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Limit-Cycles in Enzyme-Systems with Nonlinear Negative Feedback

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Abstract. A necessary criterion has been obtained for the occurrence of oscillating reactions in multi-enzyme systems with end-product inhibition. Computer-simulations strongly suggest that this criterion is also sufficient, since limit-cycles arise whenever the criterion is violated with a cycle-time in good agreement with the theoretically derived estimate.

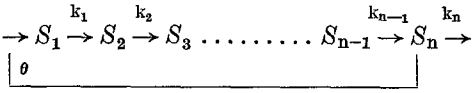
Introduction

Since Goodwin's [2] conjecture, that biological oscillations can arise naturally in self-controlled biochemical systems, much interest has been devoted to this problem. An explicit mathematical model for a biochemical oscillator was presented by Morales and McKay [6]. Their model was based on the Yates-Pardee [12] product inhibition type kinetics. Analog-computer simulations showed the model to exhibit a limit-cycle. Phaseplane studies based on Bendixons criterion carried out by Griffith [3] showed, however, that the two-state oscillator of Morales and McKay did not fulfill the requirement for the existence of a limit-cycle, thus casting some doubt on the validity of "proofs" given by means of analog-computer simulations in general. Recently, the theoretical interpretation of biochemical limit-cycles has got a firm basis due to the kinetic investigations of Walter [9], and the thermodynamic investigations of Progogine's [1] school. Thus, Walter tested the general multi-state Yates-Pardee scheme by the Rooth-Hurwitz criterion, and was able to show that the stationary state of such a scheme could not be proved stable for all values of the rate constants, contrary to Griffith's analysis of the Morales-McKay (2-state) scheme. Indeed, Walter [9], demonstrated a limit-cycle by analog-computer simulations of a system, which theoretically was shown to be unstable.

At the European Biophysics Congress, Baden 1971, Walter presented results of more than 2,500 analog-computer simulations, showing the existence of limit-cycles in a number of cases. A striking feature of hese results is that a criterion for the existence of limit-cycles previously derived by Viniegra and Martinez [8] for a rather special situation, is always fulfilled. It is the aim of the present paper to analyse these findings, and we will derive a general criterion, of which the Viniegra-Martinez formula is a special case.

The Model

The general Yates-Pardee scheme may be written;



Scheme 1

Scheme 1 shows the synthesis and decompositions by first-order kinetics of a series of substances S_i. (Such first-order kinetics may be realized in enzymatically catalyzed reactions of the Michaelis-Menten type, if the concentrations S_i are small.)

The substance S_n leaves the system at a rate proportional to its own concentration, whereas S_1 is fed into the system at a constant rate from outside; this step is inhibited, however, by the end-product S_n. Specially, S_n is thought to react with the enzyme E_1, which catalyzes the formation of S_1:

$$E_1 + \varrho\, S_n \rightleftarrows X_n\,, \tag{1}$$

where X_n is inactive.

If α is the mass-action equilibrium constant for the reaction (1) and the rate of formation of S_1 with no S_n present is c_0, we obtain the actual rate of formation of S_1 as

$$\frac{dS_1}{dt} = \frac{c_0}{1 + \alpha S^{\varrho}_n} - k_1 S_1 \tag{2}$$

if we as usual assume the rates in scheme 1 to be sufficiently slow, so that reaction (1) may be considered in equilibrium. The first term in (2) presents a nonlinear negative feedback control in the kinetic system.

The rate equations for scheme 1 are thus:

$$\begin{aligned} \frac{dS_1}{dt} &= \frac{c_0}{1 + \alpha S^{\varrho}_n} - k_1 S_1 \\ \frac{dS_i}{dt} &= k_{i-1} S_{i-1} - k_i S_i \qquad i = 2,3 \ldots\ldots n \end{aligned} \tag{3}$$

which we may write

$$S_t = F(a, S)\,, \tag{4}$$

where a is a vector with components $k_1, k_2, \ldots\ldots k_n, c_0, \alpha$. We want to find relations between the components of vector a, so that (4) exhibits a limit-cycle.

The Stationary State

The concentrations of S in the stationary state, denoted as S^0, are obtained from the set of nonlinear equations derived from (4);

$$F(a, S^0) = 0 \tag{5}$$

which immediately yields

$$S^0_i = \frac{k_{i-1}}{k_i} S^0_{i-1} \qquad i = 2,3 \ldots\ldots n \tag{6}$$

and

$$(S^0_1)^{\varrho+1} + \frac{1}{\alpha}\left(\frac{k_n}{k_1}\right)^{\varrho} S^0_1 - \frac{1}{\alpha}\left(\frac{k_n}{k_1}\right)^{\varrho} \frac{c_0}{k_1} = 0\,. \tag{7}$$

If we introduce a constant c by the equation $c_0 = ck_1S_1^0$, we can rewrite the last term in (7) in terms of c, and thus we obtain

$$S_1^0 = \frac{k_n}{k_1} \sqrt[\varrho]{\frac{c-1}{\alpha}} . \tag{8}$$

Since c by definition is a function of S^0 this is only a formal solution of Eq. (7). However, by definition

$$c_0 = ck_1S_1^0 = ck_n \sqrt[\varrho]{\frac{c-1}{\alpha}} \tag{9}$$

from which we obtain a relation for c;

$$c \sqrt[\varrho]{c-1} = \frac{c_0}{k_n} \sqrt[\varrho]{\alpha} . \tag{10}$$

If we solve either (7), or (10) and (8), for S_1^0, we can determine from (6) the stationary concentrations of Scheme 1.

Then we may investigate the stability of this stationary state; We expand the operator $F(a, S)$ from S^0 and retain first order terms.

$$S_t \simeq \left.\frac{\partial F(a, S)}{\partial S}\right|_{S=S^0} \cdot (S - S^0) . \tag{11}$$

where the derivative of $F(a,S)$ is a matrix, evaluated at the stationary concentrations S^0. The criterion for stability of the stationary state is, that the eigenvalues of this matrix have negative real parts. Since the resulting matrix is nearly a band matrix, we can find the characteristic polynomial analytically quite easily. In fact, we obtain

$$\prod_{i=1}^{n} (k_i + \lambda) - A \prod_{i=1}^{n-1} k_i = 0 , \tag{12}$$

where A derives from (11), when the feedback term (2) is differentiated with respect to S_n;

$$A = \frac{-\alpha \varrho c_0 (S^0{}_n)^{\varrho-1}}{[1 + \alpha(S^0{}_n)^{\varrho}]^2} . \tag{13}$$

To investigate the computer simulations carried out by Walter, we inspect the solutions of (12) for the case $k_i = k$; $i = 1,2 \ldots\ldots n$. Using (8) and (6) in the form

$$(S_n^0)^{\varrho} = \frac{c-1}{\alpha} \tag{14}$$

we readily obtain from (12) and (13)

$$(k + \lambda)^n = -k \cdot \frac{c-1}{c} \cdot \varrho \tag{15}$$

and thus the desired equation for the eigenvalues λ_m

$$\lambda_m = -k + k \cdot e^{i\left(\frac{\pi}{n} + \frac{2\pi}{n} \cdot m\right)} \cdot \sqrt[n]{\varrho \cdot \frac{c-1}{c}} \qquad m = 0,1 \ldots\ldots, n-1. \tag{16}$$

Thus, the eigenvalues are positioned in the complex plane on a circle with centre on the negative real axis, namely in $-k$ and with radius

$$r = k \cdot \sqrt[n]{\varrho \cdot \frac{c-1}{c}} . \tag{17}$$

Thus, if r is less than k, all eigenvalues at the stationary state have negative real parts, and thus the stationary state is stable.

Limit Cycles

A necessary condition for observing limit-cycles in Scheme 1 is that the real part of the lamda's is positive: We obtain for the eigenvalue closest to the positive halfplane,

$$\mathrm{Re}(\lambda) = -k + k \cdot \sqrt[n]{\varrho \cdot \frac{c-1}{c}} \cdot \cos\left(\frac{\pi}{n}\right). \tag{18}$$

Requiring this eigenvalue to be positive yields;

$$\varrho > \frac{c}{c-1} \cdot \sec^n\left(\frac{\pi}{n}\right) \tag{19}$$

which is the desired necessary condition for the existence of a limit-cycle.

Walter [9, 11] has observed, that the condition $\varrho > \sec^n \pi/n$ was always satisfied when limit-cycles appeared in scheme 1. This inequality was presented by Viniegra and Martinez [8], and derived from the characteristic equation (12) on the very special additional assumption that $c_0 = kS_n^0$. We remark that this assumption together with $k_i = k$, for all i, implies from (9) and (8) that the stationary state occurs for all concentrations S_i^0 equal to zero, a rather uninteresting situation. In the following we will compare the correct necessary condition (19) with analog-computer calculations of Walter, and our own digital computations of Scheme 1.

The Necessary Criterion for Instability

In order to discuss the stability of Scheme 1, we rewrite (10) and (19) as:

$$c \sqrt[\varrho]{c-1} = \frac{c_0}{k} \sqrt[\varrho]{\alpha} \tag{20}$$

$$\varrho > \frac{c}{c-1} \sec^n\left(\frac{\pi}{n}\right). \tag{21}$$

If (21) is satisfied with c computed from (20), the Scheme 1 is unstable. It is an immediate consequence that large values of the right-hand side of (20) causes c to be sufficiently large to reduce (21) to Walter's guiding inequality $\varrho > 1 \cdot \sec^n \pi/n$. From (20) it follows, moreover, that $c/(c-1)$ is always larger than one. This, then, is the explanation for the observation of Walter, that $\varrho > \sec^n(\pi/n)$ was satisfied, whenever a limit-cycle was observed for Scheme 1.

However, due to the factor $c/(c-1)$, which depends on ϱ, c_0, k and α [Eq. (20)], the particular combination of these four constants may exclude limit-cycles for values of ϱ, which are much larger than those shown in Table 1. Thus, small values of the right-hand side in (20) yields c-values close to one, and hence large values of the factor $c/(c-1)$. A typical example is shown in Table 2.

As a qualitative rule, we may conclude from (20) that Table 1 is valid if $(c_0 \sqrt[\varrho]{\alpha})/k$ is much larger than one; if, on the other hand, this expression is smaller than one, limit-cycles are likely to be excluded, since $c/(c-1)$ grows prohibitively large.

A comprehensive picture of the possibility of the occurrence of limit-cycles in Scheme 1 is given in Fig. 1. Here, a number of combinations of c_0, k, α, ϱ and n was

Table 1. Viniegra-Martinez' necessary condition for the occurrence of limit-cycles in Scheme 1. The value of ϱ must be larger than the tabulated value, according to inequality (19) with the factor $c/(c-1)$ missing. Thus ϱ must be at least 2. n is the number of species in Scheme 1

n	ϱ larger than
3	8
4	4
5	3
6	2
7	2
8	2
9 (and larger values)	1

Table 2. Example of values of the factor $\frac{c}{c-1}$, occurring in the criterion for stability (21). The values presented are calculated for the case $c_0 = 0.5$, $n = 7$ and $\varrho = 4$.

The Table shows this factor to differ considerably from one for a number of parameter combinations, thus virtually excluding a limit-cycle, since this factor is to be multiplied on the numbers occurring in the column 'ϱ larger than' in Table 1

α	$\frac{c}{c-1}$						
1	1.18	1.35	4.08	20.5	10^4	$1.6 \cdot 10^5$	
0,1	1.31	1.65	14.4	164.9	10^5	$1.6 \cdot 10^6$	
0,01	1.57	2.39	104.9	1605.0	10^6	$1.6 \cdot 10^7$	
	0.05	0.1	0.5	1.0	5.0	10.0	k

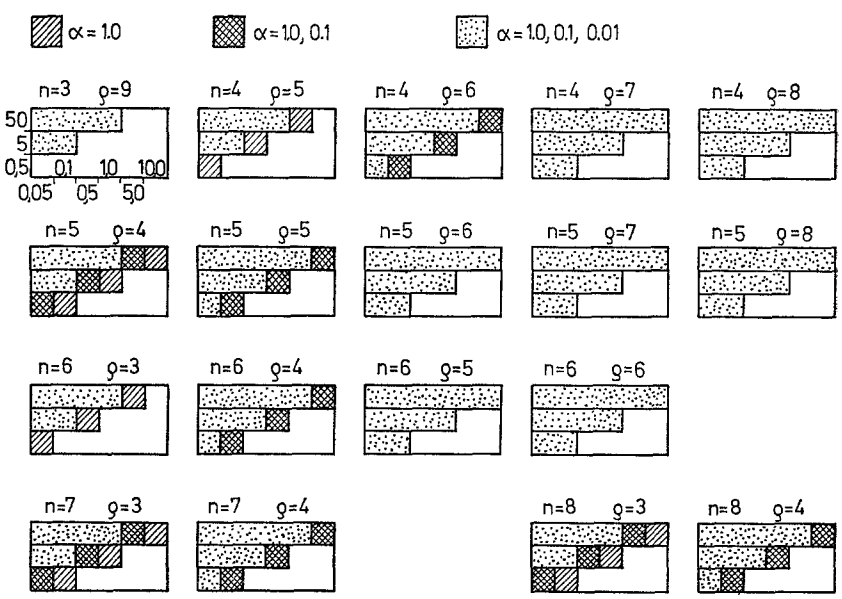


Fig. 1. Theoretical occurrence of limit-cycles in Scheme 1, calculated from the criteria (20) and (21). Ordinate is the value of c_0, abscissa the rate constant k. The system is stable (no limit-cycle) if the field is empty; limit-cycles occur according to the following signature

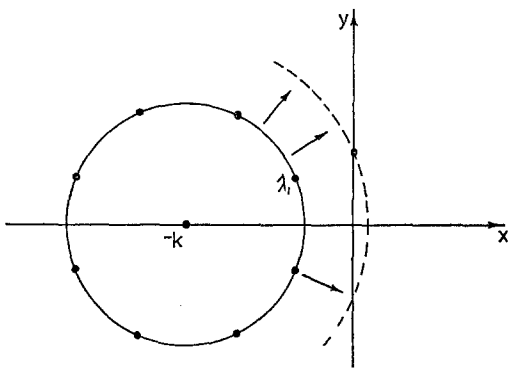


Fig. 2. This figure demonstrates Hopf-bifurcation. See text for explanation

tested for stability by solving (20) for c numerically, using Newton's method. Thus, Table 1 shows a *necessary* condition of combinations of c_0, k, α, ϱ and n for the existence of limit-cycles in Scheme 1.

Hopf-Bifurcation

Fig. 1 may be compared to analog-computer experiments published by Walter [11].

Two main results are pertinent in these experiments:

1. In no case has a limit-cycle been observed in violation of the prediction of Fig. 1.

2. Walter does not obtain limit-cycles for the smallest values ot the rate constant k ($k = 0.05$ and 0.1).

Apart from 2, the necessary condition (21) seems to be nearly sufficient, in so far that limit-cycles are observed whenever (21) is satisfied. This point is further studied by us below. We have extended the analysis of the stability of the steady state by a method due to Hopf [4].

Consider the set of differential equations (4) for some vector a, for which the radius r defined in (17) is less than the general rate constant k. Thus, the stationary state defined by (6) and (8) is a stable point, since all eigenvalues are in the negative halfplane. If one of the components of vector a is varied so that radius r grows, two (complex conjugate) eigenvalues may cross the imaginary axis (see Fig. 2).

In the first approximation then, a stable periodic motion around the stationary state occurs. For small amplitudes of this oscillation, the period of motion, T, may be computed from

$$T = \frac{2\pi}{\mathrm{Im}\,(\lambda_a)}, \tag{22}$$

where $\mathrm{Im}(\lambda_a)$ is the imaginary part of eigenvalue λ_a, crossing into the positive half plane.

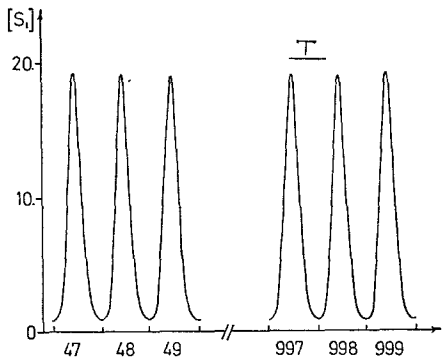


Fig. 3. Digital simulation of Scheme 1 demonstrating limit-cycle. The number of the cycle is indicated below each concentration top. No change occurs in shape, amplitude and period from cycle No. 49 to cycle No. 999. Parameters used in this simulation are: $n = 5$; $\varrho = 4$; $c_0 = 5.0$; $\alpha = 0.01$; $k = 0.1$

From (22) and (16), we thus get an estimate for the period T of the limit-cycle:

$$T \simeq \frac{2\pi}{k \cdot \sin\left(\frac{\pi}{n}\right) \cdot \sqrt[n]{\varrho \frac{c-1}{c}}}. \tag{23}$$

This approximate value for T shows the dependence of the cycle time on n, c and ϱ, and thus, from (20), on k, ϱ, c_0, n and α.

An immediate result is that for fixed n and ϱ and small values of k such that $\frac{c-1}{c}$ is close to unity, the cycle time T is nearly inversely proportional to k. This applies to case 2 above, where no limit-cycles were found in analog-computer simulations despite the fact that the stationary state is unstable. We have re-examined this area by solving (4) numerically (Hamming-Predictor Corrector program [5]). In contrast to 2 above, we find limit-cycles also for small values of the rate constant k, in particular the values 0.05 and 0.1. A typical situation is shown in Fig. 2. Note that the predicted approximate cycle time T (23), based on linearisation of (4), is fairly correct even for the actual nonlinear system's cycle time. This holds generally in all our computer experiments, and T (linear) is no more than 30% different from the actual cycle time. Moreover, we examined a large number of cases from Fig. 1, where limit-cycles had not been observed in Walter's experiments, although the stationary point was unstable due to our criterion (21). We observed limit-cycles for *all* these systems. Thus, the criterion (21) seems to be not only necessary but also sufficient. This may be stated as follows; if in Scheme 1, the stationary state is unstable, due to criterion (21), then a limit-cycle arises.

We may add that instability of stationary points for differential-equations like (4) quite often results in limit-cycles, a wellknown result in mechanical systems. However, limit-cycles do not *necessarily* arise in such cases; also, another cyclic solution may occur; the possibility of almost-periodic solutions to (4) cannot be excluded [7]. As stated, we did not find these kinds of phenomena in any of our experiments.

We conclude that digital simulations seems more reliable than analog-computation, probably mainly due to the rather smaller round-off error in the former meth-

od. Indeed, as a precaution against artifically introduced oscillations from the selected algorithm used, we varied accuracy, steplength and number of cycles integrated, but no significant change in the limit-cycles amplitude, general shape or cycle time occurred.

Conclusion

It has been shown that a set of coupled enzymatically catalysed reactions with only negative non-linear feedback may exhibit limit-cycle behaviour; no autocatalytic step is necessary. The kinetic equations considered here is a simplied form of actually occuring enzymatically catalyzed reactions; the model's advantage is, that it is possible analytically to find the eigenvalues of the stationary state, and to estimate the cycle time of the limit-cycle.

On this basis a necessary — and probably sufficient — criterion for the occurrence of a limit-cycle in this model is formulated (20 and 21).

It is hoped that it will soon be possible to extend this kind of analysis to more general cases on the basis of this work. This expectation is based on the fact that the control theory of general chemical kinetic systems

$$S_t = F(a, S) + \sigma(b, S) \tag{24}$$

may be extremely facilitated, if the eigenvalues of the operator F are known, whereas the control $\sigma(b, S)$ may be unknown to a certain extent. Thus, the study of a whole class of extensions to the present work may be possible by adding a suitable control term $\sigma(b, S)$ to the system (3).

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