Biophysical Chemistry 18 (1983) 73–87
Elsevier

BPC 00790

Review

SIMPLIFYING PRINCIPLES FOR CHEMICAL AND ENZYME REACTION KINETICS

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Received 26th January 1983
Accepted 14th April 1983

Key words: Steady-state approximation; Singular perturbation system; Simplifying principles; Tihonov's Theorems; Korzuhin's Theorem

Tihonov's Theorems for systems of first-order ordinary differential equations containing small parameters in the derivatives, which form the mathematical foundation of the steady-state approximation, are restated. A general procedure for simplifying chemical and enzyme reaction kinetics, based on the difference of characteristic time scales, is presented. Korzuhin's Theorem, which makes it possible to approximate any kinetic system by a closed chemical system, is also reported. The notions and theorems are illustrated with examples of Michaelis-Menten enzyme kinetics and of a simple autocatalytic system. Another example illustrates how the differences in the rate constants of different elementary reactions may be exploited to simplify reaction kinetics by using Tihonov's Theorem. All necessary mathematical notions are explained in the appendices. The most simple formulation of Tihonov's 1st Theorem 'for beginners' is also given.

1. Introduction

In their recent paper, Kijima and Kijima [1] stated in section 1 that "the steady-state assumption or steady-state treatment in chemical reaction kinetics (...) has been used so far without proof." And once again in section 3 "... there has been no general study on the condition when the steady-state approximation holds even on the first-order reaction."

It seems that the authors are not familiar with some important work of such authors as A.N. Tihonov, L.S. Pontryagin, A.B. Vasil'eva, V.F. Butuzov, V.M. Volosov, I.S. Gradstein and V. Vazov. Especially in Tihonov's work [2], the mathematical problems which are the very basis of the steady-state approximation (i.e., the theory of systems of ordinary first-order differential equations (SFO) containing small parameters in the derivatives) are considered in a very general manner. The theorems he proved therein, called by other Soviet authors Tihonov's Theorems, may be applied to systems with reactions of any order and with any number of components.

In appendix A we reproduce the English summary of Tihonov's paper [2], by J.L. Massera from Mathematical Reviews [3].

It is interesting to note that the theory of ordinary differential equations with slowly varying coefficients (see, for example, ref. 4) is in some sense equivalent to the theory of differential equations containing small parameters in the derivatives. For example, the equation

$$\mu \mathrm{d}x/\mathrm{d}t' = p(t')x \quad (\mu \ll 1) \tag{1}$$

by the transformation of the independent variable (time)

$$t = t'/\mu \tag{2}$$

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N.B. In different sources the name of A.N. Tihonov is often written as Tikhonov, Tichonov, Tichonoff, Tychonoff, etc.

is transformed into the form

$$dx/dt = p(\mu t)x \tag{3}$$

where now the new coefficient, $p(\mu t)$, varies slowly. However, the formulation of Tihonov's Theorems has so far been known to the author only in Russian [2,5,6] and reported in Polish [7,8]. As far as the literature in English is concerned, even in the specialist book by Murray [9], singular perturbation systems are treated heuristically with only a brief mention of Tihonov's rigorous proof [2] and a reference to Vasil'eva's work [10]. The latter seems to be the only source in English (apart from the above-mentioned summary [3]) in which Tihonov's Theorem is formulated and prove (and some similar, more sophisticated mathematical cases are discussed, everything being treated in a rather complicated manner); even there the more general theorem proven by Tihonov in ref. 2 (Tihonov's 2nd Theorem) is not reported at all.

As the steady-state approximation is widely used in scientific literature concerning, e.g., chemical relaxation (see ref. 11) and enzyme kinetics (see ref. 12), we think that it would be interesting to restate here Tihonov's Theorems (without proofs). We will follow Tihonov's original paper [2]. The notions used there are well known to the specialist. But, for the convenience of our readers, we give in appendix B the definitions of all the mathematical terms used.

In ref. 2, Tihonov proved two theorems, one for an SFO, not necessarily autonomous (see appendix C), containing a small parameter in some derivatives, and another for SFO containing several small parameters in the derivatives. We shall call them Tihonov's 1st Theorem and Tihonov's 2nd Theorem, respectively. The 1st Theorem is in reality a special case of the more complicated 2nd Theorem. However, because of its greater simplicity and wide applicability we give here also the 1st Theorem. Moreover, in appendix D we provide possibly most simple formulation of the 1st Theorem (according to ref. 5) 'for beginners'.

2. Tihonov's 1st Theorem

Consider an SFO with one small parameter μ:

$$dx/dt = f(x, z, t) \tag{4a}$$

$$\mu dz/dt = F(x, z, t) \tag{4b}$$

and its solution, determined by initial conditions:

$$x(t^0) = x^0, \quad z(t^0) = z^0 \tag{5}$$

where $x = (x_1, \ldots, x_n)$, $x^0 = (x_1^0, \ldots, x_n^0)$ and $f = (f_1, \ldots, f_n)$ are vectors in n-dimensional space, whereas $z = (z_1, \ldots, z_s)$, $z^0 = (z_1^0, \ldots, z_s^0)$, $F = (F_1, \ldots, F_s)$ are vectors in s-dimensional space. Putting $\mu = 0$ in eq. 4, one obtains the *degenerate system*

$$dx/dt = f(x, z, t): \quad x(t^0) = x^0 \tag{6a}$$

$$z = \phi(x, t) \tag{6b}$$

where $z = \phi(x, t)$ is a root of the system of algebraic equations $F(x, z, t) = 0$ or

$$F_l(x, z, t) = 0 \quad (l = 1, \ldots, s) \tag{7}$$

The system of equations

$$dz/d\tau = F(x, z, t): \quad z(t^0) = z^0 \tag{8}$$

in which both x and t are taken as parameters, is called the *adjoined system*. Of course, the point $z = \phi(x, t)$ is an isolated singular point (root) of the adjoined system, as all terms on the right-hand sides of eq. 8 are nullified at this point.

We shall assume further that all functions we use are continuous ones and that the differential equations we consider have uniquely determined solutions.

The aim of the work [2], i.e., the investigation of the solution of the SFO (eq. 4) with initial conditions (eq. 5) when $\mu \to 0$ is summarized in

Tihonov's 1st Theorem

When $\mu \to 0$ the solution of the original system (eq. 4), with conditions (eq. 5), tends to the solution of the degenerate system (eq. 6) if:

(1) the root $z = \phi(x, t)$ is the stable root of the adjoined system;

(2) the initial values z^0 lie in the domain of influence of the root $z = \phi(x, t)$ for initial values (x^0, t^0).

This asymptotic equality remains valid for all times t for which the solution of the adjoined system lies inside the stability domain D of the root $z = \phi(x, t)$.

(For the definitions of an isolated, stable root and of the domain of influence, according to Tihonov [2], see appendix B; for the conditions of existence, uniqueness and stability of solutions of an SFO see appendix C.)

This theorem remains valid also if the right-hand sides of eq. 4 depend continuously on the parameter μ, i.e., for the system

$$dx/dt = f(x, z, t, \mu) \tag{4a'}$$

$$\mu dz/dt = F(x, z, t, \mu) \tag{4b'}$$

where f and F are continuous functions of their arguments [6].

As the asymptotic procedure lowers the order of the SFO, the initial conditions (eq. 5) generally may not be fulfilled by the solutions of the asymptotic (degenerate) system. The solution of the original system may be approximated by the solution of the degenerate system for times $t \gg t_d$, where $t_d \approx |\mu \ln \mu|$ [6]. If the asymptotic system is of the second order, one may make a complete discussion of its, based e.g., on the phase-plane methods [5–8].

3. Tihonov's 2nd Theorem

Consider now an SFO with several small parameters $\mu^{(j)}$,

$$dx/dt = f(x, z^{(1)}, \ldots, z^{(m)}, t) \tag{9a}$$

$$\mu^{(j)} \cdot dz^{(j)}/dt = F^{(j)}(x, z^{(1)}, \ldots, z^{(m)}, t); \quad (j = 1, \ldots, m) \tag{9b}$$

and its solution determined by the initial conditions

$$x(t^0) = x^0, \quad z^{(j)}(t^0) = z^{0(j)} \quad (j = 1, \ldots, m) \tag{10}$$

where $x = (x_1, \ldots, x_n)$, $x^0 = (x_1^0, \ldots, x_n^0)$ and $f = (f_1, \ldots, f_n)$ are vectors in n-dimensional space, whereas $z^{(j)} = (z_1^{(j)}, \ldots, z_{s_j}^{(j)})$, $z^{0(j)} = (z_1^{0(j)}, \ldots, z_{s_j}^{0(j)})$ and $F^{(j)} = (F_1^{(j)}, \ldots, F_{s_j}^{(j)})$ are vectors in s_j-dimensional spaces, respectively (for $j = 1, \ldots, m$).

Tihonov investigated the solution of eq. 9 with the initial conditions, eq. 10, when all $\mu^{(j)} \to 0$ in such a way that

$$\mu^{(j+1)}/\mu^{(j)} \to 0 \tag{11}$$

Putting in eq. 9 $\mu^{(m)} = 0$, one obtains the *singly degenerate system of the first order*:

$$dx/dt = f(x, z^{(1)}, \ldots, z^{(m)}, t) \tag{12a}$$

$$\mu^{(j)} dz^{(j)}/dt = F^{(j)}(x, z^{(1)}, \ldots, z^{(m)}, t) \quad (j = 1, \ldots, (m-1)) \tag{12b}$$

$$z^{(m)} = \phi^{(m)}(x, z^{(1)}, \ldots, z^{(m-1)}) \tag{12c}$$

with initial conditions

$$x(t^0) = x^0; \quad z^{(j)}(t^0) = z^{0(j)} \quad (j = 1, \ldots, (m-1)) \tag{13}$$

where $z^{(m)} = \phi^{(m)}(x, z^{(1)}, \ldots, z^{(m-1)}, t)$ is a root of the system of algebraic equations $F^{(m)}(x, z^{(1)}, \ldots, z^{(m)}, t) = 0$, i.e.

$$F_{l_m}^{(m)}(x, z^{(1)}, \ldots, z^{(m)}, t) = 0 \quad (l_m = 1, \ldots, s_m) \tag{14}$$

The system of equations

$$dz^{(m)}/d\tau = F^{(m)}(x, z^{(1)}, \ldots, z^{(m)}, t); \quad z^{(m)}(0) = z^{0(m)} \tag{15}$$

in which $x, z^{(1)}, \ldots, z^{(m-1)}, t$ are taken as parameters, is called the *adjoined system of the first order*.

The system is termed *doubly degenerate* if it is a singly degenerate system for a degenerate system of the first order. The degenerate system of the first order has $(m-1)$ small parameters $\mu^{(j)}$ $(j = 1, \ldots, (m-1))$; by putting $\mu^{(m-1)} = 0$ in this system, one obtains a degenerate system of the second order and similarly the definitions of the other notions of the second order. Analogously, one defines degenerate systems of the k-th order and all other notions of the k-th order.

The behavior of the solution of the original system (eq. 9) with initial conditions, eq. 10, when all $\mu^{(j)} \to 0$ are under the condition eq. 11, is summarized in

Tihonov's 2nd Theorem

When $\mu^{(j)} \to 0$ the solution of the whole original system (eq. 9), with the initial conditions (eq. 10), tends to the solution of the degenerate (m-times degenerate) system if:

(1) the roots $z^{(j)} = \phi^{(j)}$, with the aid of which the degenerate system is defined, are stable roots of adjoined equations of the j-th order for any j $(1 \leqslant j \leqslant m)$;

(2) the initial values $z^{0(j)}$ are in the domain of influence of the roots $z^{(j)} = \phi^{(j)}$ for initial values $(x^0, z^{0(1)}, \ldots, z^{0(j-1)}, t^0)$.

The asymptotic equalities remain valid for all

times t_* for which the solution of the totally degenerate system $\bar{x}(t)$, $\bar{z}^{(j)}(t)$, lies inside the stability domains of the roots $z^{(j)} = \phi^{(j)}$ for all j.

4. Chemicals systems. Korzuhin's Theorem

Tihonov's 1st and 2nd Theorems have a direct application in the simplification of kinetic equations. From the law of mass action (LMA) one obtains generally an SFO with right-hand sides being polynomials of orders not greater than the second, i.e., the reactions are uni- and bimolecular. In some chemical reaction models trimolecular reactions are also assumed (e.g., in the 'Brusselator' model [13]), which leads to terms of the third order. But, for applying Tihonov's Theorems, neither linearity nor positiveness of variables, nor even autonomy of the SFO (see appendix B) is required.

First of all, it is necessary to take into account the reagents for which the system is closed – for such reagents (or groups of reagents) a mass conservation law (MCL) holds. Biochemical systems are very often closed for some macromolecular components, e.g., enzymes and enzymatic complexes:

$$E + \sum_c E_c = E_0 \tag{16}$$

where E_0 is the total enzyme concentration, E the actual concentration of free enzyme, E_c the actual concentration of the c-th enzyme complex and the summation is taken over all complexes present in the system. For simplicity here after we denote a reagent and its molecular concentration by the same symbol, a capital Latin letter. If one differentiates eq. 16, one obtains

$$\mathrm{d}E/\mathrm{d}t' + \sum_c \mathrm{d}E_c/\mathrm{d}t' = 0 \tag{17}$$

From eq. 17 it is seen that the kinetic equations for a group of reagents for which the MCL exists (e.g., for an enzyme and its complexes) are linearly dependent. So, if the SFO has been primarily written down for all reagents present in the system, one equation (for E or one of E_c) must be dropped and replaced by the algebraic equation expressing the MCL (eq. 16). Of course, there may be more than one group of reagents for which an MCL holds.

As far as the reagents for which the system is open are concerned, suitable flux terms, describing an exchange with the environment, must be included in the kinetic equations in addition to the terms arising from chemical reactions. Such terms are usually introduced in the form

$$J_r = k \cdot (R_{\mathrm{out}} - R) \tag{18}$$

where J_r is the flux of component R (positive when R is being supplied to the system, negative when it is flowing out of the system), R the actual concentration inside the system, and R_{out} the actual concentration outside the system, i.e., in the environment. Usually, R_{out} is assumed to be independently controllable (a so-called control variable). A special case, but one which is very often utilized, is to keep R_{out} constant:

$$R_{\mathrm{out}} = R_0 \tag{19}$$

R_0 is called the reservoir concentration. Then the flux term (eq. 18) contributes to the right-hand side of the kinetic equation for R a constant term equal to

$$J_r^+ = kR_0 \tag{20}$$

and a linear term

$$J_r^- = -kR \tag{21}$$

R_{out} may also change with time, but the time dependence $R_{\mathrm{out}}(t)$ must be known. In such a case the SFO is no longer an autonomous one, but Tihonov's Theorems still may be applied.

If there are N reagents in the system under consideration and there are g groups for which MCL are fulfilled (the h-th group composed of N_h reagents, $h = 1, \ldots, g$), then the system of kinetic equations to be solved is as follows:

$$\mathrm{d}R_i/\mathrm{d}t' = k_i + \sum_{j=1}^{N} k_i^j R_j + \sum_{j,l=1}^{N} k_i^{jl} R_j R_l + \sum_{j,l,m=1}^{N} k_i^{jlm} R_j R_l R_m \quad (i = 1, \ldots, (N-g)) \tag{22a}$$

$$R_i = R_0^{(h)} - \sum_{p_h=1}^{N_h} R_{p_h} \quad (i = (N-g+1), \ldots, N) \quad (h = 1, \ldots, g) \tag{22b}$$

where h differential equations have been replaced by algebraic ones of the type in eq. 16. Hereinafter t' denotes real time and R_i the actual concentration of the i-th component; the summation over p_h denotes summation over all N_h components belonging to the h-th group for which an MCL is fulfilled and $R_0^{(h)}$ is the total concentration of this group of reagents. The R_{p_h} are present also on the right-hand sides of eq. 22a as their summations are taken over all N components present in the system.

As usual, each kinetic constant (k_i, k_i^j, k_i^{jl}, k_i^{jlm}) is taken as having a positive or negative sign or being equal to zero if, respectively, R_i is produced, consumed or is not involved at all in the given elementary step (i.e., in reaction with R_j, R_l, etc., or the flux term). The terms k_i arise from the influx (eq. 20) or from the decomposition reactions of the zero-th order (e.g., one may assume that the reagent is among other things a substrate for an enzyme and that the enzyme is saturated with the substrate so that the reaction rate is maximal and does not depend on the concentration of the reagent). The terms $k_i^j R_j$ arise from the outflux (eq. 21) or from decomposition reactions of the first order.

An SFO like eq. 22a is called a *closed chemical system* (CCS) if the following conditions are fulfilled [5]:

(1) all R_i are positive (concentrations);

(2) the right-hand sides are of order not greater than two (all k_i^{jlm} and all higher terms are equal to zero, i.e., at most bimolecular interactions are present);

(3) no autocatalytic terms are present, i.e., all k_i', k_i'' and k_i'' possess negative signs or are equal to zero;

(4) the system is closed, i.e., there is neither influx (all $k_i = 0$) nor outflux and so the mass conservation law

$$\sum_{i=1}^{N} \rho_i R_i = \text{constant}$$

is valid, where ρ_i denotes the molecular mass of the i-th component;

(5) no elementary reactions of decomposition of the zero-th order are present, i.e., in eq. 22 k_i' and k_i^{jl} are nonnegative for all $j \neq i$, $l \neq i$;

(6) in elementary reactions all stoichiometric coefficients are equal to unity or zero, i.e., even the reaction A → 2B is treated as a nonelementary one but as a result of a chain of elementary reactions, e.g., A → B + C, C → B; so the kinetic constants in eq. 22 do not contain factors related to stoichiometric coefficients, since one assumes that in a CCS only elementary reactions occur.

Some authors (e.g., see ref. 5) define a CCS using only conditions 1–4. It is interesting to report here the theorem proven by Korzuhin (see refs. 5 and 6):

Korzuhin's Theorem

It is always possible to construct a closed chemical system of kinetic equations, in which the behavior of some variables will coincide with any desired accuracy and for any desired time period with the behavior of a given system

$$dR_i/dt' = \Psi_i(R_1, \ldots, R_N) \quad (i = 1, \ldots, N)$$

where Ψ_i are polynomials of nonnegative integer powers.

For an open system the MCL (eq. 23) is not fulfilled. But any system may always be 'extended to a closed system' by introducing coupled reservoirs of the necessary reagents. Additional variables have to be introduced to fulfill also other conditions imposed on the system (eq. 22) to be a CCS. Korzuhin's Theorem is, in some sense, the inverse of Tihonov's Theorems as it concerns the problem of construction of a whole ('original') SFO for the given system treated as a degenerate one. The SFO eq. 22a, with conditions 1–6, represents a homogeneous CCS which is in general the simplest possible nonlinear SFO. The importance of Korzuhin's Theorem lies in the fact that it demonstrates the possibility of realization of any given behavior (in particular, of stable auto-oscillations) of a part of the reagents during any given time period just in a homogeneous CCS if only the number of variables (reagents) is sufficiently large.

In heterogeneous (e.g., compartmental) systems some of the conditions 1–6 are no longer valid, therefore the SFO representing such systems are more complicated. Complex behavior may be ob-

served much more frequently in heterogeneous systems than in homogeneous ones as the number of variables may be then considerably smaller.

Korzuhin's algorithm – treating any 'non-chemical' term in the SFO as a complex chemical reaction which unfolds in a chain of elementary reactions – makes it possible to inspect quickly and effectively the different model variants for any given complex chemical or biochemical system. At the same time it allows one to understand better such extremely important phenomena as, for example, autocatalysis. We discuss later the application of Korzuhin's Theorem to a simple autocatalytic model and its connections with Tihonov's Theorems.

5. General procedure to simplify kinetic equations

Small parameters appear in (bio)chemical kinetic systems in a natural way if one represents variables in a normalized dimensionless form. To do this one puts in eq. 22

$$R_i = r_i R_i^{(0)} \tag{23}$$

where r_i denotes the dimensionless normalized concentration of the i-th component and $R_i^{(0)}$ is a constant having the dimension of concentration. As $R_i^{(0)}$ one may take, for example, the total concentration of a group of reagents for which an MCL is fulfilled (as E_0 in eq. 16 and $R_0^{(h)}$ in eq. 22b), the Michaelis constant for an enzyme, the inhibition constant for an inhibitor, constant concentration in the environment (reservoir value, as R_0 in eq. 20), initial concentration R_i^0, or concentration $\bar{R}_i$ that the given component would have in the steady state, etc. Small parameters appear also in a kinetic system if some elementary reaction steps are rapid as compared to others.

Taking into account eq. 23, one obtains eq. 22 in the form

$$\mathrm{d}r_i/\mathrm{d}t' = \kappa_i + \sum_{j=1}^{N} \kappa_{ij} r_j + \sum_{j,l=1}^{N} \kappa_{ijl} r_j r_l + \sum_{j,l,m=1}^{N} \kappa_{ijlm} r_j r_l r_m \quad (i = 1, \ldots, (N-g)) \tag{24a}$$

$$r_i = 1 - \sum_{p_h=1}^{N_h} r_{p_h} \quad (i = (N-g+1), \ldots, N) \tag{24b}$$

where now all kinetic constants have the dimension [time]$^{-1}$

$$\kappa_i = k_i / R_i^{(0)} \tag{25a}$$

$$\kappa_{ij} = k_{ij} R_j^{(0)} / R_i^{(0)} \tag{25b}$$

$$\kappa_{ijl} = k_{ijl} R_j^{(0)} R_l^{(0)} / R_i^{(0)} \tag{25c}$$

As a rule, the values for $R_i^{(0)}$ in eqs. 24 are such taken that the dimensionless variables r_i are between 0 and 1 and of the order of unity at most. Then the most important term in any equation of the form of eq. 24a is the one for which the constant (κ_i, κ_{ij}, κ_{ijl}, ...) has the greatest absolute value, γ_i. Dividing the i-th equation by γ_i one obtaines from eq. 24a

$$T_i \cdot \mathrm{d}r_i/\mathrm{d}t' = a_i' + \sum_{j=1}^{N} a_{ij}' r_j + \sum_{j,l=1}^{N} a_{ijl}' r_j r_l + \sum_{j,l,m=1}^{N} a_{ijlm}' x_j x_l x_m \quad (i = 1, \ldots, (N-g)) \tag{26}$$

where

$$T_i = 1/\gamma_i \tag{27}$$

denotes the i-th characteristic time and where all constants

$$a_i' = \kappa_i / \gamma_i \tag{28a}$$

$$a_{ij}' = \kappa_{ij} / \gamma_i \tag{28b}$$

$$a_{ijl}' = \kappa_{ijl} / \gamma_i \tag{28c}$$

are now less or much less than unity and only the one for which κ was selected for defining the time T_i (eq. 27) is exactly equal to unity.

If we are interested in the behavior of the system in times of a certain specified order, say of the order of T, we introduce the dimensionless time variable t by taking T as the unit of time:

$$t = t'/T \tag{29}$$

Then, changing the differentiation with respect to t' by differentiation with respect to dimensionless time, t

$$\frac{\mathrm{d}}{\mathrm{d}t'} = \frac{\mathrm{d}t}{\mathrm{d}t'} \cdot \frac{\mathrm{d}}{\mathrm{d}t} = \frac{1}{T} \cdot \frac{\mathrm{d}}{\mathrm{d}t} \tag{30}$$

denoting

$$T_i/T = m_i \tag{31}$$

and eliminating from the right-hand sides of eqs. 26 the variables $r_{N-g+1},\ldots,r_N$ by using eqs. 24b, one obtains finally the system

$$m_i \cdot \mathrm{d}r_i/\mathrm{d}t = a_i + \sum_{j=1}^{P} a_{ij} r_j + \sum_{j,l=1}^{P} a_{ijl} r_j r_l + \sum_{j,l,m=1}^{P} a_{ijlm} r_j r_l r_m \quad (i = 1,\ldots,P) \tag{32}$$

where $P = (N - g)$.

In short-hand notation

$$m_i \dot{r}_i = \Psi_i(r_1,\ldots,r_P) \tag{33}$$

where hereinafter the dotted variable denotes differentiation with respect to the dimensionless time variable t and Ψ_i denotes the right-hand side of the i-th equation, eqs. 32.

If the time scale T we are interested in is such that some m_i are much greater than unity, say these q with indexes $i = (p+1), (p+2),\ldots, P$, then, by dividing the corresponding equation by m_i, one finds that the time derivatives of these variables

$$\dot{r}_i = \frac{1}{m_i} \cdot \Psi_i(r_1,\ldots,r_P) \quad (i = (p+1),\ldots,P) \tag{34}$$

are close to zero, since $1/m_i \ll 1$. They are called *very slow* or *reservoir variables*, i.e., changing only with characteristic times $T_i \gg T$. For time duration of order T they remain practically unchanged and may be replaced by their initial values

$$r_i(0) = r_i^0 = A_i, \quad (i = (p+1),\ldots,P) \tag{35}$$

(where $(P - p) = q$) and be treated as parameters. This is equivalent to treating the system as an open one [6]. In this way eqs. 33 are reduced to

$$m_i \dot{r}_i = \Psi_i(r_1,\ldots,r_p; A_{p+1},\ldots,A_P) \quad (i = 1,\ldots,p) \tag{36}$$

The system may now be further reduced using Tihonov's Theorems. The equations are classified in such a way that the first class contains all, say n, equations whose relative time scale parameter m_i is of the order (equal to) unity (i.e., the kinetic equations for variables for which characteristic times T_i are of the same order as T) – these are so called *slow* or *basic variables*

$$\dot{r}_i = \Psi_i(r_1,\ldots,r_p; A_{p+1},\ldots,A_P) \quad (i = 1,\ldots,n) \tag{37a}$$

The remaining s equations ($s = p - n$) are classified into m classes in such a way that into successive classes go the equations having smaller and smaller parameters m_i (i.e., the kinetic equations for variables for which characteristic times T_i are smaller and smaller than the time unit T). Into the j-th class ($j = 1,\ldots,m$) go s_j equations. The small parameters (much less than unity) we will denote further by $\mu^{(j)}$ or just by μ. So

$$\mu^{(j)} \dot{r}_{l_j}^{(j)} = \Psi_{l_j}^{(j)}(r_1,\ldots,r_n; r_{n+1},\ldots,r_p; A_{p+1},\ldots,A_P) \quad (l_j = 1,\ldots,s_j; j = 1,\ldots,m) \tag{37b}$$

where

$$n + \sum_{j=1}^{m} s_j = n + s = p \tag{37c}$$

and $\mu^{(j)}$ fulfill eqs. 11, i.e., they are small parameters of greater and greater degree. These groups of variables are called *quick variables* – after a time of order even much smaller than that of T they reach their (quasi)stationary values. In the following the quick, basic and reservoir variables will be denoted by z, x and y, respectively.

The separation of reservoir variables is often made to some extent automatically, based on the fact that the components having very great concentrations as compared to others (that is exactly why they are called 'reservoir variables') change much slower than other components. If we consider the simplest bimolecular reaction $A + X \xrightarrow{k} B$, it is easy to see that in any moment the instantaneous transformation rates are $\mathrm{d}X/\mathrm{d}t \propto -\exp(-t/t_X)$ and $\mathrm{d}A/\mathrm{d}t \propto -\exp(-t/t_A)$ where $t_X = 1/kA$ and $t_A = 1/kX$ are instantaneous time constants for X and A, respectively. If one now assumes that $A \gg X$ then $t_A \gg t_X$, i.e., in any instant A changes much slower than X. So, if one assumes that some variables are reservoir ones, it is equivalent to treat them as parameters – they may be replaced in eqs. 22 by their constant (initial) values (cf. eqs. 35). The differential equations for these variables may be dropped from the very beginning before introducing dimensionless concentrations and time variables and separating basic and quick variables. In such a case the constant values of these reservoir variables, treated as parameters, are often useful to define the time

unit T_i and/or the small parameters $\mu^{(j)}$ or are included directly in kinetic constants.

The system eqs. 37, when taken together with the necessary initial conditions, is identical with eqs.9 and 10 (or with eqs. 4 and 5 for $m = 1$) and Tihonov's Theorem may be used to simplify it. The conditions of continuity and of uniqueness of solutions (see appendix C) are of course fulfilled for the SFO, eqs. 32), and thus also for eqs. 37, whereas the stability of a solution (see appendices B and C) must be verified in each particular case. However, if the system eqs. 4b or 4b′ is linear in quick variables z (this is the case when reactions between quick reagents, e.g., between enzyme complexes, may be neglected), then the algebraic system eqs. 7 is a linear one and therefore it has the unique solution; the adjoined system eqs. 8 has then the unique stationary root. This root is stable if there are no bifurcations, i.e., no reactions of decomposition of the type $Z_s \rightarrow Z_j + Z_l$ of the quick reagents [6]. In such a case, before applying Tihonov's 1st Theorem one must verify only a condition concerning the initial values, namely, whether they lie in the domain of influence of the root (eq. 6b).

6. Examples

As the first example, let us take the simplest enzymatic reaction – the mechanism of Michaelis and Menten:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} (ES) \xrightarrow{k_2} P + E \tag{38}$$

with four components: substrate S, enzyme E, enzyme-substrate complex (ES) and product P. In this mechanism no fluxes are taken into account and so there exist two MCL:

$$E + (ES) = E(0) = E_0 \tag{39a}$$

$$S + P + (ES) = S(0) = S_0 \tag{39b}$$

As a result, there are only two independent differential kinetic equations. The characteristic time constants for substrate and enzyme are, respectively,

$$T_1 = 1/k_1E_0; \quad T_2 = 1/k_1S_0 \tag{40}$$

If we are interested in times of order T_2 we obtain [14]

$$dx/dt = -x + (k - \beta)z + xz \tag{41a}$$

$$\mu dz/dt = x - kz - xz \tag{41b}$$

with initial conditions

$$x(0) = 1; \quad z(0) = 0 \tag{41c}$$

where

$$t = t'/T_2 = k_1E_0t'; \quad x(t) = S/S_0; \quad z(t) = (ES)/E_0 \tag{42a}$$

are dimensionless variables and the constants are defined as follows

$$\mu = T_1/T_2 = E_0/S_0; \quad \beta = k_2/k_1S_0;$$

$$K_M = (k_{-1} + k_2)/k_1; \quad k = K_M/S_0 \tag{42b}$$

t' denotes real time and K_M the Michaelis constant.

If one assumes $S_0 \gg E_0$ then $T_2 \gg T_1$ and $\mu \ll 1$. In such a case the enzyme is the fast component whereas the substrate is the slow one. The degenerate system is here reduced to one equation obtained from eq. 41b. The root

$$\bar{z} = x/(k + x) \tag{43}$$

(here and in eq. 44 x is treated as a parameter!) obtained from eq. 41b when $\mu = 0$ is the stable root of the adjoined system

$$dz/d\tau = x - kz - xz \tag{44}$$

since for any value of x the right-hand side and so the time derivative in eq. 44 is negative for $z > \bar{z}$ (z will therefore decrease until the derivative will be equal to 0, i.e., until $z = \bar{z}$) and positive for $z < \bar{z}$ (in such a case z will increase until $z = \bar{z}$). When the system starts from the initial value $z(0) = 0$, z will increase until $z = \bar{z}$, so the initial conditions (eq. 41c) lie in the domain of influence of the root $z = \bar{z}$.

The assumptions of Tihonov's 1st Theorem are fulfilled and the steady-state approximation may be used: putting $z = \bar{z}$ in eq. 41a, a single equation with one variable x is obtained

$$dx/dt = -\beta x/(k + x) \tag{45}$$

which is easily integrable and gives the transcendental algebraic equation for $x(t)$:

$$x + k \cdot \ln x = 1 - \beta t \tag{46}$$

Returning to the dimensional variables one obtains from eq. 45 the well known Michaelis-Menten expression

$$dS/dt' = -k_2 S E_0/(K_M + S) \tag{47}$$

The steady-state approximation fails, however, for very short times (of the order $t \ll 1/\mu$). One may see that the initial conditions (eq. 41c) can no longer be satisfied in a consistent manner (the authors of ref. 1 speak about induction period, T_0). If we do not want to use the quasi-stationary approximation, the solution of the system eq. 41, is very complicated, based on coordinate transformation of the rate equations and subsequent solution of an integral equation [14].

It has been demonstrated that the steady-state approximation for the Michaelis-Menten mechanism may be applied either when $E_0 \ll S_0$ (irrespective of the values of rate constants) or when the rate constants of decomposition of the enzyme-substrate complex (i.e., k_{-1} and k_2) are much greater than the rate constant of its formation (i.e., k_1) even if the concentrations of enzyme and substrate are of the same order, $E_0 \approx S_0$: the applications of Tihonov's 1st Theorem to the Michaelis-Menten mechanism in different cases are discussed in refs. 5 and 6.

As the second example, let us consider the simplest autocatalytic process, describing an exponential growth

$$dx/dt = x \tag{48}$$

It is not a closed chemical system in the sense of the definition given in section 4. But, following Korzuhin's Theorem, it is possible to find a closed chemical system, the behavior of which will coincide with any desired accuracy with the behavior of eq. 48. One may show that this is the case, for example, for the following system:

$$\begin{aligned} &A + Z_3 \xrightarrow{k_1} Z_1 + Z_2 \\ &B + Z_2 \xrightarrow{k_2} Z_3 + C \\ &Z_1 \xrightarrow{k_3} Z_2 \\ &A + B + C + Z_1 + Z_2 + Z_3 = M_0 = \text{constant} \end{aligned} \tag{49}$$

We assume that A and B are present at very high concentration, i.e., that they are reservoir variables and may be treated as parameters (cf. section 5).

The kinetic equations for Z_1, Z_2 and Z_3 are

$$dZ_1/dt' = -k_3 Z_1 + k_1 A Z_3 \tag{50a}$$

$$dZ_2/dt' = k_3 Z_1 - k_2 B Z_2 + k_1 A Z_3 \tag{50b}$$

$$dZ_3/dt' = k_2 B Z_2 - k_1 A Z_3 \tag{50c}$$

Writing eq. 50 in terms of dimensionless variables

$$z_i = Z_i/M_0 \quad (i = 1, 2, 3) \tag{51}$$

one will observe that the characteristic time constants for Z_1, Z_2, and Z_3 are equal

$$T_1 = 1/k_3; \quad T_2 = T_3 = 1/k_2 B \tag{52}$$

Introducing dimensionless time

$$t = t'/T = k_1 A t' \tag{53}$$

and assuming that

$$k_1 A/k_2 B = \mu; \quad k_1 A/k_3 = \mu \tag{54}$$

(for simplicity we assume that both quotients have identical values) one finds that all m_i (cf. eqs. 32) are equal to μ and so eqs. 50 in dimensionless form becomes

$$dz_1/dt = -z_1 + z_3 \tag{55a}$$

$$dz_2/dt = z_1 - z_2 + z_3 \tag{55b}$$

$$dz_3/dt = z_2 - z_3 \tag{55c}$$

One may demonstrate that when $\mu \to 0$, the solution $z_3(t)$ of the system, eqs. 55, coincides with the solution $x(t)$ of eq. 48 (cf. ref. 5). By changing the variable

$$x = \mu z_3 \tag{56}$$

one obtains from eqs. 55

$$dx/dt = -x + z_2 \tag{57a}$$

$$\mu dz_1/dt = x - z_1 \tag{57b}$$

$$\mu dz_2/dt = x + z_1 - z_2 \tag{57c}$$

The adjoined system is in this case

$$dz_1/d\tau = x - z_1 \tag{58a}$$

$$dz_2/d\tau = x + z_1 - z_2 \tag{58b}$$

where x is no longer a function of time but a parameter. Putting $\mu = 0$, one obtains the root of the adjoined system

$$\bar{z}_1 = x; \quad \bar{z}_2 = 2x \tag{59}$$

As eqs. 58 are a linear SFO, it is easy to integrate. The result is:

$$z_1(\tau) = \bar{z}_1 + (z_1^0 - \bar{z}_1)\exp(-\tau) \tag{60a}$$

$$z_2(\tau) = \bar{z}_2 + [(z_1^0 - \bar{z}_1)\tau + (z_2^0 - \bar{z}_2)]\exp(-\tau) \tag{60b}$$

One sees immediately from eqs. 60 that the root, eqs. 59, is stable: z_1 and z_2 quickly tend to the steady-state values $\bar{z}_1$ and $\bar{z}_2$, irrespective of their initial values z_1^0 and z_2^0, i.e., all possible initial values lie in the domain of influence of the root.

The assumptions of Tihonov's 1st Theorem are thus fulfilled and, when $\mu \to 0$, solutions of the system, eq. 57, tend to the solution of the degenerate system

$$dx/dt = -x + z_2 \tag{61a}$$

$$z_1 = x \tag{61b}$$

$$z_2 = 2x \tag{61c}$$

and so x (and also z_3) behaves as if it were produced in the autocatalytic process (eq. 48).

To demonstrate that the assumptions of Tihonov's Theorem are fulfilled, it is not necessary to integrate the adjoined system. In more complicated cases this may be practically impossible. Instead, one may use the Routh-Hurwitz criterion for this purpose (cf. appendix C). In the above example, the adjoined system (eqs. 58) has the unique root (the uniqueness of a root may be in general checked with the aid of Lipschitz conditions, cf. appendix C). The characteristic equation (eq. C8) in this case is

$$\det\begin{vmatrix} -1-\lambda & 0 \\ 1 & -1-\lambda \end{vmatrix} = 0 \tag{62}$$

i.e.

$$\lambda^2 + 2\lambda + 1 = 0 \tag{63}$$

So we have (eq. C9)

$$b_0 = 1; \quad b_1 = 2; \quad b_2 = 1 \tag{64}$$

and further

$$D_0 = 1; \quad D_1 = 2; \quad D_2 = \begin{vmatrix} 2 & 1 \\ 0 & 1 \end{vmatrix} = 2 \tag{65}$$

i.e., they are all positive. The Routh-Hurwitz criterion shows that the root is stable and, as it is also unique, any initial conditions must lie in its domain of influence. If there is more than one root of the adjoined system, similar considerations must be made for all roots separately, as the coefficients of the characteristic equation and consequently the Routh-Hurwitz determinants depend on the value of the root (cf. eqs. C7–C10).

From the above considerations, one may see that to sustain an autocatalytic process 'inexhaustible' reservoirs of 'building' materials (A and B) for the reagents Z_1 and Z_2 must exist. In reality the system, eqs. 49, models an autocatalytic system only for the times for which A and B are practically constant (of the order $t < 1/\mu$). After a sufficiently long time any reservoir will in reality be exhausted. One ought also to note that the autocatalytic process gives a byproduct C.

As the third example, let us consider a case when small parameters arise in the system because of the differences in rate constants (cf., the remark concerning the applicability of the steady-state approximation to the Michaelis-Menten mechanism in this section). This will also demonstrate another method of separation of quick and basic (slow) variables. Let us assume that the reaction

$$R_1 + R_2 \xrightarrow{k} R_3 \tag{66}$$

takes place in the system and that this reaction is a quick one, i.e.

$$k = 1/\mu \quad \text{where} \quad \mu \ll 1 \tag{67}$$

The reagents R_1, R_2 and R_3 may participate also in slow reactions (i.e., with rate constants much less than k). Assume that in appropriately defined dimensionless variables the kinetics of the system are given by

$$\dot{x}_i = g_i(r, x) \tag{68a}$$

$$\dot{r}_1 = -\frac{1}{\mu} r_1 r_2 + G_1(r, x)$$

$$\dot{r}_2 = -\frac{1}{\mu} r_1 r_2 + G_2(r, x) \tag{68b}$$

$$\dot{r}_3 = \frac{1}{\mu} r_1 r_2 + G_3(r, x)$$

where $r = (r_1, r_2, r_3)$ and $x = (x_1, \ldots, x_n)$; the terms G_k and g_i denote contributions of slow reactions, x denotes slow variables.

The SFO in which the terms of the order less than $1/\mu$ (e.g., the contributions of slow reactions)

are neglected, is called a *truncated system*. Usually, the truncated system has certain linear integrals, some of which represent conservation laws. These integrals are slow variables for the original SFO and taking them as new variables leads to separation of quick and slow variables.

It is easy to see that in the example under consideration there exist two such lineary independent integrals of the truncated system

$$x_{n+1} = r_1 + r_3 \tag{69a}$$

$$x_{n+2} = r_1 - r_2 \tag{69b}$$

By introducing these integrals into eq. 63 and denoting r_1 by z, one obtains

$$\dot{x}_i = g_i(z, x) \quad (i = 1, \ldots, n)$$

$$\dot{x}_{n+1} = G_1(z, x) + G_3(z, x) \tag{70a}$$

$$\dot{x}_{n+2} = G_1(z, x) - G_2(z, x)$$

$$\mu \dot{z} = -z(z - x_{n+2}) + \mu G_1(z, x) \tag{70b}$$

where now $x = (x_1, \ldots, x_n, x_{n+1}, x_{n+2})$. The system obtained is identical with eqs. 4′ and Tihonov's 1st Theorem may be applied if the assumptions are fulfilled. The above example illustrates how the differences in rate constants of elementary reactions may be exploited to simplify reaction kinetics by using Tihonov's Theorem.

7. Concluding remarks

The method proposed by Kijima and Kijima [1] may not be applied directly (without linearlization) even to a Michaelis-Menten mechanism (eq. 41) because of the nonlinear term xz. Their method may be classified between graph-theoretical methods, however, the word 'graph' does not appear in the article. Because of introducing two kinds of arrows (edges or branches in graph-theoretical terminology) their graphs correspond to so-called colored graphs (two 'colors' of edges) and because each edge has given direction and 'value' (the rate constant) they belong to the class of graphs called *labelled signal flow graphs* (SFG) or labelled directed graphs [15]. The theory of graphs, developed primarily for the analysis of electrical networks, has been extensively used for simplifying kinetic (bio)chemical systems (see, for example, refs. 16–19). It is interesting to note that the 'diagrams' used by Hill [18] and other authors are also labelled SFG and therefore the general theory developed for SFG may be applied to these diagrams [20].

Tihonov's Theorems may be applied to systems in which reactions of the second (and even higher) order take place. If some rate constants are equal to zero, i.e., some reaction steps are irreversible, the fundamental assumption of ref. 1 is not fulfilled. In contrast, Tihonov's Theorems may be even easier to apply in such cases (because of the smaller number of terms) than in the case when all reactions are reversible and no general assumptions about the number of components in the system are necessary.

The fast components 'forget' their initial values. These values are necessary only to check if the assumptions of Tihonov's Theorems are fulfilled (i.e., to check if they are lying in the domain of influence of the root). However, sums of initial concentrations of some groups of fast components (e.g., the total initial enzyme concentration E_0) may determine some characteristic constants of the system, e.g. small parameters $\mu^{(j)}$.

The obvious conclusion from Tihonov's Theorems is that the steady-state approximation works well for times (much) greater than the characteristic time constant used as the unit of time (i.e., the time constant of the components which may be thought to be slow in the time scale we are interested in), however, not so great that the reagents assumed to be reservoir variables are exhausted.

The fact that the characteristic time constant for a given reagent is small, compared to the time scale we are interested in, constitutes a criterion for fast equilibration which is even more important in practice than the fact that some elementary reactions are relatively quick (i.e., some reaction rate constants are much greater than others). Only relations between characteristic time scales T_i of different reagents and their relations to the time scale of observation we are interested in (which is taken as the normalizing unit of time T) are of importance. In section 5 we have subdivided all the reagents into at least three classes, called reservoir (very slow), y, basic (slow), x, and quick, z,

$$\dot{y}_k = \mu \Phi_k(x, y, z, t, \mu) \quad (k = l, \ldots, q) \tag{71a}$$

$$\dot{x}_i = f_i(x, y, z, t, \mu) \quad (i = 1, \ldots, n) \tag{71b}$$

$$\dot{z}_l = \frac{1}{\mu} F_l(x, y, z, t, \mu) \quad (l = 1, \ldots, s) \tag{71c}$$

The concentration hierarchy in the system is usually in agreement with the time hierarchy [6], i.e.

$$y_k \ll x_i \ll z_l \tag{72}$$

As we have demonstrated, if we are interested in the behavior of the system in periods of the order of the time scale characteristic for x_i, then we put $\dot{y}_k = 0$ (cf. eqs. 34 and 35) and we eliminate the variables z_l using Tihonov's Theorem. However, if we are interested in the transient phenomena (i.e., in time interval $0 < t < \mu$), then the variables z_l ought to be considered as basic ones and x_i as reservoir ones. Oppositely, if we are interested in the evolution of the system ($t \gg 1$), then the variables y_k ought to be treated as basic ones and x_i as quick ones. Real systems generally have more than three characteristic time scales (i.e., some groups of quick variables, $z_l^{(j)}$ $(j = 1, \ldots, m)$ and then Tihonov's 2nd Theorem may be a useful mathematical tool.

In conclusion, it may be said that the mathematical foundations of the steady-state approximation have been well established four more than 30 years.

Appendix A

The abstract of Tihonov's work [2] from Mathematical Reviews [3]

Tihonov, A.N. Systems of differential equations containing small parameters in the derivatives. Mat. Sbornik N.S. 31 (73), 575–586 (1952). (Russian)

Consider a system (1) $\mathrm{d}x/\mathrm{d}t = f(x, z, t)$, $\mu \mathrm{d}z/\mathrm{d}t = F(x, z, t)$, where x, f are n-vectors, z, F m-vectors, and f, F satisfy suitable regularity assumptions. Assume $z = \phi(x, t)$ is an isolated solution of $F(x, z, t) = 0$ whose points are (asymptotically) stable equilibrium points of the adjoined system $\mathrm{d}z/\mathrm{d}\tau = F(x, z, t)$ (x, t being considered here as parameters, the point (x, t) belonging to a bounded open region D). Assume that the initial conditions (x^0, z^0, t^0) are such that the solution $z(\tau)$ of the adjoined system $\mathrm{d}z/\mathrm{d}\tau = F(x^0, z, t^0)$, $z(0) = z^0$, satisfies $\lim_{\tau \to \infty} z(\tau) = \phi(x^0, t^0)$. Then the solution of (1) through the initial point (x^0, z^0, t^0) tends, as $\mu \to 0$, to the solution of the degenerate system $\mathrm{d}x/\mathrm{d}t = f(x, \phi(x, t), t)$ as long as the point (x, t) does not leave the region D. A similar result holds for systems containing several small parameters $\mu_1, \ldots, \mu_m$ tending to 0 in such a way that $\mu_{j+1}/\mu_j \to 0$. *J.L. Massera* (Montevideo).

Appendix B

B.1. Mathematical definitions (from ref. 2)

The norm of an s-dimensional vector $v = (v_1, \ldots, v_s)$ will be denoted by $|v|$ and defined as

$$|v| = \sqrt{\sum_{l=1}^{s} v_l^2} \tag{B1}$$

(Euclidian norm).

The root $z = \phi(x, t)$ of the adjoined system of equations $F(x, z, t) = 0$ will be called the *isolated root* if there exists an ϵ for which this system may not be fulfilled by any other vector $z' = (z_1', \ldots, z_s')$ with the property

$$|z' - \phi(x, t)| < \epsilon \quad (z' \neq \phi) \tag{B2}$$

The isolated singular point (root) $\bar{z} = (\bar{z}_1, \ldots, \bar{z}_s)$ of the *adjoined system*

$$\mathrm{d}z/\mathrm{d}\tau = F(x, z, t); \quad z(t^0) = z^0 \tag{B3}$$

(where x and t are taken as parameters) is called a *stability point* if for any ϵ there exists a $\delta(\epsilon)$ such that trajectories starting in a point M belonging to the $\delta(\epsilon)$ neighborhood of the singular point tend to this singular point without leaving the ϵ neighborhood when $\tau \to \infty$, i.e.

(1) the trajectory of any point $\bar{z} = (z_1, \ldots, z_s)$ belonging to the $\delta(\epsilon)$ neighborhood of the point $\bar{z}$ tends to $\bar{z}$ when $\tau \to \infty$:

$$\lim_{\tau \to \infty} z(\tau) = \bar{z} \quad \text{if} \quad |z(0) - \bar{z}| < \delta(\epsilon) \tag{B4}$$

(2) trajectories of points from the $\delta(\epsilon)$ neighborhood of the point $\bar{z}$ do not leave the ϵ neighborhood of this point:

$$|z(\tau) - \bar{z}| < \epsilon \quad \text{for any } \tau, \text{ if } |z(0) - \bar{z}| < \delta(\epsilon) \tag{B5}$$

The isolated root $z = \phi(x, t)$ of the system $F(x, z, t) = 0$ is called *stable* in some bounded region D of the space (x, t), if for all points belonging to $\bar{D}$ the points $z = \phi(x, t)$ are singular stability points of the system (eq. B3).

The *domain of influence* of the stable root $z = \phi(x, t)$ in the subspace $x =$ constant, $t =$ constant is defined as a set of all points z^0 from which trajectories of the adjoined system (eq. B3) tend to $z = \phi(x, t)$ when $\tau \to \infty$.

The root $z^{(m)} = \phi^{(m)}(x, z^{(1)}, \ldots, z^{(m-1)}, t)$ of the adjoined system of the first order $F^{(m)}(x, z^{(1)}, \ldots, z^{(m)}, t) = 0$ (where $x, z^{(1)}, \ldots, z^{(m-1)}, t$ are treated as parameters) is called an *isolated root of the first order*, if there exists such ϵ that this system may not be fulfilled by any other vector $z'^{(m)}$ for which

$$|z'^{(m)} - \phi^{(m)}| < \epsilon \quad (z'^{(m)} \neq \phi^{(m)}) \tag{B6}$$

The root of the first order $z^{(m)} = \phi^{(m)}(x, z^{(1)}, \ldots, z^{(m-1)}, t)$ is called a *stable root* in some closed bounded region $\bar{D}_1$ if for all $(x, z^{(1)}, \ldots, z^{(m-1)}, t)$ of this region $z^{(m)} = \phi^{(m)}$ is a stable singular point of the adjointed system.

Appendix C

C1. Other mathematical definitions

An ordinary differential equation of the first order or a system of first-order ordinary differential equation (SFO)

$$\mathrm{d}z/\mathrm{d}\tau = F(z, \tau) \quad (z = z_1, \ldots, z_s) \tag{C1}$$

is called *autonomous* if the right-hand sides do not depend explicitly on the independent variable τ, i.e.

$$\mathrm{d}z/\mathrm{d}\tau = F(z) \tag{C2}$$

where F and z are vectors of s-dimensional space $(s = 1, 2, \ldots)$.

C2. Existence and uniqueness of solutions of SFO

The system (eqs. C1) has a unique solution $z = z(\tau)$ through every point $(z = z_0, \tau = \tau_0)$ in the $(s+1)$-dimensional domain D if for $(z, \tau) \in D$ functions $F(z, \tau)$ are single-valued, bounded, continuous and if the *Lipschitz conditions*

$$|F(\tau; z_1, \ldots, z_s) - F(\tau; v_1, \ldots, v_s)| < M \cdot \sum_{l=1}^{s} |z_l - v_l| \tag{C3}$$

are fulfilled for some M independent of z and v. Then $z(\tau)$ is a continuous function of the given value $z_0 = z(\tau_0)$. Each solution extends to the boundary of D.

The Lipschitz conditions are satisfied, in particular, whenever $F(z, \tau)$ has bounded and continuous derivatives, $\partial F/\partial z_l$, $(l = 1, \ldots, s)$ in D.

The root of an SFO is a *stable* root if all perturbations δz around this root diminish to zero when $\tau \to \infty$

$$\delta z \xrightarrow[\tau \to \infty]{} 0 \tag{C4}$$

To investigate if a root $\bar{z}$ of the autonomous SFO (eq. C2) is stable, one linearizes the system around $\bar{z}$; by putting

$$z_l = z_l + \delta z_l \quad (l = 1, \ldots, s) \tag{C5}$$

one obtains

$$\mathrm{d}(\delta z_l)/\mathrm{d}\tau = \sum_{j=1}^{s} a_{lj} z_l \quad (l = 1, \ldots, s) \tag{C6}$$

where the matrix $A = \|a_{l,l}\|$ is given by

$$a_{lj} = \partial F_l/\partial z_j|_{z=\bar{z}} \quad (l, j = 1, \ldots, s) \tag{C7}$$

The root $z = \bar{z}$ is stable if all eigenvalues λ_l, $(l = 1, \ldots, s)$, of the matrix A have negative real parts. λ_l are the zeros of the characteristic equation

$$\det|a_{lj} - \lambda\delta_j^l| = 0 \tag{C8}$$

when $\delta_j^l = 0$ for $l \neq j$, $\delta_j^l = 1$ for $l = j$ (Kronecker's delta).

The characteristic equation, after resolving the determinant in eq. C8, is a polynomial of the s-th order:

$$b_0\lambda^s + b_1\lambda^{s-1} + \ldots + b_{s-1}\lambda + b_s = 0 \tag{C9}$$

Given $b_0 > 0$, all roots λ_l of the characteristic polynomial (all eigenvalues of the system eqs. C2 have negative real parts if and only if the determi-

nants

$$D_0 = b_0$$

$$D_1 = b_1$$

$$D_2 = \begin{vmatrix} b_1 & b_0 \\ b_3 & b_2 \end{vmatrix}$$

$$D_3 = \begin{vmatrix} b_1 & b_0 & 0 \\ b_3 & b_2 & b_1 \\ b_5 & b_4 & b_3 \end{vmatrix} \qquad \text{(C10)}$$

$$\vdots \quad \vdots \quad \vdots \quad \vdots$$

$$D_s = \begin{vmatrix} b_1 & b_0 & 0 & \dots & 0 \\ b_3 & b_2 & b_1 & \dots & 0 \\ \dots & \dots & \dots & \dots & \dots \\ b_{2s-1} & b_{2s-2} & b_{2s-3} & \dots & b_s \end{vmatrix} \quad (\text{where } b_m = 0 \text{ if } m > s)$$

are all positive (Routh-Hurwitz criterion). This is true only if all b_l and either all even-numbered D_l or all odd-numbered D_l are positive (Liénard-Chipart test; [21].

Appendix D

D1. Tihonov's Theorem for Beginners

Consider a system of p first-order ordinary differential equations. Let us assume that s of the p equations have a small parameter μ, multiplying the time derivatives:

$$dx_i/dt = f_i(x_1,\dots,x_n, z_1,\dots,z_s) \quad (i = 1,\dots,n) \qquad \text{(D1a)}$$

$$\mu dz_l/dt = F_l(x_1,\dots,x_n, z_1,\dots,z_s) \quad (l = 1,\dots,s) \qquad \text{(D1b)}$$

where $(n + s) = p$.

Eq. D1a is called the *degenerate system*, eq. D1b the *adjoined system*.

D2. Tihonov's Theorem

The solution of the whole (original) system (eqs. D1) tends to the solution of the degenerate system when $\mu \to 0$ if the following conditions are fulfilled:

(a) the solution $\bar{z}_1 = \phi_1(x_1,\dots,x_n, z_1,\dots,z_s),\dots,\bar{z}_s = \phi_s(x_1,\dots,x_n, z_1,\dots,z_s)$ is an isolated root of the algebraic system

$$F_l(x_1,\dots,x_n, z_1,\dots,z_s) = 0 \quad (l = 1,\dots,s) \qquad \text{(D2)}$$

(i.e., in the small neighborhood of this root there are no others roots);

(b) the solution $\bar{z}_1,\dots,\bar{z}_s$ is a stable isolated singular point of the adjoined system (i.e., any perturbation throwing the system out of this point, diminishes with time to zero) for all values of $(x_1,\dots,x_n)$ which are treated here just as parameters and not as functions of time;

(c) the initial values $(z_1^0,\dots,z_s^0)$ are in the domain of influence of the stable singular point of the adjoined system (i.e., the system will evolve in such a way that $(z_1,\dots,z_s)$, when started from $(z_1^0,\dots,z_s^0)$, will tend to $(\bar{z}_1,\dots,\bar{z}_s)$;

(d) the solutions of the whole system (eqs. D1) and of the adjoined system (eq. D1b) are unique and the right-hand sides (f_i and F_l) are continuous functions.

Note added in proof (Received 20th June 1983)

Professor Benno Hess has recently called the author's attention to the publication of Reich and Sel'kov [22], in which the authors outline the simplest case of using Tihonov's 1st Theorem (however, without formulation of the theorem itself) with one rapid and one slow variable.

Acknowledgements

The author is greatly indebted to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. for the scholarship for staying in the Max-Planck-Institut für Biophysikalische Chemie in Göttingen and would like to express his gratitude to Professors L. De Mayer, M. Eigen and H. Strehlow for the kind hospitality he received during his stay.

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