A New Model System for the Study of Complex Dynamical Enzyme Reactions. II. Oscillations in a Reaction-Diffusion-Convection System

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A nonlinear enzyme reaction in a chemical gradient with an artificial feed-back loop is modified by the application of a constant temperature gradient leading to laminar convective motion of the fluid at an electrode. The complete system is shown to undergo a bifurcation into a limit cycle as a function of the applied temperature gradient. The effect of other parameters on the oscillation is described. More complicated types of behavior are expected in parameter space.

Keywords: Dissipative structures – enzyme kinetics – temperature gradient – reaction-diffusion-convection systems – oscillations

1. Introduction

In subcellular reacting systems it is obvious that the medium between cell wall and nucleus, between membranes of the organelles, the plasma, is neither well-stirred, in which case it could be modeled as a continuous flow stirred tank reactor (CSTR), nor is it undisturbed and motionless, in which case it could properly be approximated by looking at non-stirred reaction-diffusion set-ups widely used in the study of chemical waves [1]. Plasma streaming and slow laminar convective motion can readily be observed under the microscope [2], leading to mixing of the contents of the cell on a time scale much slower than the scale of enzyme catalyzed reactions. What are the impacts of such fluid motion on the self-organized coupling of, say, a membrane bound reaction to a plasma based reaction? Are gradients being upheld, disturbed, or do they actively take part in the formation of spatial and temporal structures regulating the cell's metabolism?

Studies of chemical waves in excitable and oscillatory media have revealed the possibility that adding convective forces may lead to spontaneous formation of pretty complex structures [3], but in most experiments these effects have been tried to avoid, and so far no quantitative results on laminar convective motion as a bifurcation parameter are available. Recently, however, it has been suggested that well-controlled flows might add quantifiable features to chemical structures [4].

In the foregoing article we have introduced a new system, where an enzyme reaction was coupled to a local feedback loop via diffusion [5]. This reaction-diffusion system indicated interesting dynamical solutions, but stable oscillatory behavior, for instance, could not be realized in a reproducible manner. In this contribution we extend the system by explicitly evoking a temperature gradient in the reaction cell, leading to laminar convective motion of the liquid at the surface of our working electrode. With this set-up we succeeded in finding experimental conditions for which an oscillatory current-time response was observed in chronoamperometric experiments. We describe the extended system and present a qualitative bifurcation study of various parameters. For the first time a convection including parameter has been found to act as bifurcation parameter in an unstirred enzyme system. Impacts on simulations of the system of partial differential equations for this new type of model are discussed.

2. Experimental

The set-up used is basically the same as in our previous paper [5]. Initial concentrations of the participating chemicals are given in the caption of the figures. Again the solution was kept at $T_1 = 19.0 \pm 0.1$ °C. This time, however, as a modification, the nitrogen atmosphere above the solution including the teflon coating of the working electrode was kept at a temperature T_2 (see Fig. 1 a) different from T_1 in the solution. This applied temperature gradient was al-

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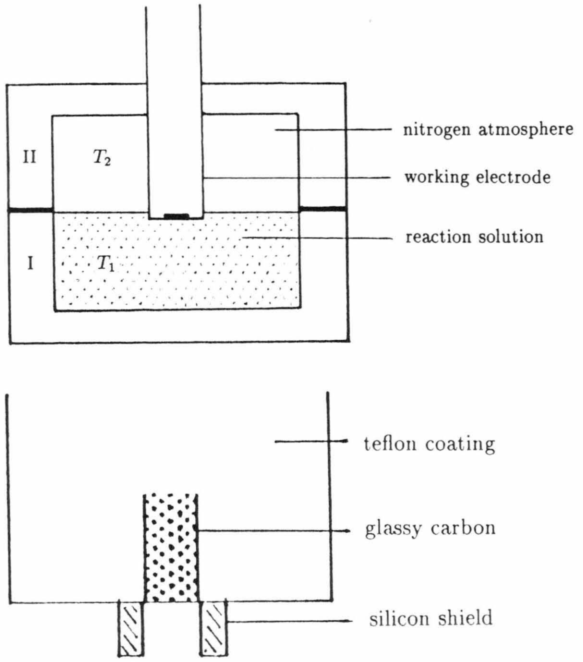

Fig. 1 a) Set-up for electrochemical experiments with two temperature baths, I and II, to control a temperature gradient $\Delta T = T_2 - T_1$ between gaseous and liquid phase. Counter and reference electrode have been omitted for clarity. b) Shielding device to eliminate convective flow parallel to the working electrode's surface. The diameter of the glassy carbon active area is 2 mm.

lowed to stabilize for one hour and was controlled during the whole chronoamperometric experiment. Different from our previous experiments, the reaction volume was 19 ml, just enough to cover the glassy carbon working electrode as shown in Figure 1 a.

3. Results

The introduction of a temperature gradient was motivated by the empirical finding that it served to stabilize long-term current measurements under potentiostatic conditions. To start with, we again investigated the system containing 2.0 mM 1,4-benzoquinone at pH = 5.0. We performed chronoamperometric experiments at a constant potential of -300 mV and found successively higher current values at corresponding times with increasing temperature gradient $\Delta T = T_2 - T_1$. As an example, twenty minutes after the potential pulse was set the current at $\Delta T = 0.0$, 0.5, 1.0, and 1.5 °C was found to be 2.56, 2.60, 2.92 and 3.26 μA, respectively. This is the expected result for increasing convective motion of the solution right in front of the electrode (cf. [6]).

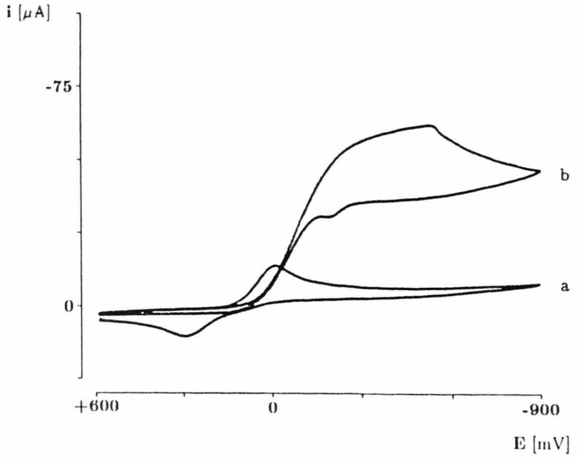

Fig. 2. Cyclic voltammograms of a solution containing 2.0 mM p-benzoquinone and 4.8 mM H_2O_2, with no enzyme (a), and with a peroxidase concentration of 0.21 μM (b). Scan rate is 10 mV/sec in both experiments. In both cases $T_1 =$ 19.0 °C, and $\Delta T = 1.2$ °C.

To distinguish between axial and horizontal components we compared current values at $\Delta T = 1.2$ °C with and without a cylindrical shielding device to avoid horizontal flow as indicated in Figure 1 b. The values were 2.65 μA with and 3.2 μA without the shield, i.e. a major component of the current increase is a convective flow parallel to the electrode surface. Applying gradients larger than $\Delta T = 0.5$ °C, current-time recordings yielded stable and reproducible steady state behaviour, an example of which can be seen in Fig. 4 b (with $\Delta T = 1.2$ °C). Note that the duration of the experiment was two hours. Instead of trying to carefully avoid convection participation in long-term measurements we thus have shown that a reliable background for our reaction, as far as a stable steady state is concerned, can be set up by explicitly taking this factor into account as part of the system. At some small distance from the electrode surface convection certainly overlaps with diffusion and has to be taken care of in appropriate model equations for the system.

The next step is to add the kinetic part and thus complete the system. To characterize it, we show a cyclic voltammogram of the solution without and with enzyme at a scan rate of 10 mV/sec (Figure 2). The cyclic voltammograms do not differ from recordings in the absence of a temperature gradient (cf. Figs. 1 and 2 in [5]). At this scan rate there is no

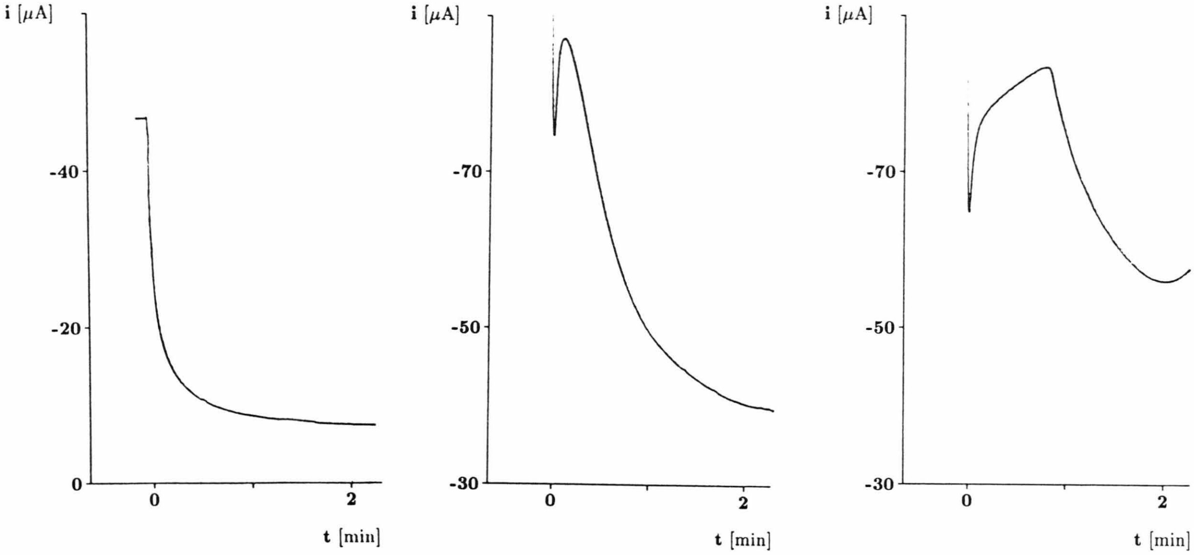

Fig. 3. Detail of chronoamperometric experiments at $E = -300$ mV of a solution containing 2.0 mM p-benzoquinone and 0.21 μM peroxidase, with a) no hydrogen peroxide, b) 2.4 mM H_2O_2, and c) 4.8 mM H_2O_2. In all cases $T_1 = 19.0$ °C, and $\Delta T = 1.2$ °C.

oxidation peak detectable in the catalytic case, the reduction peak is slightly shifted to negative potentials, the current shows catalytic enhancement and the nonlinear behavior as well as a small shoulder at $E = -200$ mV during the back scan, and at -160 mV the anomalous part of the anodic branch is left. The inhibitory phase starts at -550 mV, catalytic enhancement drops, although the scan rate is slow enough to allow maximum kinetic activity. We therefore argue that at these conditions there is a domain of nonlinearity in the substrate-rate equation, or, more strictly speaking, a non-monotonous force-flux characteristics, and thus at potentials smaller than -160 mV we expect an interesting region of parameter space with the possibility of an instability of the steady state.

The series in Fig. 3 shows the first two minutes of the chronoamperometric experiment for the system at a hydrogen peroxide concentration of 0, 2.4, and 4.6 mM. Figure 3a corresponds to cyclic voltammogram 2a (no kinetics), 3c corresponds to cyclic voltammogram 2b (full system), and 3b lies just in between. These three concentrations of substrate lead to three qualitatively different types of dynamical response. In Fig. 3a is, of course, the slow decay of the current proportional to $\sqrt{t^{-1}}$ (following the sudden potential step to -300 mV) into the steady state current, there are no irregularities at all. This decay is caused by a decreasing benzoquinone gradient at the electrode. In Fig. 3b substrate for the enzyme is present, hydroquinone is consumed and benzoquinone is recycled. There is a catalytic cycle, and therefore an enhanced steady state current is to be expected and indeed can be seen to be approached. However, the current response shows a significant abnormality, namely one peak in the decay after only a few seconds. This peak indicates a dynamical obstacle in the course of the reaction, most likely a regulatory function of the enzyme, which under these conditions is circumvented. Figure 3c finally shows a most exciting third possibility of our system. Again the current starts with a rapid decrease, then stops and turns to increase, but instead of the exceptional maximum during its fall into the steady state exhibits a relaxational type of large peak and after approximately two minutes starts to rise again. Note that in Fig. 2 at 10 mV/sec it takes two minutes in the cyclic voltammogram from -300 mV on the cathodic branch back to -300 mV on the anodic branch. At a scan rate of 5 mV/sec the current can be seen to rise again on the anodic branch, approximately two minutes past the point $E = -300$ mV on the first scan. The two minima observed in the chronoamperometric experiment of Fig. 3c and during the cyclic voltammogram at 5 mV/sec are ob-

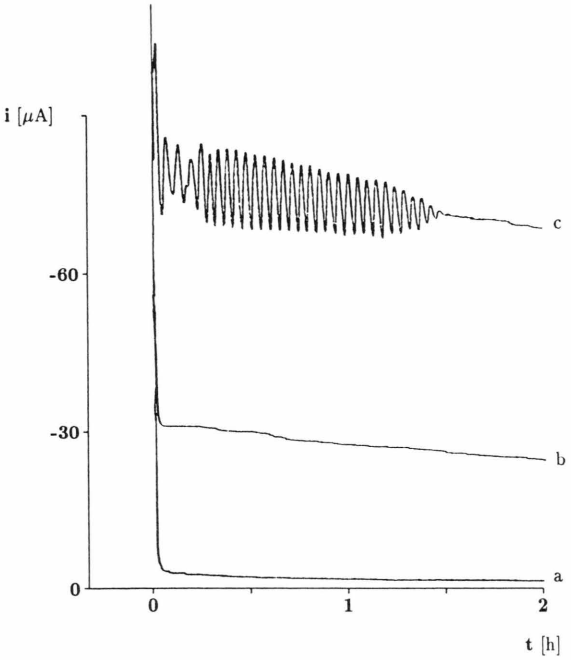

Fig. 4. Bifurcation of a catalytic steady state into oscillatory behavior as a function of an increasing concentration of H_2O_2. Chronoamperometry carried with parameters as in Figures 3a–c.

viously closely related, and the nontrivial behavior in the cyclic voltammogram is not simply caused by the sweeping potential.

Figure 4 now is a compact presentation of the dynamics of our system at a constant temperature gradient for the same conditions as in Figure 3. There are the two types of steady state, one uncatalyzed, one catalyzed, where the latter slowly shifts, probably because of some irreversible side reaction. During this intervall of time the solution turns yellow and red. Figure 4c once again shows a strong catalytic enhancement compared to Fig. 4b, meaning that the enzyme is still able to increase its turnover of substrate, but in addition this increase of the hydrogen peroxide concentration led to a change of the governing dynamical regime. The system switched from steady state to an oscillatory mode. The oscillation enters with one large peak (see above) and then, after a small number of transitory irregular peaks, displays a sustained, only slightly damped behavior; finally it ends up in a steady state. This experiment showed good reproducibility; overall behavior, mean frequency and amplitude stay pretty much the same from one experiment to another. The duration of the oscillatory mode, however, can vary between runs, sometimes it lasted longer than two hours, Fig. 4c is a typical result. The finding that small and permanent external disturbances were able to damp the oscillation into a steady state in less than one hour led us to the assumption that further improvement of the set-up will lead to stabilization of the oscillation, and therefore the underlying attractor of the idealized state space (no side reaction, constant concentration of hydrogen peroxide) is a limit cycle. Under these conditions the steady state has lost its stability and undergone a bifurcation.

In this sense we define the system of Fig. 4c as our oscillating reference system and now turn to qualitatively describe the changes which can be observed when altering one of the other reaction parameters. It turns out that our system has a large number of bifurcation parameters which can force it back into a steady state (apart from the trival solution of a parameter set zero). To give a preliminary orientation in this high dimensional parameter space we start with only a few steps in each direction.

An enzyme concentration of 0.079 μM yielded a stable steady state. There is a small minimum visible at the very beginning, but the damping is strong enough to prevent any peak. Increasing the concentration we witness the soft onset of oscillatory behavior with small amplitude, but we have not characterized the exact type of instability yet. As an example, at 0.184 μM for about one hour we observe a type of oscillation clearly different from the one shown in Figure 4c. This new type is of lower frequency, smaller average amplitude, and the size of peaks varies in an irregular manner. At some point the system suddenly switches into the simple large amplitude mode in which it rests for more than another hour. Our impression is that the first four peaks in Fig. 4c belong to this second type of oscillation, rather than to a mere transition into the "limit-cyclical" behavior. For at 0.236 μM the second type has disappeared, the simple oscillation is completely developed from the very beginning and it reproducibly lasts for more than two hours. Close inspection reveals a small shoulder before the maximum is reached and in one or two instances even a small second peak in the relaxation region (the minimum in contrast is sinusoidal).

Another important and easy to control parameter is the potential held constant during the experiments, i.e. the feed-back rate and the gradient control. At −100 mV the current drops in an almost exponential

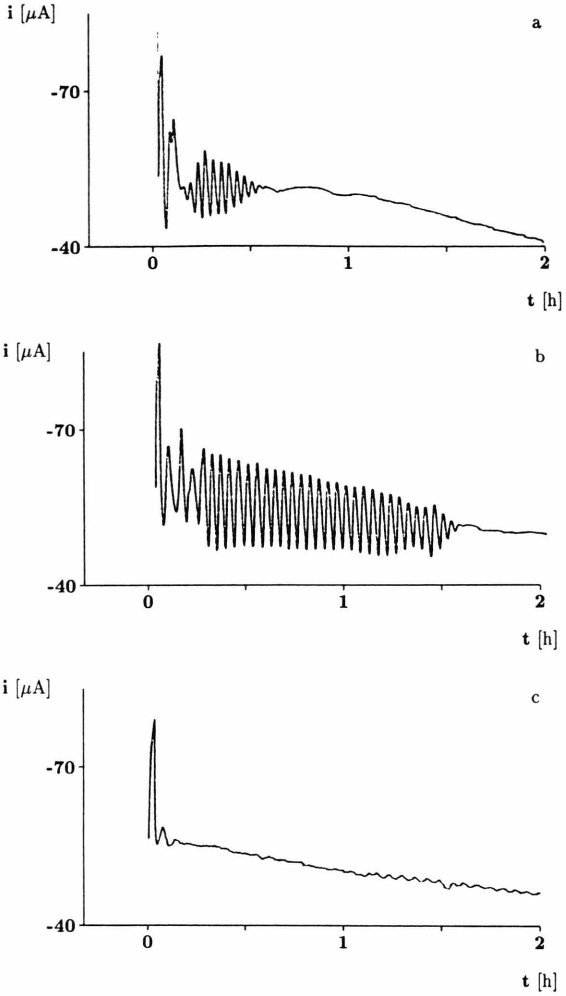

Fig. 5. Dynamics of the system at $E = -300$ mV with parameters as in Fig. 3c, except that a) $\Delta T = 0.5$ °C, b) $\Delta T = 1.0$ °C, and c) $\Delta T = 1.8$ °C.

way to the enhanced steady state, which then also slowly shifts. No signs of a focus or an oscillation in the vicinity can be detected. Applying -500 mV we observe the immediate onset of oscillations, but the average amplitude is significantly smaller than at $E = -300$ mV and a steady state is reached in about one and a half hour. Yet, this experiment revals a special feature of our system, which might become more important as our investigation continues. Namely, the trace of oscillations can be separated into three different sections. A first section of almost sinusoidal type, where successive peaks are of different amplitude, but look similar, a second section with a duration of about thirty minutes, which is completely irregular and, as described for low enzyme concentrations, the third section of simple large amplitude oscillations following a sudden switch. This last type is strongly damped in the experiment at $E = -500$ mV. It is too early to draw conclusions from these observations now, and better parameter sets have to be found for closer inspection of the different regimes as well as the transitions with fine tuned parameter changes. What is more important in our context is to collect empirical data on the influence of convective motion on the dynamical behavior.

Fig. 5 shows the system's behavior at three different values of ΔT. To complete the picture compare Fig. 3c of [5] for the same system, where $\Delta T = 0.0$ °C. At $\Delta T = 1.0$ °C the curve looks like Fig. 4c, i.e. the oscillation is stable within some finite range of parameter. A decrease of ΔT yields Fig. 5a, there are but a small number of peaks, the oscillation is not stable any more, strong damping results in a steady state. Moving in the other direction, at $\Delta T = 1.8$ °C a similar thing happens, but the damping is even stronger. The steady state in this case seems less stable, there are small remainders of an oscillation visible, but this could well be only an indication of a nearby bifurcation point. Even after several more hours no peaks arise.

It can be concluded that convective motion, as produced by a temperature gradient in our system, can be a necessary prerequisite to yield sustained oscillations when coupled with a complex, nonlinear reaction-diffusion system. On the other hand this convection cannot be the source of the oscillation (as for example in unstable convective rolls, cf. [7], or in patterns induced by Marangoni type convection [3]), because with the same gradient the oscillatory mode can be switched on and off by means of kinetic parameters (concentrations). It is only under the given special conditions for reaction and diffusion that an instability occurs.

Two more experiments may confirm that this special type of laminar convection plays an integral part in structure formation. We performed experiments with the reference system, but added a cylindrical shield as shown in Figure 1b. In this case, where horizontal convection is prevented in front of the electrode no oscillations arise, there are only two peaks with strong damping at the beginning. This is in agreement with our finding that the increase in steady state current as a function of increasing temperature gradient is mainly due to horizontal motion. It also appears that the task of convection may exceed the mere formation of a well-defined reproducible border of the diffusion layer as assumed in the diffusion layer approach for forced convection [6], because all our efforts using a rotating disk electrode (a technique widely applied to avoid inhomogeneities of the diffusion layer) failed to produce anything but one small peak followed by a steady state.

A possible mechanism for the onset of oscillations in electrode reactions is the interplay of surface corrosion and passivation known for a number of metals in either acidic or alkaline solution [8]. We interrupted an experiment at various phase angles of the limit cycle and compared the structure of the surface using a stereo microscope, but did not find any differences between steady state and oscillations, or between various phases of the oscillations. No precipitation was ever observed and no corrosion of the surface occurred. Also, after the oscillation had vanished and the experiment had been stopped, simple stirring of the solution was enough to restore an initial distribution that spontaneously started to oscillate again after another pulse to −300 mV. This seems quite unlikely in case the enzyme had been adsorbed at the surface. And, most convincingly, using a gold electrode with different diameter (1 mm) we could easily find a parameter set for which the system exhibited a related type of oscillations.

4. Discussion

What are the implications of these results? A simple new technique has been presented to introduce convective motion in an electrochemical setup. A temperature gradient is easy to handle and can be used as a control parameter. Although the nature of this convection cannot be quantified at present our results show that a major component of the velocity field vector must be parallel to the electrode's surface. Forced convection at a rotating disk electrode has a significant axial (perpendicular to the electrode's surface) and an angular component, the two techniques could thus contribute different pieces of information for the same system. The temperature gradient did not cause any disturbances in the cyclic voltammogram, not even at slow scan rates, it rather served to yield reproducible long-term behavior and therefore allowed to expand chronoamperometric experiments to up to several hours, long enough to observe the formation of dissipative structures.

Cyclic voltammetry and chronoamperometry have proved to be valuable tools in the search for complex dynamics of biochemical systems. Whereas cyclic voltammetry was used to find the domain of the nonlinearity leading to abnormal kinetics, only continuous measurement of a product dependent variable led to the actual detection of an oscillatory island in parameter space. The oscillation is due to a bifurcation of the catalytic steady state, several parameters have been shown to act as bifurcation parameters. Most remarkably, no sustained oscillations were ever found in the absence of a finite positive temperature gradient. We assume that only the complete reaction-diffusion-convection system is capable of the full dynamical complexity. Convection, although laminar, seems to play an integral part in structure formation when used to switch the dynamics of the system as shown in Figure 5. On the other hand given the qualitative difference between electrode materials and the quantitative difference in surface area of experiments with glassy carbon and gold, respectively, we are quite positive that coupling of reaction kinetics, diffusion and convection should suffice to describe the nature of the oscillations observed in our experiments.

5. Outlook

We have introduced a new artificial one-enzyme system for the study of self-organized dissipative structures. The temporal structures shown arise beyond the onset of a dynamical instability and are due to collective changes of concentrations in the diffusion layer of the electrode. Recordings of sustained oscillations turned out to be reproducible within narrow ranges of amplitude and frequency, the set-up might therefore be able to allow for distinction of periodic, complex periodic, and perhaps even chaotic dynamics. Together with periodic changes of the current in potentiostatic experiments we reproducibly find sections of irregular behavior, especially for parameter values between simple steady state and large amplitude oscillation. Although long-time observation is limited by slow irreversible shifts of the system it seems promising to further investigate the parameter space for complex and chaotic oscillations.

So far there is no well-suited set of differential equations for this new system with its special feature of coupling of reaction, diffusion, and convection resulting in chemical oscillations. We have recently started to investigate one-dimensional model equations for reaction-diffusion systems with electrochemical boundary conditions and a catalytic cycle, and are currently comparing several possible control mechanisms for the enzyme like forward inhibition and backward activation to account for the nonlinear kinetic effects described in Part I [5]. The oscillation is independent of electrode material as long as it is inert and of the exact geometry, so we expect a simple one-dimensional reaction-diffusion equation to form the core of a valid mathematical description. However, the oscillation is dependent on the nature of convection, so this will be the dominant part of the investigation of an extended reaction-diffusion-convection equation of the type

$$\frac{\partial b}{\partial t} = F + D_b \cdot \frac{\partial^2 b}{\partial \chi^2} - v_\chi \cdot \frac{\partial b}{\partial \chi}$$

for one spatial dimension χ. The question to be answered is: how can laminar convective motion at the electrochemical diffusion layer turn a stable reaction-diffusion system unstable?

As the system might well turn out to be a good model for intracellular organization of enzyme reactions where coupling occurs between a membrane bound enzyme and a plasma based enzyme, such a study is expected to yield results of more general interset than just a mathematical description for a specific in vitro reaction.

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