

## Application of the Fowler-Warten Method to a Stiff Enzymatic Reaction

Aso, Yoichi

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Tsuji, Yumiko

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Hayashi, Katsuya

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

<https://doi.org/10.5109/23485>

---

出版情報：九州大学大学院農学研究院紀要. 21 (1), pp.33-45, 1977-02. Kyushu University  
バージョン：  
権利関係：

## Application of the Fowler-Warten Method to a Stiff Enzymatic Reaction

Yoichi Aso, Yumiko Tsuji\* and Katsuya Hayashi

Laboratory of Sericultural Chemistry, Faculty of Agriculture,  
Kyushu University 46-02, Fukuoka 812

(Received September 14, 1976)

The rate equations are very often to be solved in the simulation and the optimization of the biochemical reaction system. Numerical computations of the equation by the digital computer have been frequently confronted with its stiffness. The computations of the stiff system require a great deal of computation time in comparison with those of the other systems. From this point of view, usual techniques are of no practical use for the stiff systems.

The Fowler-Warten method, which is one of the explicit integration formula for stiff system, was modified and applied to the three-step enzymatic reaction. By the present method, the computation of the time-course can be performed with rapidity and small error.

### INTRODUCTION

In chemical kinetics, a chemical reaction is first represented by a suitable reaction scheme and a rate equation usually in the form of simultaneous nonlinear ordinary differential equation is derived from the postulated scheme. For estimation of the rate constants, the experimental data are treated with the solution of rate equation.

When the rate equation is in the form of simple linear differential equation, the rate equation can be easily solved by analytical method. Practically, the rate equation to be solved has more complicated form. In such case, even if the rate equation is in linear form, the analytical solution becomes quite difficult, because it needs finally to solve a characteristic equation formulated by algebraic equation of high degrees. For nonlinear rate equation, with the exception of a few special cases, the analytical solution should be essentially abandoned due to its mathematical nature. Thus, the differential equations in the rate equation must be solved numerically using an integration formula with a digital computer.

In the digital computation, several problems have been pointed out in connection with the error and the execution time in computation. In digital computer, a numerical value is expressed by a numerical word with a finite size and a continuous value, such as time or the concentration of chemical species, is discretized. Consequently, the round-off or truncation error results from the computation procedures. The problems on the computation error have been

---

\* Present address: Seika Junior Women's College, Fukuoka 815

extensively studied (Henrici, 1962). Since the execution time in computation is closely related to the numerical stability of the integration formula, the problem on the saving of it has been generally discussed on the basis of the mathematical behavior of the integration formula.

It has been well recognized that in many biochemical reaction systems the values of the rate constants or concentrations of chemical species were greatly separate one another. For the differential equation describing such systems, a considerably large computation time will be required when conventional techniques are adopted for numerical computation. Such the troublesome equation appears not only in biochemical system but also in many other systems and is called "stiff" (Dahlquist, 1974). If an enormous computation time is required for solving the differential equation, the computational analysis of the biochemical system will turn into an expensive and actually impractical one. Thus, for solving the rate equation of the biochemical systems, it is indispensable to establish the algorithm or programming procedure which allows to solve numerically the stiff differential equation with a reasonable computation time and computation error (Reich, 1969).

The present paper deals with the application of the Fowler-Warten method (Fowler and Warten, 1967) to the numerical solution of biochemical stiff rate equation with minor modifications.

## METHOD

The fundamental problems on the numerical solution of stiff equation with practical computation time and reasonable computation error are closely related to the setting of stepsize in the numerical integration. The selection and setting of an appropriate stepsize are constrained by the numerical stability of the differential equation to be solved. The Fowler-Warten method has been considered to be the most useful one for solving the stiff equation. However, some modifications were required for the Fowler-Warten method on its application to analysis of biochemical system.

In general, the rate equation of an enzymatic reaction in which  $n$  distinct chemical species are involved can be expressed in the form of simultaneous ordinary differential equations written in vector notation,

$$\frac{d}{dt}\boldsymbol{x} = \boldsymbol{f}[x_i; i=1, 2, \dots, n] \quad (1)$$

where  $\boldsymbol{x} = (x_1, x_2, \dots, x_n)'$  is the concentration of species and the prime indicates transposed matrix. The function  $\boldsymbol{f}$  is generally nonlinear for  $x_i$ . Usually, these differential equations cannot be solved analytically but numerically, with the exception of some special cases. Let  $\varepsilon_i$  be the perturbation on the solution  $x_i$ . Then, the equation (1) may be transformed into

$$\dot{\boldsymbol{x}} + \dot{\boldsymbol{\varepsilon}} = \boldsymbol{f}[x_i + \varepsilon_i; i=1, 2, \dots, n] \quad (2)$$

where  $\boldsymbol{\varepsilon} = (\varepsilon_1, \varepsilon_2, \dots, \varepsilon_n)'$  and the symbol dot represents the corresponding deriva-

tive with respect to time  $t$ . The right side of the equation (2) can be expanded in a Taylor series as

$$\mathbf{f}[x_i + \varepsilon_i; i=1, 2, \dots, n] \simeq \mathbf{f}[x_i; i=1, 2, \dots, n] + \mathbf{J}\varepsilon + \mathcal{O}(\varepsilon^2) \quad (3)$$

where  $\mathbf{J}$  is so-called Jacobian matrix with element  $J_{ij} = \partial f_j / \partial x_i$  ( $i, j=1, 2, \dots, n$ ). If the perturbation  $\varepsilon$  is tolerably small, a new differential equation (variational equation) of  $\varepsilon$  is approximately set up as

$$\dot{\varepsilon} = \mathbf{J} \cdot \varepsilon \quad (4)$$

If eigenvalues  $\lambda_j$  ( $j=1, 2, \dots, n$ ) of the matrix  $\mathbf{J}$  are all distinguishable, a solution of the equation (4) becomes equal to a linear combination of  $\exp[\lambda_j t]$ ,

$$\varepsilon_i = \sum_{j=1}^n c_{ij} \cdot \exp[\lambda_j t] \quad (i, i=1, 2, \dots, n) \quad (5)$$

where  $c_{ij}$  is a constant. If the real parts of all  $\lambda_j$  are negative,  $\varepsilon_i$  tends to diminish with increase in time  $t$ . This is the basic requirement for differential equations to be solved stably, even if some small error arises during numerical computations. These situations, however, are more complicated in case that function  $\mathbf{f}$  is nonlinear, because  $\mathbf{J}$  varies depending upon the time  $t$ .

The stiffness of differential equation is characterized by that the maximum value is extremely different from the minimum value in  $\lambda_j$ . This means that the perturbation-sensitive and -insensitive components exist together in the same solution  $x_i$  of the equation (1).

Time  $t$  is forced to be quantized in a numerical computation as

$$t_{i+1} = t_i + h \quad (j=0, 1, 2, \dots) \quad (6)$$

where  $t_0$  is an initial time and  $h$  is a stepsize between successive discrete times. By this replacement, the differential equation is transformed into the corresponding difference equation (as described later) and is solved step by step in unit  $h$ . The stepsize  $h$  is restricted according to conditions on the stability. This restriction has a great effect on the computation time with a digital computer. The conditions on the stability are determined by applying an integration formula to the differential equation,

$$\dot{x} = \lambda \cdot x \quad (7)$$

where  $\lambda$  is a negative complex constant. For example, in the Euler method,

$$x(t_{i+1}) = x(t_i) + hf[t_i, x(t_i)] \quad (j=0, 1, 2, \dots) \quad (8)$$

is adopted as the integration formula, where  $h = t_{j+1} - t_j$  (positive real value) and  $f = dx/dt$ . If the equation (8) is substituted for the equation (7), the following equation,

$$x(t_{j+1}) = (1 + h\lambda)x(t_j) \quad (9)$$

is derived, and a solution of the equation (9) is obtained as

$$x(t_n) = (1+h\lambda)^n x(t_0) \quad (10)$$

The approximated solution  $x(t_n)$ , as the same as that of the original equation (7), is accessible to 0 for  $t \rightarrow \infty$  under the condition,

$$|1+h\lambda| < 1 \quad (11)$$

According to the condition (11), the region of  $h\lambda$  on a complex plane is shown in Fig. 1. Excepting that the constraint is in the closed region as shown by

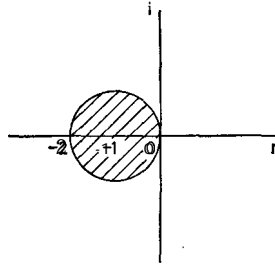


Fig. 1. Stability region for Euler's method.  $i$  represents the imaginary axis, and  $r$  represents the real axis.  $h\lambda$  is in the shadowed portion (within the circle with a radius 1).

the following inequality, this situation is the same as that in the 4th order Runge-Kutta-Gill method.

$$\left| 1+h\lambda + \frac{1}{2!}(h\lambda)^2 + \frac{1}{3!}(h\lambda)^3 + \frac{1}{4!}(h\lambda)^4 \right| < 1 \quad (12)$$

Let us consider a system of homogeneous linear differential equation,

$$\dot{x} = Ax \quad (13)$$

where  $x = (x_1, x_2, \dots, x_n)'$ ,  $A$  is an  $n \times n$  coefficient matrix with real values. For the Euler method, similarly to the equation (8),

$$x(t_{j+1}) = x(t_j) + hAx(t_j) \quad (14)$$

And, similarly to the equation (9), the simultaneous difference equation is

$$x(t_{j+1}) = (I+hA)x(t_j) \quad (15)$$

where  $I$  is a unit matrix. A solution of the equation (15) is

$$x(t_n) = (I+hA)^n x(t_0) \quad (16)$$

where  $x(t_0)$  is the initial value. If one of the eigenvalues of  $(I+hA)$  is  $\lambda'$ , the constraint condition for stepsize  $h$ , as stated above, is  $|\lambda'| < 1$ . Considering that the corresponding eigenvalue of  $A$  is  $\lambda_j$ , then,

$$\lambda'_j = 1+h\lambda_j \quad (17)$$

and the constraint condition for  $h\lambda_j$  is

$$|\lambda'_j| = |1 + h\lambda_j| < 1 \quad (18)$$

If  $\lambda_j$  is a (negative) real value, the value of a stepsize  $h$  should be in the following region.

$$0 < h < -\frac{2}{\lambda_j} \quad (19)$$

If all of  $\lambda_j$  are distinguishable, the solution of the equation (13) is

$$\mathbf{x}_i = c_{i1}\exp[\lambda_1 t] + c_{i2}\exp[\lambda_2 t] + \cdots + c_{in}\exp[\lambda_n t] \quad (20)$$

where  $c_{ij}$  ( $i, j=1, 2, \dots, n$ ) is a constant. The component of the solution (20) involving the  $\text{Max}[\lambda_j]$  is rapidly accessible to 0 with increasing in time  $t$ . The upper limit of the stepsize  $h$ , however, is fixed according to the condition (19) during the computation. It is said again that stiff system has wide separation between the eigenvalues of the coefficient matrix  $A$ . This type of problem has been recently considered and the integration formulas with wider stability region have been designed (Curtis and Chance, 1972; Dahlquist, 1974; Hemker, 1972).

Fowler and Warten (1967) have suggested an exponentially fitted method which is based upon one of those integration formulas. The practical algorithm of the Fowler-Warten method can be summarized as follows:  $\dot{\mathbf{x}} = \mathbf{f}(t, \mathbf{x})$  is the differential equation to be solved.

- Step 1.  $\dot{\mathbf{x}}_a(t) = \{\mathbf{x}(t) - \mathbf{x}(t-h_0)\} / h_0$
- Step 2.  $\mathbf{d}_1 = \dot{\mathbf{x}}(t) - \mathbf{x}, (t)$
- Step 3.  $\mathbf{x}_p(t+\delta) = \dot{\mathbf{x}}(t) + \delta\mathbf{x}(t) \quad (\delta \leq h/4)$
- Step 4.  $\dot{\mathbf{x}}_p(t+\delta) = \mathbf{f}[t+\delta, \mathbf{x}_p(t+\delta)]$
- Step 5.  $\mathbf{E}_\delta = \delta\{\dot{\mathbf{x}}_p(t+\delta) - \dot{\mathbf{x}}_p(t)\} / 2$
- Step 6.  $\mathbf{U}_\delta = \{u_1 + u_2 | \mathbf{x}_p(t+\delta) | \} / 2,$   
 $\mathbf{L}_\delta = \{l_1 + l_2 | \mathbf{x}_p(t+\delta) | \} / 2$
- Step 7.  $\mathbf{d}_2 = \{\dot{\mathbf{x}}_p(t+\delta) - \dot{\mathbf{x}}_p(t)\} / \delta$
- Step 8.  $\begin{cases} \lambda_j = d_{2j} / d_{1j} & (d_{1j} \neq 0) \\ \lambda_j = 0 & (d_{1j} = 0) \end{cases} \quad (j=1, 2, \dots, n)$
- Step 9.  $\begin{cases} c_{1j} = \{\exp[\lambda_j h] - 1\} / \lambda_j h, & c_0 = \exp[\lambda_j h] \quad (\lambda_j < 0) \\ c_{1j} = 1 + \lambda_j h / 2, & c_0 = 1 + \lambda_j h \quad (\lambda_j \geq 0) \end{cases} \quad (j=1, 2, \dots, n)$
- Step 10.  $\mathbf{x}_c(t+h) = \mathbf{x}(t) + h\dot{\mathbf{x}}_a(t) + hc_1\mathbf{d}_1$
- Step 11.  $\dot{\mathbf{x}}_c(t+h) = \mathbf{f}[t+h, \mathbf{x}_c(t+h)]$
- Step 12.  $\mathbf{E}_h = h\{\dot{\mathbf{x}}_c(t+h) - \{\dot{\mathbf{x}}_a(t) - c_0\mathbf{d}_1\}\}$
- Step 13.  $\begin{cases} \mathbf{U}_h = u_1 + u_2 | \mathbf{x}_c(t+h) | \\ \mathbf{L}_h = l_1 + l_2 | \mathbf{x}_c(t+h) | \end{cases}$

In the above description,  $\delta$  and  $h$  are controllable stepsizes,  $\mathbf{x}_c(t+h)$  is the

computed solution at time  $(t+h)$ ,  $E_\delta$  and  $E_h$  are the error estimates for which the  $\delta$  and  $h$  are controlled,  $u_1, u_2, l_1$ , and  $l_2$  are 0.0005, 0.0075, 0.0001, and 0.00005, respectively and  $U_\delta, L_\delta, U_h$ , and  $L_h$  are associated with the limit on number line as shown in Fig. 2. Just after the value of  $E_\delta$  (or  $E$ ) is estimated in Step

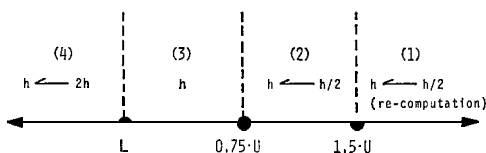


Fig. 2. Schematic representation of limits,  $L$ ,  $0.75 U$ , and  $1.5 U$ , on the number line.

5 (or Step 12), the procedures are divided into the following four with the same numbering in Fig. 2.

- 1) The stepsize  $\delta$  (or  $h$ ) is halved, and the re-computation from Step 3 or (Step 9) is executed.
- 2) The stepsize  $\delta$  (or  $h$ ) is halved at the next time step.
- 3) The stepsize  $\delta$  (or  $h$ ) is not changed.
- 4) The stepsize  $\delta$  (or  $h$ ) is doubled after seven time steps. However, once one of the procedures from 1) to 3) is selected during the seven time steps, this doubling is cancelled.

As will be stated in the following section, it was found that an intact application of the Fowler-Warten method to the computation of the present enzymatic reaction model was led to an unfavorable result. A few modifications were, therefore, attempted.

First, in the present procedure it is checked after Step 3 and Step 10 whether the solutions  $x_p$  and  $x_c$  satisfy the non-negative constraint condition or not because the solutions are (relative or absolute) concentrations of chemical species. If one of the solutions is negative value, the above case 1) is selected as the next procedure. Second, it should be noted that the conservation law of mass is realized in a closed chemical system. Therefore, the difference between sum of computed concentrations and sum of the corresponding initial concentrations is monitored. Namely, the relative error  $\epsilon_t(\%)$  at time  $t$  is newly defined as

$$\epsilon_t = 100 |S_t - S_i| / S_i \quad (21)$$

where  $S_t$  and  $S_i$  are the sum of concentrations at time  $t$  and at initial time, respectively. To keep the  $\epsilon_t$  small, the error estimates  $E_\delta$  and  $E_h$  are transformed into  $F \cdot E_\delta$  and  $F \cdot E_h$ , respectively, where  $F$  is called "factor". Additionally, the independent variable  $t$  is calculated, without use of the integration formula, by  $t_0 + nh$  ( $n=0, 1, \dots$ ) for speed-up of computation. The flow chart of the essential part of the program with these modifications is shown in Fig. 3. The program was written in FORTRAN (more than the level of JIS 7000) and in conversational mode which allows the adjustments of some parameters

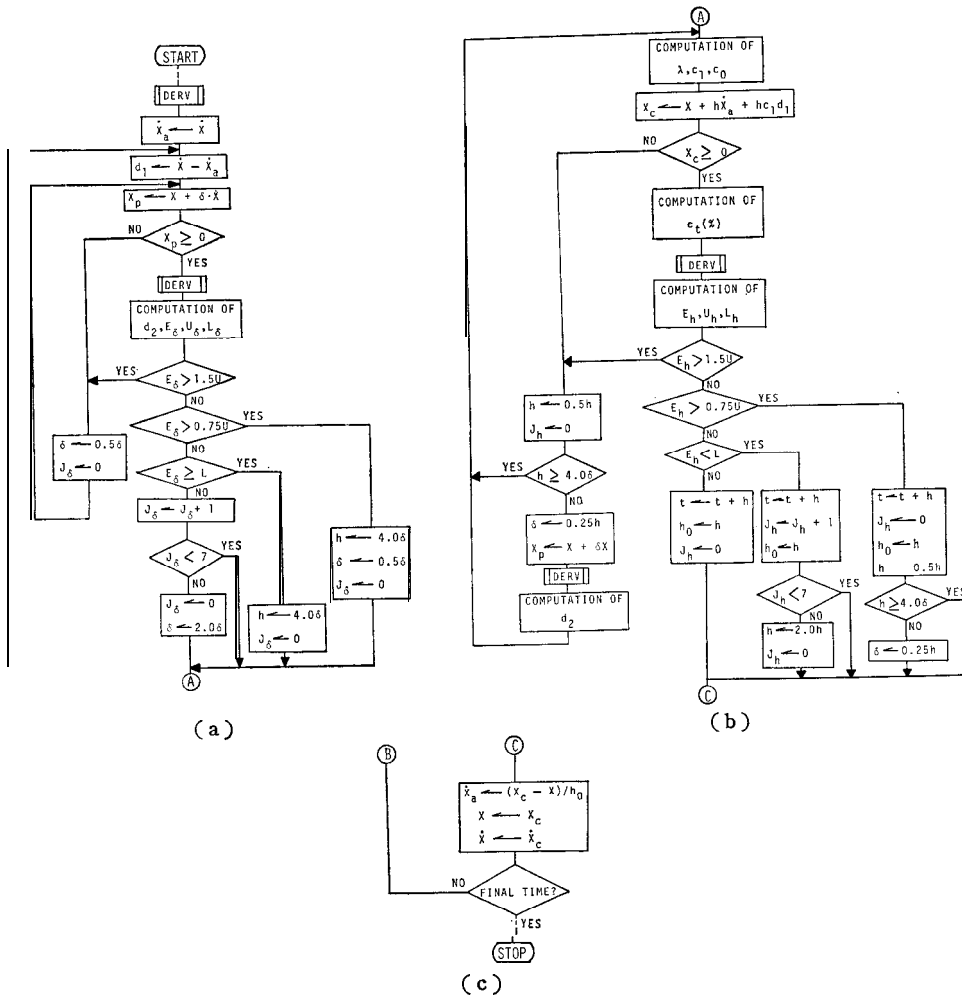
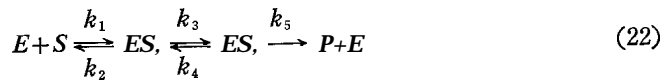


Fig. 3. Flow chart of the essential part of the program.

by one's judgement. Computations were performed with FACOM 230-75 in the Computer Center, Kyushu University.

COMPUTATIONS AND DISCUSSION

The three steps enzymatic reaction,



was subjected to the digital computation, where  $E, S, ES_1, ES_2,$  and  $P$  are enzyme, substrate, enzyme-substrate complex 1, enzyme-substrate complex 2 and



product, respectively, and  $k_j$  ( $j=1, 2, \dots, 5$ ) is the rate constant on the corresponding reaction path. The intermediates  $ES$ , and  $ES$ , are characterized by their intramolecular conversion. The rate equations of this reaction scheme are expressed by the simultaneous ordinary differential equations,

$$\left. \begin{aligned} \frac{d(E)}{dt} &= -k_1(E)(S) + k_2(ES_1) + k_5(ES_2) \\ \frac{d(S)}{dt} &= -k_1(E)(S) + k_2(ES_1) \\ \frac{d(ES_1)}{dt} &= k_1(E)(S) + k_4(ES_2) - (k_2 + k_3)(ES_1) \\ \frac{d(ES_2)}{dt} &= k_3(ES_1) - (k_4 + k_5)(ES_2) \\ \frac{d(P)}{dt} &= k_5(ES_2) \end{aligned} \right\} \quad (23)$$

where  $(E)$ ,  $(S)$ ,  $(ES_1)$ ,  $(ES_2)$  and  $(P)$  are concentrations of the corresponding chemical species. The initial condition and the rate constants were identical, for comparison, with those adopted in the previous paper (Okamoto *et al.*, 1975). The initial conditions were

$$\left. \begin{aligned} (E)_0 &= 1.0 \times 10^{-6} \text{ M}, \\ (S)_0 &= 1.0 \times 10^{-4} \text{ M}, \\ (ES_1)_0 &= 0.0 \text{ M}, \\ (ES_2)_0 &= 0.0 \text{ M}, \\ (P)_0 &= 0.0 \text{ M} \end{aligned} \right\} \quad (24)$$

and the rate constants were

$$\left. \begin{aligned} k_1 &= 5 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}, \\ k_2 &= 5 \text{ sec}^{-1}, \\ k_3 &= 10^3 \text{ sec}^{-1}, \\ k_4 &= 10^2 \text{ sec}^{-1}, \\ k_5 &= 0.12 \text{ sec}^{-1} \end{aligned} \right\} \quad (25)$$

In the practical computation, the dimension of these rate constants,  $\text{sec}^{-1}$ , was converted into  $\text{min}^{-1}$ : for instance,  $k_1 = 3 \times 10^7 \text{ M}^{-1} \text{ min}^{-1}$ ,  $k_2 = 3 \times 10^2 \text{ min}^{-1}$ , etc.

The computed time-course of the equation (23) with the usual 4th order Runge-Kutta-Gill method is shown in Fig. 4. It is observed in Fig. 4 that the solutions can be separated into two, highly and lower sensitive, parts for time in the early stage of the reaction, i.e., rapid formations of  $ES_1$  and  $ES_2$ , rapid decrease of  $E$ , and relatively slow formation of  $P$  took place together in the early stage. The stepsize  $h$  went down to about  $0.48 \times 10^{-4} \text{ min}$  in the case of the 4th order Runge-Kutta-Gill method with a non-negative constraint condition (Okamoto *et al.*, 1975), and about 284,400 computation steps were consumed

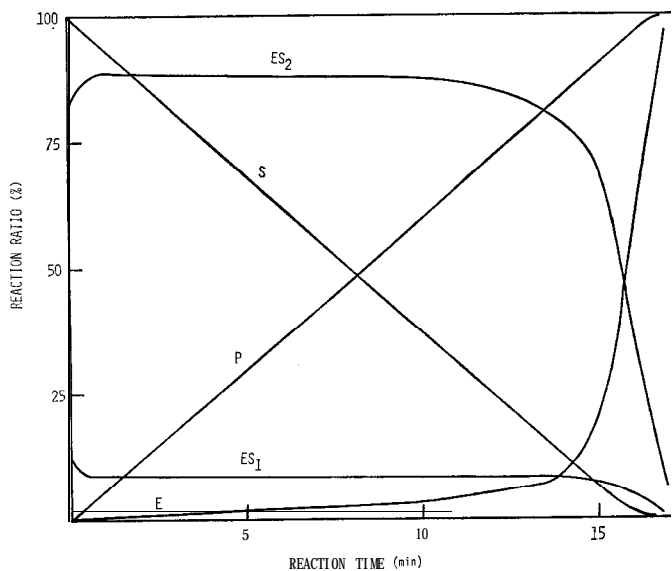


Fig. 4. Calculated time-courses of the enzymatic reaction with substrate. The concentrations (the ordinate) are normalized to the relative ratios:  $E = (E)/(E)_0$ ,  $ES_1 = (ES_1)/(E)_0$ ,  $ES_2 = (ES_2)/(E)_0$ ,  $S = (S)/(S)_0$ , and  $P = (P)/(S)_0$ .

until 81.7 % formation of product.

In the present computation, the deviation  $\epsilon_i$  is defined as an index of the relative error

$$\epsilon_i = 100 | (E)_0 - \{ (E)_c + (ES_1)_c + (ES_2)_c \} | / (E)_0 \quad (26)$$

where subscript c indicates the computed value. Figure 5 shows that the deviation  $\epsilon_i$  from the conservation law of mass have highly propagated with steps in use of the Fowler-Warten method without modifications.  $\epsilon_i$  has grown up to about 8,000 % during 5,000 computation steps. It is clear from Fig. 6 that the

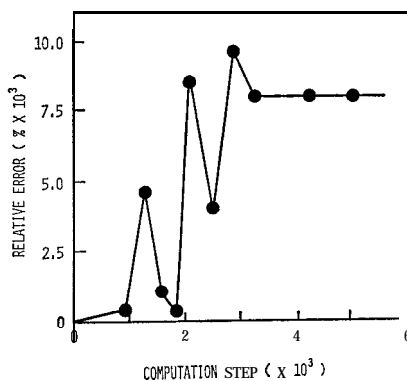
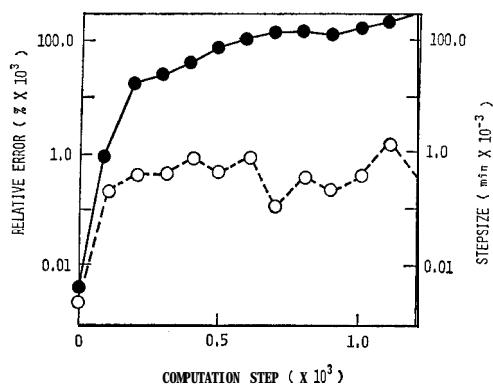


Fig. 5. Error propagation.



**Fig. 6.** Relationship between the error propagation and the stepsize increase. The stepsize is expressed by the broken line, and the computation error by the solid line. The ordinates are both logarithmic.

propagation of  $\varepsilon_t$  caused by the unreasonable increase in stepsize  $h$ . When the stepsize  $\delta$  and  $h$  were always halved independently of the values of  $E_\delta$  and  $E_h$ , the error  $\varepsilon_t$  decreased obviously. Then the stepsizes were set small from the first step. However,  $\varepsilon_t$  has still highly grown up. It was found that this behavior was caused by setting the extremely small error estimates  $E_\delta$  and  $E_h$  in computation.

This trouble could be avoided conveniently, as stated in the previous section, by multiplying  $E_\delta$  and  $E_h$  by factor  $F$ . The error decreased remarkably with increase in the value of the factor  $F$  as shown in Fig. 7. Figure 8 shows the mode of variation of the stepsize  $h$  and the error  $\varepsilon_t$  for  $F=10^9$ . The initial stepsize decreased rapidly in the early computation steps. Then the stepsize  $h$  and the error  $\varepsilon_t$  were grown up to nearly constant values. It should be noted that in Figs. 5-9, the abscissae do not express the practical reaction times but the numbers of computation step. The number of computation step is related to the computation time. Therefore, the practical reaction time was computed by using the stepsize as

$$t_n = t_0 + \sum_{i=1}^n h_i \quad (27)$$

The percentage of product formation vs. number of computation step, as the index of the computation speed, was plotted with several  $F$  values in Fig. 9. In the case of  $F=1$ , about 50 % of product was formed at the 1,900th computation step. In other case of  $F=10^9$ , however, only about 12 % of product was formed at the 10,000th computation step.

The effects of the factor  $F$  on number of computation steps (at 81.7 % product formation) and the error  $\varepsilon_t$  are summarized in Table 1. It is shown in Table 1 that the computation speed (not CPU time but computation step) was greatly shortened with reasonable error by the modified Fowler-Warten method as compared with the explicit 4th order Runge-Kutta-Gill method. The more stiff rate equation with  $k_1=5 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$  was also solved in short time by an

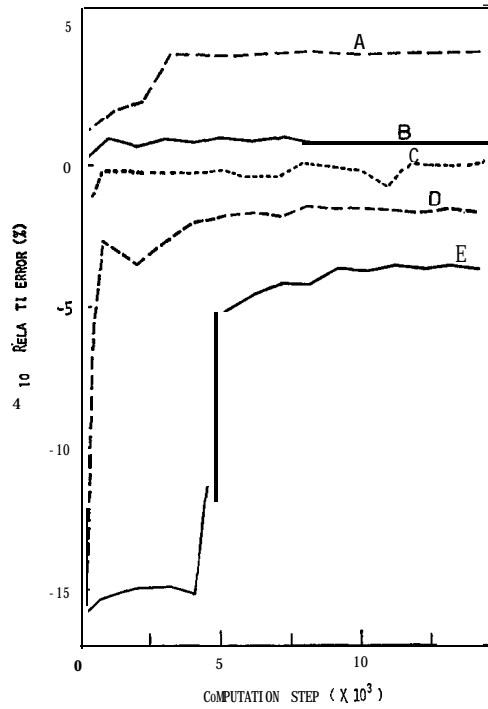


Fig. 7. Relationship between the factor and the error propagation. Values of the factor were from top to bottom 1 (A),  $10^3$  (B),  $10^5$  (C),  $10^7$  (D), and  $10^9$  (E).

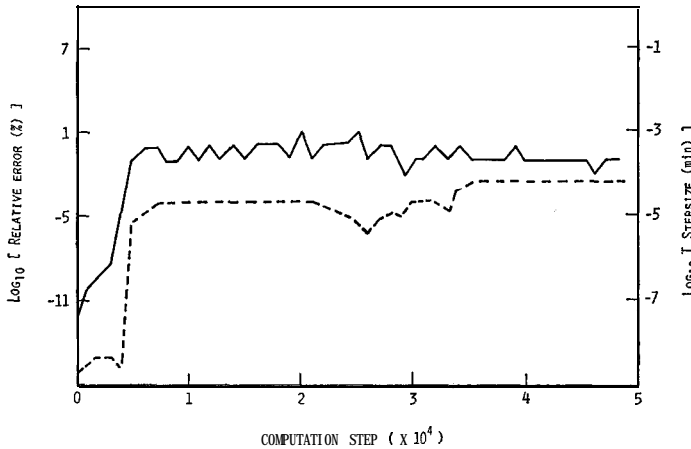
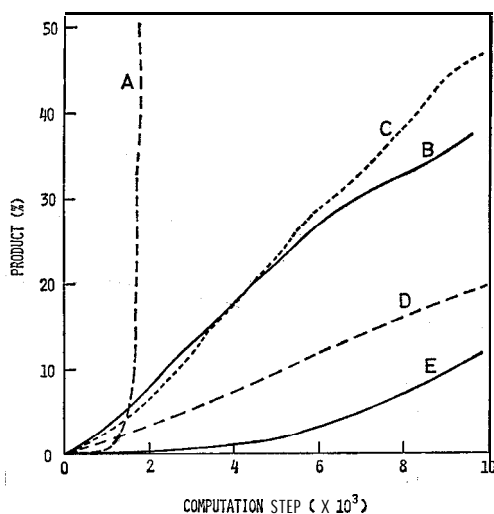


Fig. 8. Error and stepsize for the value of the factor, 109. The stepsize is expressed by the broken line, and the error by the solid line.

appropriate adjustment of the value of the factor  $F$ . As the Fowler-Warten method is an explicit formula, the computation procedure has the virtue of



**Fig. 9.** Relationship between the factor and the computation speed. The computation speed is expressed by the percentage of the product formation. Values of the factor (A-E) were the same as in Fig. 7.

**Table 1.** Effects of the factor on computation speed and error.

Factor	Computation steps	Relative error (%)
1	2150	8000
$10^{+3}$	17600	40
$10^{+5}$	26700	0.8
$10^{+7}$	35900	0.02
$10^{+9}$	49500	0.0027
Runge-Kutta-Gill Method	284400	—

The computation step and the relative error are those just at the time when 81.7% of product is formed.

simplicity. The techniques for the automatic evaluation of factor  $F$  and a suppression of the propagation of the error  $\epsilon_i$  are being investigated.

## REFERENCES

- Curtis, A. R. and E. M. Chance 1972 Numerical methods for simulation and optimization. *In* "Analysis and Simulation of Biochemical Systems,\* Vol. 25, organized by H. C. Hemker and B. Hess, North-Holland Pub. Corn., Amsterdam, pp. 39-57
- Dahlquist, G. 1974 Problems related to the numerical treatment of stiff differential equations. *In* "International Computing Symposium 1973," North-Holland Pub. Corn., Amsterdam, pp. 307-314
- Fowler, M. E. and R. M. Warten 1967 A numerical integration technique for ordinary differential equations with widely separated eigenvalues. *IBM J. Res. Develop.*, **11**: 537-543
- Hemker, P. W. 1972 Numerical methods for differential equations in system simulation and in parameter estimation. *In* "Analysis and Simulation of Biochemical Systems," Vol.

- 25**, organized by H. C. Hemker and B. Hess, North-Holland Pub. Corn., Amsterdam, pp. 59-80
- Henrici, P. 1962 **Discrete Variable Methods in Ordinary Differential Equations**. John Wiley & Sons, Inc., New York
- Okamoto, M., Y. Aso, D. Koga and K. Hayashi 1975 Note on steady-state approximation of enzyme kinetics. **J. Fac. Agr., Kyushu Univ., 19: 125-138**
- Reich, J. G. 1969 Aims, applications and achievements, an introductory essay. **FEBS Letters, 2: S3-S8**