the assumed above n-compartment model as a stochastic one and thus it will make possible the use of the *a priori* information about the error either caused by measurements or by different factors.

Let y_i satisfy the following statistic model:

$$y_i = C(t_i) + \nu_i, \quad i = 1, 2, \ldots, m,$$

where ν_i are independent identically distributed normal random variables, i.e. $\nu_i \in \mathbb{N}(0, \sigma^2)$.

Let $\{x_j\}_1^n$ be observations on $\{\xi_i\}_1^m$ — random variables. Let employing some method an estimator $\hat{y} = \hat{y}(x_1, x_2, \ldots, x_n)$ of the unknown parameter θ be obtained. It is well-known that every reasonable method along with the estimator $\hat{y}$ supplies an estimator of the error δ of $\hat{y}$. Again, any such method achieves an asymptotic, normal distribution for the estimator, i.e. $\hat{y} \in N(\theta, \delta)$. Then, according to the usual statistical philosophy, we may write $\theta \in N(\hat{y}, \hat{\delta})$.

We use the method of non-linear regression to estimate the unknown parameters C_i and k_i (see eq. (1)) from the data (t_i, y_i), i.e. we estimate the components of the vector

$$\theta^\top = (C_1, \ldots, C_n, k_1, \ldots, k_n)$$

as well as its error $\sigma^2\omega$.

Therefore for the unknown vector θ we have

$$\theta \in N(\hat{\theta}, \sigma^2\omega). \tag{11}$$

Let us consider also the vector U of the urine measurement. The components U_i are usually obtained as follows: During the trial all the excreted urine is accumulated. At the moment τ_i, the drug amount U_i is measured. Obviously, the obtained $U_1, U_2, \ldots, U_N$ are dependent.

In order to take into account the error in the data of U_i, let introduce the drug amount x_i excreted in the interval $[\tau_{i-1}, \tau_i]$, $i = 1, 2, \ldots, N$, where $\tau_0 = t_{\text{lag}}$. We assume that

$$x_i = \hat{x}_i + \varepsilon_i,$$

where $\varepsilon_i \in N(0, \sigma_1^2)$. The parameter σ_1 is supposed to be equal to the error of the method of measurement of the corresponding drug. Then, the measured cumulative amounts (the components of the vector U) are obtained as follows:

$$U_k = \sum_{i=1}^{k} x_i, \quad k = 1, 2, \ldots, N. \tag{12}$$

Now for U we get

$$U \in N(\hat{U}, \sigma_1^2 K K^{\mathsf{T}}), \tag{13}$$

where K is the matrix

$$K = \begin{Vmatrix} 1 & 0 & 0 & \ldots & 0 \\ 1 & 1 & 0 & \ldots & 0 \\ 1 & 1 & 1 & \ldots & 0 \\ \ldots & \ldots & \ldots & \ldots & \ldots \\ 1 & 1 & 1 & \ldots & 1 \end{Vmatrix}.$$

The computation of $\operatorname{cov}[U] = \sigma_1^2 K K^{\mathsf{T}}$ is given in detail in Appendix I.

The problem is to estimate the error of $U^{\infty} = U^{\mathsf{T}} W$.

The vectors U and W are random but independent, because the observations of the random vectors U and θ are made at different places (urine and plasma) and besides not at the same time. Therefore, if we consider the random vectors

$$\xi = \theta - \hat{\theta} \quad \text{and} \quad \eta = U - \hat{U},$$

they would be also independent. From (11), (13) it follows

$$\xi \in N(0, \sigma^2) \quad \text{and} \quad \eta \in N(0, \sigma_1^2 I_N), \tag{14}$$

(I_N is the unit matrix of order N).

The vector θ influences the components of W by the function $\varphi(t)$, i.e.

$$W_i = \frac{\varphi(t_i/\theta)}{\sum_{j=1}^{N} [\varphi(t_j/\theta)]^2}, \quad i = 1, 2, \ldots, N.$$

Let now present U in the form

$$U \approx \hat{U} + \sigma_1 K \eta,$$

and also to present W by Taylor series in the vicinity of $\hat{\theta}$, neglecting the terms of second and higher order:

$$W(\theta) \approx \hat{W} + \Omega\xi,$$

where $\hat{W} = W(\hat{\theta})$ and $\Omega = \underset{\theta}{\operatorname{grad}}\, W(\hat{\theta})$.

Now for the estimate of $U^\infty = U^\top W$ we obtain

$$U^\infty \in N(\hat{U}^\infty, \operatorname{cov}[\hat{U}^\infty]),$$

where

$$U^\infty = (\hat{U} + \sigma_1 K\eta)^\top (\hat{W} + \Omega\xi).$$

The main result is the next a little bit complicated formula:

$$\operatorname{cov}[U^\infty] = \sigma^2 A\omega A^\top + \sigma_1^2 \operatorname{tr}[K^\top \hat{W}\hat{W}^\top K] + \sigma^2\sigma_1^2 \operatorname{tr}[K^\top \Omega\omega\Omega^\top K]. \tag{15}$$

In Appendix II the components of the matrix Ω and $\operatorname{cov}[\hat{U}^\infty]$ are computed.

3. EXAMPLES AND COMPARISON WITH OTHER METHODS

With the above proposed method we have estimated the maximum cumulative amount of Sulfamethxozole (free) after single oral administration of two tablets of Biseptol (Polfa). Each of them contains 400 mg Sulfamethxozole (SMZ) and 80 mg Trimetoprim.

As an example, we show the results of an experiment for six healthy volunteers. Formally, their names are noted by AAA, BBB, CCC, EEE, YYY, ZZZ.

In Table 1 the plasma concentrations of SMZ in [mg/ml] measured in the moments of time t_i in [h], $i = 1, 2, \ldots, 7$.

TABLE 1. The plasma concentrations of SMZ [mg/ml] measured in the moments of time t_i [h] ($i = 1, 2, \ldots, 7$)

t [h]	0.5	1	1.5	3	6	10	24
AAA	1	10	18	41	45	33	11
BBB	26	40	38	37	27	20	4
CCC	20	33	45	46	36	24	9
EEE	4	11	12	33	40	26	9
YYY	25	40	41	48	47	29	14
ZZZ	17	32	48	52	41	45	14

The cumulative amounts U_i of SMZ excreted till the moment of time τ_i, $i = 1, 2, \ldots, 6$, are given in Table 2.

It turns out that the data of Table 1 show a good approximation with a relation of the form (1) for $n = 2$:

$$C(t) = C_1 e^{-k_1 t} + C_2 e^{-k_2 t},$$

TABLE 2. The cumulative amounts U_i [mg] of SMZ excreted till the moment of time τ_i [h] $(i = 1, 2, \ldots, 6)$

τ [h]	3	6	12	24	48	72
AAA	67.00	101.76	139.76	197.52	200.01	203.85
BBB	14.40	61.90	81.85	123.65	136.97	148.02
CCC	19.76	45.40	78.05	119.85	134.80	135.14
EEE	1.87	8.14	22.64	38.39	59.39	60.39
YYY	20.20	36.44	46.30	69.40	86.65	92.25
ZZZ	15.29	40.89	74.79	110.39	134.39	139.59

where $k_1 > k_2 > 0$ (k_1 — rate constant of absorption, k_2 — rate constant of elimination) and $C_1 < 0$, $C_2 > 0$.

The estimate of parameters $\theta^{\mathsf{T}} = (C_1, C_2, k_1, k_2)$ was made employing a program of non-linear regression 3R of BMDP [4]. On Table 3 the obtained estimates for these parameters, for $t_{\text{lag}} = [\ln(-C_1/C_2)]/(k_1 - k_2)$ and also for the residual mean square (RMS), are presented.

TABLE 3. The estimate of parameters $\theta^{\mathsf{T}} = (C_1, k_1, C_2, k_2)$ made employing a program of non-linear regression 3R of BMDP [5]

θ	C_1	k_1	C_2	k_2	t^*_{lag}	RMS**
AAA	−114.21	0.3986	96.926	0.0957	0.54	10.43
BBB	−108.68	3.5684	46.315	0.0886	0.25	3.35
CCC	−74.94	1.2496	62.198	0.0897	0.16	5.26
EEE	−115.61	0.3137	106.258	0.1119	0.42	23.27
YYY	−60.39	0.8218	59.762	0.0705	0.13	16.62
ZZZ	−83.537	1.1625	64.664	0.0548	0.23	39.59

The estimated $\hat{U}^{\infty}$ and its standard deviation $\delta = (\text{cov}[\hat{U}^{\infty}])^{1/2}$ are shown in Table 4.

TABLE 4. The estimated $\hat{U}^{\infty}$ and its standard deviation $\delta = \left(\text{cov}[\hat{U}^{\infty}]\right)^{1/2}$

Voll.	AAA	BBB	CCC	EEE	YYY	ZZZ
$\hat{U}^{\infty}$	219.66	141.22	134.37	52.86	91.21	148.41
δ	24.11	14.35	14.12	5.59	9.81	20.29

For comparison, the method given in [3] was chosen, because this method does not require equal intervals Δt. Briefly, the essence of this method is as follows:

Let the pharmacokinetics of the drug is described by an one-compartment model without absorption, i.e.

$$C(t) = C_2 e^{-k_2 t}.$$

Then after integration in the interval $[O,t]$ with initial condition $U(0) = 0$ one obtains

$$U(t) = U^{\infty}(1 - e^{-k_2 t}).$$

After one more integration in the same interval follows

$$\int_0^t U(t)\,dt = U^{\infty}t - \frac{1}{k_2}U^{\infty}(1 - e^{-k_2 t}).$$

Now, substituting $U^{\infty}(1-e^{-k_2 t}) = U(t)$ and dividing by t, one obtains the straight line $Y = aX + b$, where

$$Y = \frac{\int_0^t U(t)\,dt}{t}, \quad X = \frac{U(t)}{t}, \quad b = U^{\infty} \quad \text{and} \quad a = -\frac{1}{k_2}.$$

Now it is clear that U^{∞} may be estimated as an intercept of the straight line. This is done after the approximation of the experimental data $U_i = U(\tau_i)$, using simple linear regression.

Our data U_i (Table 2) are measured after oral treatment, i.e. there is a phase of absorption. In order to avoid this phase and use one-compartment model (see the so-called flip-flop phenomenon" [5]), it is reasonable to neglect the first observation of U, i.e. for $t = 3$. For moments of time, long after the maximum of the function

$$C(t) = C_1 e^{-k_1 t} + C_2 e^{-k_2 t}, \quad k_1 > k_2,$$

is achieved, it may be simplified as follows:

$$C(t) \approx C_2 e^{-k_2 t}.$$

As it is seen from Table 1, the maximum of plasma concentration for all volunteers is achieved before the third hour.

In Figs. 1–6 by $*$ are denoted the measured U_i; by a solid line the approximate curves $U(t)$ are presented; the original measurements U_i are given by $*$; our estimator is presented by a solid curve and the estimator of [3] — by a dashed one. Our method shows better approximation than the compared method as it could be seen in Figs. 2, 3, 5. In the case of volunteer AAA (Fig. 1) the other model is better. In the case of ZZZ (Fig. 6) the both models are adequate. In the case of EEE (Fig. 4) the both models do not give sufficient approximation of the experimental data.

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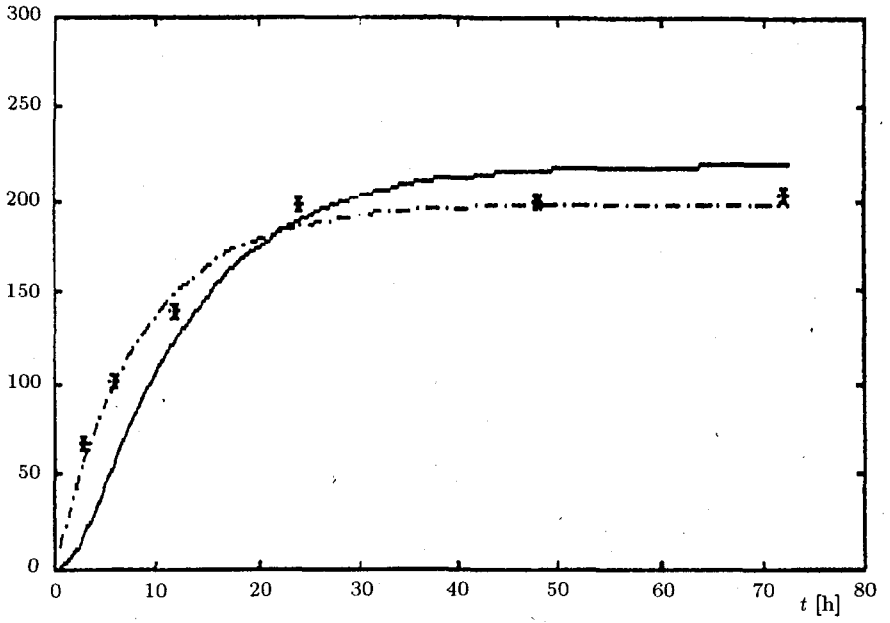

Fig. 1. The approximate curves $U(t)$ for the volunteer AAA
$*$ — original measurements U_i; —— — our estimator; – · – · – — the estimator of [3]

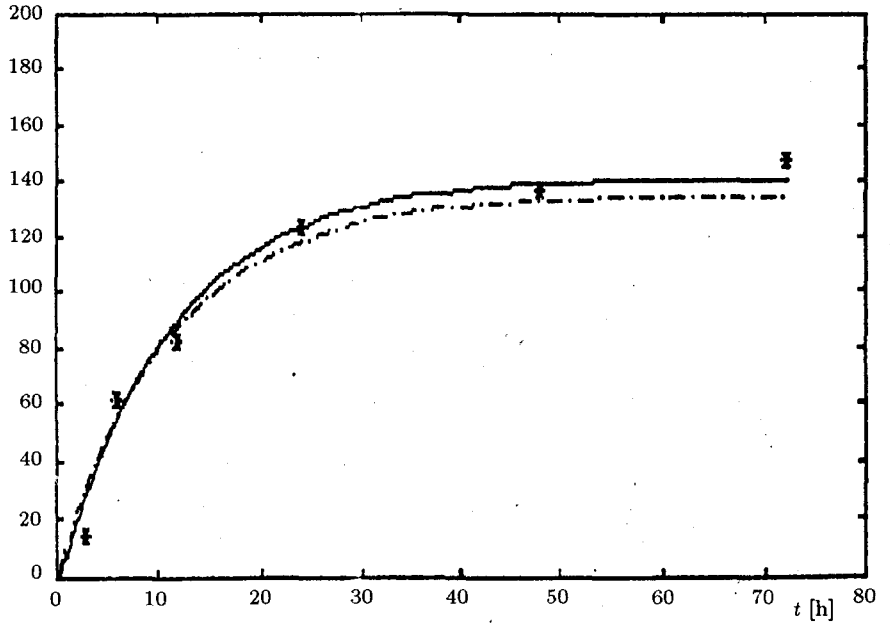

Fig. 2. The approximate curves $U(t)$ for the volunteer BBB
$*$ — original measurements U_i; —— — our estimator; – · – · – — the estimator of [3]

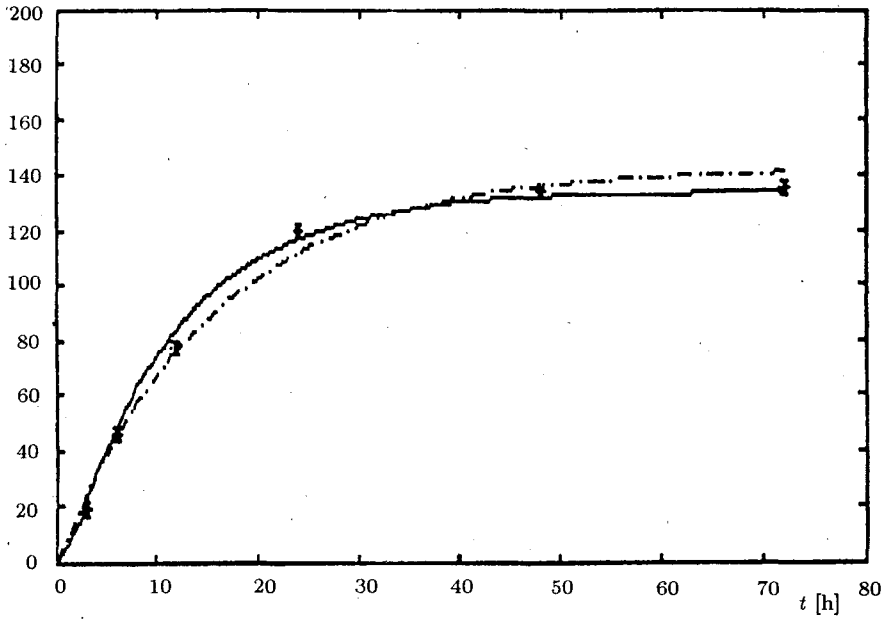

Fig. 3. The approximate curves $U(t)$ for the volunteer CCC
$*$ — original measurements U_i; —— — our estimator; – · – · – — the estimator of [3]

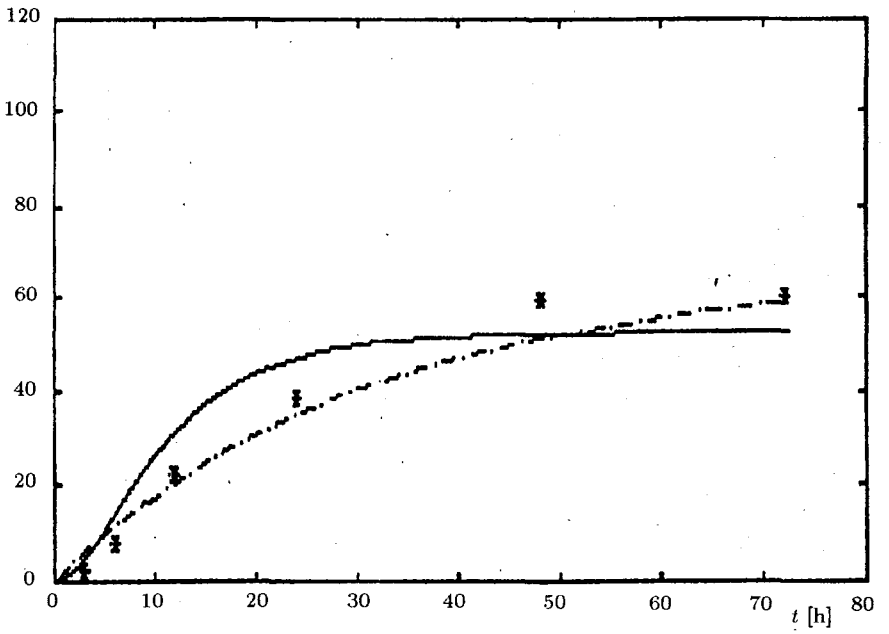

Fig. 4. The approximate curves $U(t)$ for the volunteer EEE
$*$ — original measurements U_i; —— — our estimator; – · – · – — the estimator of [3]

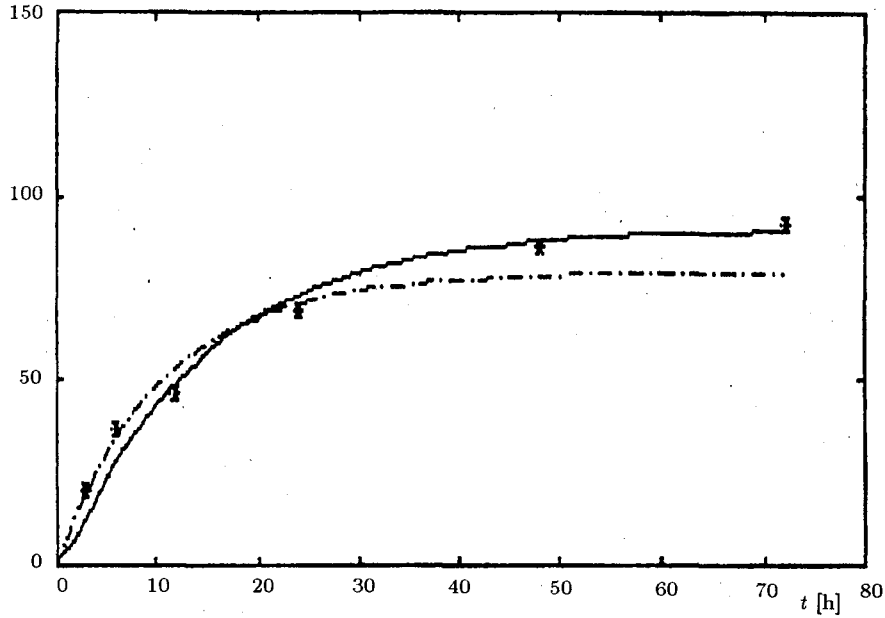

Fig. 5. The approximate curves $U(t)$ for the volunteer YYY
$*$ — original measurements U_i; —— — our estimator; – · – · – — the estimator of [3]

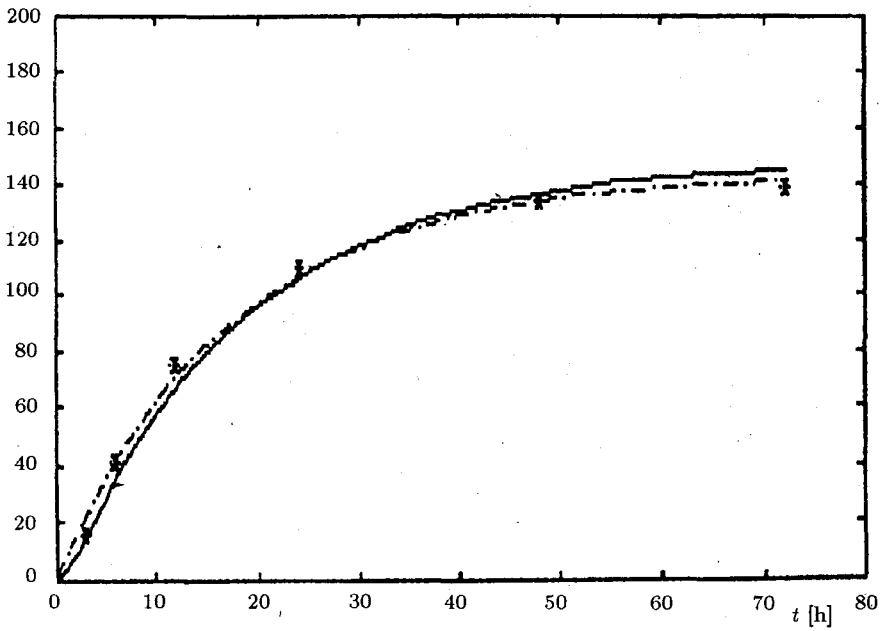

Fig. 6. The approximate curves $U(t)$ for the volunteer ZZZ
$*$ — original measurements U_i; —— — our estimator; – · – · – — the estimator of [3]

APPENDIX I. Calculation of cov[U]

Let consider first

$$\operatorname{cov}[U_k, U_l] = E[(U_k - \bar{U}_k)(U_l - \bar{U}_l)] = E[U_k U_l] - \bar{U}_k EU_l - \bar{U}_l EU_k + \bar{U}_l \bar{U}_k,$$

i.e.

$$\operatorname{cov}[U_k, U_l] = E[U_k U_l] - EU_k EU_l. \tag{A1.1}$$

Let assume $k \leq l$. Then

$$E[U_k U_l] = E\left[\sum_{i=1}^{k} x_i \left(\sum_{j=1}^{k} x_j + \sum_{j=k+1}^{l} x_j\right)\right] \tag{A1.2}$$

$$= E\left[\left(\sum_{i=1}^{k} x_i\right)^2\right] + E\left[\sum_{i=1}^{k} x_i \sum_{j=k+1}^{l} x_j\right] = \sum_{i=1}^{k} E[x_i^2] + E\left[\sum_{i \neq j}^{k} x_i x_j\right] + EU_k EU_l.$$

But as x_i are independent random variables, from (A1.2) it follows

$$E[U_k U_l] = k\sigma_1^2 + EU_k EU_l. \tag{A1.3}$$

Now from (A1.1) and (A1.3) one obtains

$$\operatorname{cov}[U_k, U_l] = \sigma_1^2 \min(k, l).$$

Then

$$\operatorname{cov}[U] = \begin{Vmatrix} 1 & 1 & 1 & \dots & 1 \\ 1 & 2 & 2 & \dots & 2 \\ 1 & 2 & 3 & \dots & 3 \\ \dots & \dots & \dots & \dots & \dots \\ 1 & 2 & 3 & \dots & N \end{Vmatrix} \sigma_1^2 = KK^{\mathsf{T}} \sigma_1^2,$$

where

$$K = \begin{Vmatrix} 1 & 0 & 0 & \dots & 0 \\ 1 & 1 & 0 & \dots & 0 \\ 1 & 1 & 1 & \dots & 0 \\ \dots & \dots & \dots & \dots & \dots \\ 1 & 1 & 1 & \dots & 1 \end{Vmatrix}.$$

APPENDIX II. Calculation of Ω and cov[U^∞]

Let consider first $\operatorname{grad}_\theta \varphi(t/\theta)$, where

$$\theta^{\mathsf{T}} = (C_1, \dots, C_n, k_1, \dots, k_n) :$$

$$\frac{\partial \varphi(t/\theta)}{\partial C_i} = -\frac{\dfrac{e^{-k_i t}}{k_i} \sum_{j=1}^{n} \dfrac{C_j}{k_j} e^{-k_j t_{\text{lag}}} - \dfrac{e^{-k_i t_{\text{lag}}}}{k_i} \sum_{j=1}^{n} \dfrac{C_j}{k_j} e^{-k_j t}}{\left(\sum_{j=1}^{n} \dfrac{C_j}{k_j} e^{-k_j t_{\text{lag}}}\right)^2} \tag{A2.1}$$

and

$$\text{(A2.2)}\qquad \frac{\partial\varphi(t/\theta)}{\partial k_i}$$

$$= \frac{\dfrac{C_i e^{-k_i t}}{k_i^2}\displaystyle\sum_{j=1}^{n}\frac{C_j}{k_j}\left((1+k_i t)e^{-k_j t_{\text{lag}}} - (1+k_i t_{\text{lag}})e^{-[(k_j-k_i)t+k_i t_{\text{lag}}]}\right)}{\left(\displaystyle\sum_{j=1}^{n}\frac{C_j}{k_j}e^{-k_j t_{\text{lag}}}\right)^2},$$

where $i = 1, 2, \ldots, n$. If $t = \tau_l$ $(l = 1, 2, \ldots, N)$, then according to (A2.1) we calculate the elements of the first n rows of the matrix $\underset{\theta}{\text{grad}}\,\varphi(t/\hat{\theta})$, whose order is $[2n \times N]$. For the elements of the next rows, from $(n+1)$ to $(2n)$, we use (A2.2).

Now let present the function $\varphi(t/\theta)$ by Taylor series in the vicinity of $\hat{\theta}$, neglecting the terms of second and higher order:

$$\text{(A2.3)}\qquad \varphi(t/\theta) = \varphi(t/\hat{\theta}) + [\underset{\theta}{\text{grad}}\,\varphi(t/\hat{\theta})]^{\mathsf{T}}\xi.$$

Then from (9) and (A2.3), for the components of $\hat{W} = W(\hat{\theta})$, it follows:

$$\text{(A2.4)}\qquad W_1(\hat{\theta}) = \frac{\varphi(t_i/\hat{\theta}) + [\underset{\theta}{\text{grad}}\,\varphi(t_i/\hat{\theta})]^{\mathsf{T}}}{\left[\displaystyle\sum_{j=1}^{N}\varphi(t_j/\hat{\theta}) + [\underset{\theta}{\text{grad}}\,\varphi(t_j/\hat{\theta})]^{\mathsf{T}}\xi\right]^2}, \quad i = 1, 2, \ldots, N.$$

Now we are ready to calculate the components of $\Omega = \underset{\theta}{\text{grad}}\,W(\hat{\theta})$. For this purpose we compute the derivative of $W(\hat{\theta})$ with respect to $\xi = (\theta - \hat{\theta})$ thus getting

$$\Omega = \underset{\theta}{\text{grad}}\,W(\hat{\theta}) = \left(\frac{dW(\hat{\theta})}{d\xi}\right)_{\xi=0}.$$

The derivative in (A2.4) with respect to ξ, for $\xi = 0$, gives us the component Ω_{lp} of the matrix $\Omega = \underset{\theta}{\text{grad}}\,W(\hat{\theta})$:

$$\Omega_{lp} = \frac{\left[\displaystyle\sum_{i=1}^{N}\varphi^2(t_i/\hat{\theta})\right][\underset{\theta}{\text{grad}}\,\varphi(t/\hat{\theta})]_{lp}^{\mathsf{T}} - 2\varphi(t_i/\hat{\theta})\displaystyle\sum_{i=1}^{N}\left\{\varphi(t_i/\hat{\theta})[\underset{\theta}{\text{grad}}\,\varphi(t_i/\hat{\theta})]^{\mathsf{T}}\right\}}{\left[\displaystyle\sum_{i=1}^{N}\varphi^2(t_i/\hat{\theta})\right]^2},$$

where $l = 1, 2, \ldots, N$ and $p = 1, 2, \ldots, 2n$.

In order to calculate $\text{cov}[\hat{U}^{\infty}]$, let us first consider the estimator of $U^{\infty} = U^{\mathsf{T}}W$, i.e.

$$\hat{U}^{\infty} = \left(\hat{U} + \sigma_1 K\eta\right)^{\mathsf{T}}\left(\hat{W} + \Omega\xi\right) = \hat{U}^{\mathsf{T}}\hat{W} + \hat{U}^{\mathsf{T}}\Omega\xi + \sigma_1\eta^{\mathsf{T}}K^{\mathsf{T}}\hat{W} + \sigma_1\eta^{\mathsf{T}}K^{\mathsf{T}}\Omega\xi$$
$$= U^{\infty} + \hat{U}^{\mathsf{T}}\Omega\xi + \sigma_1\eta^{\mathsf{T}}K^{\mathsf{T}}\hat{W} + \sigma_1\eta^{\mathsf{T}}K^{\mathsf{T}}\Omega\xi.$$

Then

$$\operatorname{cov}[U^{\infty}] = E\Big\{ \left[\hat{U}^{\top}\Omega\xi + \sigma_1\eta^{\top}K^{\top}\hat{W} + \sigma_1\eta^{\top}K^{\top}\Omega\xi\right] \times \left[\hat{U}^{\top}\Omega\xi + \sigma_1\eta^{\top}K^{\top}\hat{W} + \sigma_1\eta^{\top}K^{\top}\Omega\xi\right]^{\top}\Big\}.$$

Let denote by A the matrix $A = \hat{U}^{\top}\Omega$. Hence

$$\begin{aligned}\operatorname{cov}[U^{\infty}] &= E\left[A\xi\xi^{\top}A^{\top}\right] + \sigma_1 E\left[A\hat{W}^{\top}\xi^{\top}K\eta\right] + \sigma_1 E\left[A\xi\xi^{\top}\Omega^{\top}K\eta\right] \\ &+ \sigma_1 E\left[\eta^{\top}K^{\top}\hat{W}\xi^{\top}A^{\top}\right] + \sigma_1^2 E\left[\eta^{\top}K^{\top}\hat{W}\hat{W}^{\top}K\eta\right] + \sigma_1^2 E\left[\eta^{\top}K^{\top}\hat{W}\xi^{\top}\Omega^{\top}K\eta\right] \\ &+ \sigma_1 E\left[\eta^{\top}K^{\top}\Omega\xi\xi^{\top}A^{\top}\right] + \sigma_1^2 E\left[\eta^{\top}K^{\top}\Omega\xi\hat{W}^{\top}K\eta\right] + \sigma_1^2 E\left[\eta^{\top}K^{\top}\Omega\xi\xi^{\top}\Omega^{\top}K\eta\right].\end{aligned}$$

From (14) it follows

$$E\xi = 0, \quad E\eta = 0, \quad E\xi\xi^{\top} = \sigma^2\omega, \quad E\xi\eta = 0, \quad E\eta\eta^{\top} = \sigma_1^2 KK^{\top}.$$

Hence

$$E\left[A\xi\xi^{\top}A^{\top}\right] = \sigma^2 A\omega A^{\top}, \quad E\left[A\xi\xi^{\top}\Omega^{\top}K\eta\right] = E\left[\eta^{\top}K^{\top}\Omega\xi\xi^{\top}A^{\top}\right] = 0$$

and

$$E\left[A\xi\hat{W}^{\top}K\eta\right] = E\left[\eta^{\top}K^{\top}\hat{W}\xi^{\top}A^{\top}\right] = 0.$$

As we have

$$E_{\eta}\left[K^{\top}\hat{W}\xi^{\top}\Omega^{\top}K\right] = 0,$$

then

$$E\left[\eta^{\top}K^{\top}\hat{W}\xi^{\top}\Omega^{\top}K\eta\right] = E\left\{\eta^{\top}E_{\eta}\left[K^{\top}\hat{W}\xi^{\top}\Omega^{\top}K\eta\right]\right\} = 0.$$

Finally, we obtain the desired result:

$$\operatorname{cov}\left[U^{\infty}\right] = \sigma^2 A\omega A^{\top} + \sigma_1^2 \operatorname{tr}\left[K^{\top}\hat{W}\hat{W}^{\top}K\right] + \sigma^2\sigma_1^2 \operatorname{tr}\left[K^{\top}\Omega\omega\Omega^{\top}K\right].$$

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