

MATHEMATICAL MODELING OF AMPEROMETRIC ENZYME ELECTRODES

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SUMMARY

The advantages of computer assisted enzyme electrode design are demonstrated for the case of electrodes with cycling reactions.

1. Introduction

The number of really existing amperometric enzyme electrodes is increasing.

The arrangements become more and more complicated:

- several enzymes are combined to enzyme systems
- non-stationary operational modes are used
- enzymes with non-linear kinetics are applied

The designer of new enzyme electrodes can be much supported by making available suitable mathematical models:

- calculation of all concentration profiles involved in the enzyme membrane (understanding of the basic principles)
- prediction of the dynamic behaviour of the sensor, e.g. current/time behaviour, response time, linear measuring range.
- optimizing of the sensor design (cost saving use of enzymes, achievement of given device parameters, use of commercial enzymes).

This aim can be realized by a user-friendly software package which operates in the laboratory on a personal computer. The corresponding mathematical fundamentals are available:

- one-dimensional reaction/diffusion systems are proved to be good
- linear models have been investigated extensively, numerous explicit formulae have been derived
- numerical methods and the tools for software development (compiler, libraries, graphics) are international standard.

2. The Basic Model

We consider an enzyme electrode with a cycling reaction.

An enzyme pair homogeneously distributed in a membrane cycles a substrate, A, an a product, B, and therefore causes a relatively large concentration change of an electro-active cosubstrate, O, (cofactor mode) or an electrochemically active coproduct, P. The use of two counteracting enzymes generates a continuous consumption/regeneration cycle for A and B, and this results in an increased consumption of the non-regenerated cofactor or an increased production of the coproduct beyond the usual stoichiometric limitations. This effect is called chemically-amplified response. The dynamic behaviour of the sensor in the coproduct mode can be described by a reaction/diffusion system. The two irreversible enzyme reactions are assumed to depend linearly on the concentrations A and B, respectively.

$$\begin{aligned} A_t &= D_A A_{xx} - k_1 A + k_2 B & A(0,t) &= A^0 & A_x(d,t) &= 0 & A(x,0) &= 0 \\ B_t &= D_B B_{xx} + k_1 A - k_2 B & B(0,t) &= 0 & B_x(d,t) &= 0 & B(x,0) &= 0 \\ O_t &= D_O O_{xx} - k_1 A & O(0,t) &= O^0 & O_x(d,t) &= 0 & O(x,0) &= O^0 \\ P_t &= D_P P_{xx} + k_1 A & P(0,t) &= 0 & P(d,t) &= 0 & P(x,0) &= 0 \end{aligned}$$

where $A(x,t)$, D_A and A^0 denote the concentration of A at point x ($0 \leq x \leq d$) and time t, the diffusion coefficient of A and the bulk concentration of A, respectively. The sensor side of the membrane corresponds to $x = d$.

The letter d stands for the thickness of the membrane. Perfect stirring is assumed.

For $D_A = D_B = D$ the solution can be given explicitly / 1 /

$$\begin{aligned} A(x,t) &= A^0 \frac{\pi-4}{\pi} \sum_{n=0}^{\infty} \frac{\sin((n+0.5)x\pi/d)}{2n+1} \left[\frac{k_1 + (k_2+u)e^{-(k+u)t}}{k+u} + \frac{k_2}{k} (e^{-ut} - e^{-(k+u)t}) \right] \\ B(x,t) &= A^0 \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{\sin((n+0.5)x\pi/d)}{2n+1} \left[\frac{k_1 + (k_2+u)e^{-(k+u)t}}{k+u} - \frac{k_1 e^{-ut}}{k} - \frac{k_2 e^{-(k+u)t}}{k} \right] \\ P(x,t) &= A^0 \frac{\theta k_1}{\pi^2} \sum_{m=1}^{\infty} \frac{\sin(m\pi(d-x)/d)}{2n+1} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \frac{4m}{4m^2 - (2n+1)^2} * \\ &* \left[\frac{k_2+u}{k+u} \left(\frac{1 - e^{-zt}}{z} - \frac{e^{-(+u)t} - e^{-zt}}{z - k - u} \right) + \frac{k_2}{k} \left(\frac{e^{-(k+u)t} - e^{-zt}}{z - k - u} - \frac{e^{-ut} - e^{-zt}}{z - u} \right) \right] \end{aligned}$$

$$\text{where } k = k_1 + k_2 \quad \text{and} \quad u = D(2n+1)^2 \pi^2 / (4d^2) \quad z = D_P m^2 \pi^2 / d^2$$

The stationary solutions are as follows

$$A(x) = A^0 \left(\frac{Q_1^2 \cosh(Q(d-x))}{\cosh(Qd)} + Q_2^2 \right) / Q^2$$

$$B(x) = A^0 \frac{D_A Q_1^2}{D_B Q^2} \left(1 - \frac{\cosh(Q(d-x))}{\cosh(Qd)} \right)$$

$$P(x) = A^0 \frac{D_A Q_1^2}{D_P Q^2} \left[\frac{Q_1^2}{Q^2} \left[1 - \frac{\cosh(Q(d-x))}{\cosh(Qd)} + \frac{x}{d} \left(\frac{1}{\cosh(Qd)} - 1 \right) \right] + Q_2^2 x(d-x)/2 \right]$$

$$\text{with } Q_1^2 = k_1/D_A, \quad Q_2^2 = k_2/D_B \quad \text{and} \quad Q^2 = Q_1^2 + Q_2^2$$

The ratio of the current change measured with the cycling electrode ($k_2 > 0$) to the current measured with the monoenzyme arrangement ($k_2 = 0$) represents the gain in sensitivity, G , of the sensor. In the stationary case we have

$$G_{st} = \frac{Q_1^4}{Q^4} \left[\left(\frac{1}{\cosh(Qd)} - 1 \right) - \frac{Q_1^2 Q_2^2 d^2}{2Q^2} \right] / \left(\frac{1}{\cosh(Q_1 d)} - 1 \right) \approx \frac{Q_1^2 Q_2^2 d^2}{2Q^2}$$

3. The double cycle

The most interesting generalization of the basic arrangement is the double cycle. The coproduct, L , produced by the first cycle of A and B is at the same time the substrate of a second consumption/regeneration cycle.

This use of a second pair of counteracting enzymes results in an increased influx of the second cycle and, therefore, in a doubly increased consumption of the monitored substance, O (cofactor mode). Considering the stationary operational mode with the same assumptions as in the basic case we obtain no changes for the concentrations of A and B and:

$$0 = D_L L_{xx} + k_1 A - k_3 L + k_4 C \quad L(0) = 0 \quad L_x(d) = 0$$

$$0 = D_C C_{xx} + k_3 L - k_4 C \quad C(0) = 0 \quad C_x(d) = 0$$

$$0 = D_O O_{xx} - k_3 L \quad O(0) = O^0 \quad O(d) = 0$$

Again, the solution can be formulated explicitly / 3 /.

The gain in sensitivity is now the ratio of the current change measured with the double cycle arrangement to the signal measured with the enzyme sequence electrode defined by $k_2 = k_4 = 0$. For high enzyme loading factors ($Q_1, Q_3 \gg 1$) the amplification G_{st} can be approximated quite accurately by

$$\tilde{G}_{st} = \frac{Q_1^2 Q_3^2}{Q_2^2 Q_5^2} \left[\frac{d^4 Q_2^2 Q_4^2}{12} + \frac{d^2}{2} \left(\frac{Q_1^2 Q_4^2}{Q_2^2} + \frac{Q_2^2 Q_3^2}{Q_5^2} \right) - \frac{Q_1^2 Q_4^2}{Q_4^4} - \frac{Q_2^2 Q_3^2}{Q_5^4} \right]$$

We note the formula shows the multiplying effect of the double cycle arrangement.

4. Sensor design (teaching example)

Problem: Find the cheapest enzyme loading factors (k_1, k_2) for a stationary sensor with a cycling reaction which realizes a given amplification factor, G^0 , if the second enzyme (k_2) is $f > 0$ times more expensive than the first enzyme (represented by k_1). The other parameters are assumed to be fixed.

Solution: Using the above approximate formula one can minimize the cost expression $k_1 + f^2 k_2$ with the constraint

$$G^0 = \frac{k_1 k_2 d^2}{2D(k_1 + k_2)}$$

The minimum reads

$$k_1 = 2DG^0 (1 + f)/d^2 \quad k_2 = 2DG^0 (1 + f)/(d^2 f)$$

Discussion: The cheaper first enzyme has to be used with an enzyme loading factor which is f times greater than that of the second enzyme.

5. References

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- /3/ T.Schulmeister and F.Schubert, *Mathematical Models of Amperometric Enzyme Electrodes with Chemically-Amplified Response*, 5. Intern.Bucher Sym. on Oxidoreductases and their Perspectives in Application, Berlin, Dec. 1988.