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## ELECTROCHEMICALLY ASSISTED CATALYST FOR ENZYMATIC GLUCOSE OXIDATION

P. GROS, A. BERGEL and M. COMTAT

Université Paul Sabatier

Laboratoire de Génie Chimique et Electrochimie URA CNRS 192

118, route de Narbonne 31062 TOULOUSE cedex, FRANCE

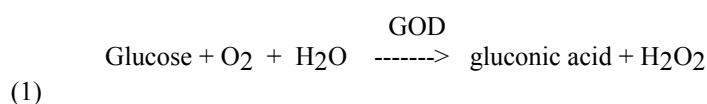
**Abstract** - A catalyst was made by entrapping glucose oxidase in a polymeric film deposited on an electrode surface. Its efficiency was studied by measuring the transformation of glucose by oxygen into gluconic acid. A numerical model was elaborated and validated with respect to experimental data. The electrochemical step affected the enzymatic catalysis in two ways: the reactant oxygen was regenerated, and the product, hydrogen peroxide which is a glucose oxidase inhibitor, was consumed. By monitoring these two concentrations, the electrochemical assistance significantly enhanced catalyst efficiency.

### INTRODUCTION

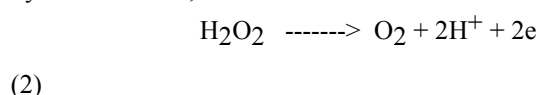
The modification of electrode surfaces represents an important domain of applied electrochemistry, whose techniques and application fields are continuing to widen. Until now, electrode surface modifications have essentially been used to increase the selectivity and the detection performances of electrochemical sensors (Ivaska, 1991; Imisides et al., 1991), but they could also offer promising opportunities for synthesis processes. As a general rule, the method consists in immobilizing proven homogeneous catalysts on the electrode in order to use their intrinsic properties in association with the new possibilities given by the electrochemical intervention: fine concentration control of some species, continuous electrochemical regeneration of some reactants, or easy extraction of the catalyst from the solution at the end of the process (Linford, 1987). Compared to a classic catalysis, this kind of electrochemical assistance allows the micro-environment of the catalyst to be monitored finely, by intervening directly in the core of the catalytic body.

A modified electrode was designed here, in order to assist electrochemically an enzymatic transformation. The enzymatic catalyst glucose oxidase was immobilized by entrapment inside a polymeric film formed at the electrode surface. Polymerization was performed by electrochemical oxidation of the pyrrole monomer, which is an increasingly common method used for electrode modification (Foulds and Lowe, 1986; Bartlett and Whitaker, 1987; Fortier et al., 1990). Among other advantages, a particular interest of the method is that it allows the thickness of the polymeric film to be controlled precisely (Holdcroft and Funt, 1988).

The entrapped glucose oxidase enzyme catalysed the oxidation of glucose by dissolved oxygen into gluconic acid:



When no potential was applied, the electrode only acted as an inert support, as would be the case with classic immobilized enzyme. However, when a suitable potential was applied, the hydrogen peroxide, which was produced by the enzymatic reaction, underwent oxidation at the electrode:



In this case, the electrochemical reaction (2) should enhance the enzymatic reaction rate (1) in two ways:

- by regenerating the reactant oxygen inside the catalytic film,
- by consuming the hydrogen peroxide, which would have drastically inhibited and denatured the glucose oxidase.

The aim of this work is to quantify the enhancement effect of electrochemical assistance on a catalytic reaction. Several groups have already discussed the interest of associating an electrochemical step with an enzymatic reaction. Most of the studies consisted of experimental approaches aimed at demonstrating the feasibility of such processes (Lortie et al., 1992; Cantet et al., 1995). A few specific electrochemical reactors have also been investigated (Fassouane et al., 1990; Grimes and Drueckhammer, 1993; Devaux et al., 1995) and a few theoretical works have dealt with modelling (Bauer et al., 1981; Bergel and Comtat, 1986; Labrune and Bergel, 1992). Nevertheless, most of the systems considered concerned homogeneous reactions or, when immobilized enzymes were used, this was only a means of retaining the enzyme near the electrode, without any regard to optimization (Mellor et al., 1992). To our knowledge, this is one of the first theoretical investigations dealing with the immobilisation of an enzyme in a polymer film in a view to the elaboration of a bioelectrochemical reactor.

The first part of the study is devoted to the elaboration of a theoretical model. The experimental data obtained with several modified electrodes with different film thicknesses were used to validate the model. The confrontation of the experimental and theoretical approaches achieved in this first part also led to a better description of the enzyme distribution inside the polymeric film. The model was used in the second part to quantify the improvement of the transformation reaction induced by the electrochemical regeneration of oxygen. This is discussed on the basis of the curves plotting the effectiveness factor as a function of the Thiele modulus. Furthermore, as predicted by the theoretical concentration profiles of hydrogen peroxide inside the catalytic film, the experiments conducted over longer periods of time in a batch reactor confirmed that the stability of glucose oxidase was increased when the hydrogen peroxide concentration in the film decreased.

## EXPERIMENTAL SECTION

### General

Glucose oxidase 220 U mg<sup>-1</sup> (E.C. 1.1.3.4) type VII from *Aspergillus niger*, β-D-glucose, potassium perchlorate and pyrrole were purchased from Sigma. All electrolytic solutions were prepared in aqueous phosphate buffer 0.1 mol l<sup>-1</sup> pH 7.0. The electrode modifications were carried out in a one-compartment cell at room temperature using a 0.03 cm<sup>2</sup> area platinum rotating disk working electrode. Enzymatic glucose oxidation was achieved in a laboratory scale batch reactor with a 4 cm<sup>2</sup> area platinum working electrode. In all cases an EGG model 362 potentiostat-galvanostat was used and connected to a Sefram x-y recorder. All potentials were measured versus a saturated calomel electrode (SCE). A large surface area platinum grid was used as counter electrode and placed opposite the working electrode to obtain homogeneous polymeric films. The glucose oxidase activity was measured spectrophotometrically according to the Sigma protocol. The unit is defined as the amount of enzyme necessary to catalyse the oxidation of one micromole of β-D-glucose in one minute at 35°C with oxygen as electron acceptor.

### Preparation of the modified electrode

The modified electrode was prepared by anodic electropolymerization of pyrrole 0.1 mol l<sup>-1</sup> in phosphate buffer also containing perchlorate ions 0.1 mol l<sup>-1</sup> as counter anions and glucose oxidase 1.5 mg ml<sup>-1</sup>. The solution was first deaerated and a nitrogen flux was maintained during experiments to avoid the oxidation of the monomer by dissolved oxygen. The working electrode was immersed in the solution one minute before starting and one minute after the end of electropolymerization to ensure the adsorption of the glucose oxidase at the platinum and polymer

surfaces. Polypyrrole films were grown galvanostatically with a current density value of  $600 \mu\text{A cm}^{-2}$  to keep a constant polymerization rate during electrolysis. The thickness of the polymeric films  $\delta_p$  was varied between 10 nm and 1  $\mu\text{m}$  by changing the electrolysis time and was controlled during electropolymerization by means of the charge passed (Holdcroft and Funt, 1988). The polypyrrole films were electrochemically overoxidised after being synthesised to obtain an inert and non-conducting matrix. This was performed by cycling the potential of the working electrode twice between  $-0.3\text{V/SCE}$  and  $1.2\text{V/SCE}$  at the sweep rate of  $5 \text{ mV s}^{-1}$  in the deaerated phosphate buffer.

### Glucose oxidation

The rotating disk electrode modified by a polypyrrole film containing the glucose oxidase was immersed in a three electrode cell containing 5 ml of aerated phosphate buffer. The rotation speed of the working electrode was 500 rpm and its potential was held at  $0.55\text{V/SCE}$  to ensure fast oxidation of the hydrogen peroxide produced by the enzymatic reaction. When a stable background current was reached ( $< 100 \text{ nA}$ ),  $125 \mu\text{l}$  of a  $200 \text{ mmol l}^{-1}$  glucose solution was injected to finally obtain a  $5 \text{ mmol l}^{-1}$  glucose solution. The current stabilized at a steady value in about thirty seconds.

In the case where the enzymatic reaction was achieved in the batch reactor,  $2.5 \text{ ml}$  of a  $20 \text{ mmol l}^{-1}$  glucose solution was introduced into the reactor. The solution was stirred with a magnetic bar. As soon as the modified electrode was immersed in the glucose solution, its potential was held at  $0.4\text{V/SCE}$ . Under this condition the glucose solution was not found to be directly oxidised at the platinum electrode during at least 10 hours. The variation of the glucose concentration was measured enzymatically by using the Glucose [HK] reagent purchased from Sigma.

### THEORY

Figure 1 represents the modified electrode structure highlighting the different locations of the enzyme entrapped in the polymeric matrix. Glucose oxidase was assumed to be uniformly distributed inside the polypyrrole film. Previous experimental studies showed that the enzyme could also be adsorbed at the electrode and at the polymer surfaces (Bartlett and Caruana, 1992; Hämmerle et al., 1992; Gros and Bergel, 1995). Glucose and oxygen diffused from the bulk of the solution through the polymeric film and were simultaneously involved in the enzymatic reaction. Depending on whether an electrode potential was applied or not, the hydrogen peroxide produced by the reaction underwent oxidation at the electrode or not.

Modelling was based on the steady state mass balance equations for the three species in the polymer. These differential equations involved both the mass transport of the species in the matrix and the enzymatic reaction kinetics. Assuming an enzymatic catalysis of the "ping-pong" type, the steady state mass balance equations for glucose, oxygen and hydrogen peroxide are respectively:

$$D_g^p \frac{d^2 C_g}{dz^2} - \frac{r_{\max}}{1 + K_g/C_g + K_o/C_o} = 0 \quad (3)$$

$$D_o^p \frac{d^2 C_o}{dz^2} - \frac{r_{\max}}{1 + K_g/C_g + K_o/C_o} = 0 \quad (4)$$

$$D_h^p \frac{d^2 C_h}{dz^2} + \frac{r_{\max}}{1 + K_g/C_g + K_o/C_o} = 0 \quad (5)$$

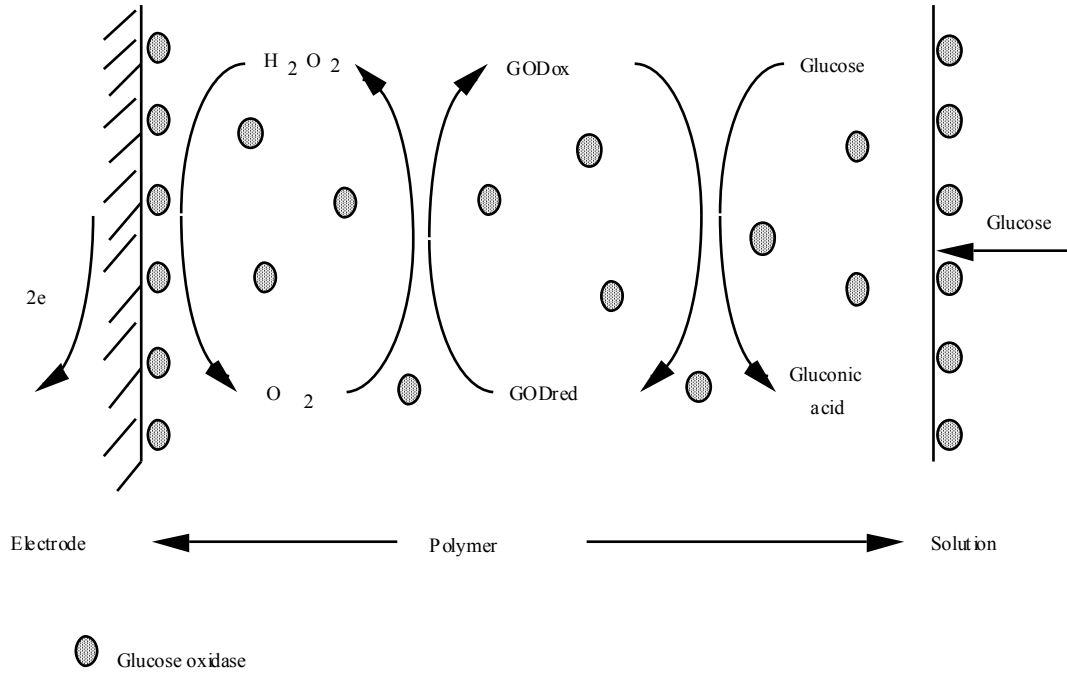


Figure 1. General kinetic scheme for the polypyrrole glucose oxidase (GOD) modified electrode.

The boundary conditions at the electrode took account of both the electrochemical conditions and the adsorption of glucose oxidase at the platinum surface. In the case where no potential was applied at the electrode, only the enzymatic catalysis due to the adsorbed glucose oxidase was considered in the equations:

$$D_g^p \frac{dC_g}{dz} \Big|_{z=0} = \frac{r_{\max}^{ad}}{1 + K_g/C_g|_{z=0} + K_o/C_o|_{z=0}} \quad (6)$$

$$D_o^p \frac{dC_o}{dz} \Big|_{z=0} = D_g^p \frac{dC_g}{dz} \Big|_{z=0} \quad (7)$$

$$D_h^p \frac{dC_h}{dz} \Big|_{z=0} = - D_g^p \frac{dC_g}{dz} \Big|_{z=0} \quad (8)$$

In the case of electrochemically assisted enzymatic catalysis, experiments showed that applying an electrode potential of at least 0.4 V/SCE allowed the electrochemical oxidation of hydrogen peroxide to be mass transport controlled. In this case, the hydrogen peroxide concentration at the electrode surface is zero:

$$C_h|_{z=0} = 0 \quad (9)$$

Furthermore the mass flux densities of oxygen and hydrogen peroxide at the electrode surface are equal and opposite, according to equation 2:

$$D_o^p \frac{dC_o}{dz} \Big|_{z=0} = - D_h^p \frac{dC_h}{dz} \Big|_{z=0} \quad (10)$$

The expression of the current induced by the electrochemical step took account of the hydrogen peroxide flux arising from the polymer, and of the hydrogen peroxide produced at the platinum surface by the adsorbed enzyme:

$$I = 2FA \left( D_h^p \frac{dC_h}{dz} \Big|_{z=0} + \frac{r_{\max}^{ad}}{1 + K_g/C_g|_{z=0} + K_o/C_o|_{z=0}} \right) \quad (11)$$

The adsorption of the enzyme at the polypyrrole surface was considered in the corresponding boundary conditions. Taking account of a linear concentration profile in the hydrodynamic diffusion layer, the equations are:

$$D_g^p \frac{dC_g}{dz} \Big|_{z=dp} + \frac{r_{\max}^{ad,p}}{1 + K_g/C_g|_{z=dp} + K_o/C_o|_{z=dp}} = \frac{D_g^b}{\delta_d} (C_g^b - C_g^s) \quad (12)$$

$$D_o^p \frac{dC_o}{dz} \Big|_{z=dp} + \frac{r_{\max}^{ad,p}}{1 + K_g/C_g|_{z=dp} + K_o/C_o|_{z=dp}} = \frac{D_o^b}{\delta_d} (C_o^b - C_o^s) \quad (13)$$

$$D_h^p \frac{dC_h}{dz} \Big|_{z=dp} - \frac{r_{\max}^{ad,p}}{1 + K_g/C_g|_{z=dp} + K_o/C_o|_{z=dp}} = \frac{D_h^b}{\delta_d} (C_h^b - C_h^s) \quad (14)$$

The glucose concentration in the bulk of the solution  $C_g^b$  was freely chosen. The oxygen  $C_o^b$  and hydrogen peroxide  $C_h^b$  concentrations were assumed to be always equal to  $0.24 \text{ mmol l}^{-1}$  and  $0 \text{ mmol l}^{-1}$  respectively.

Using dimensionless variables (Leypoldt and Gough, 1984) three principal dimensionless numbers were introduced (Coulson et al., 1979; Villermaux, 1993): the Thiele modulus relating to glucose species which is the ratio of the potential enzymatic reaction rate without internal diffusion limitation to the maximum diffusion rate in the polymer:

$$\Phi_g^2 = \frac{r(C_g^s, C_o^s) \cdot \delta_p^2}{\alpha_g \cdot D_g^p \cdot C_g^s} \quad (15)$$

the mass transfer Biot number for all three species, which represents the ratio of the external diffusion (in the solution) to the internal diffusion (in the polypyrrole film):

$$B_{ij} = \frac{D_j^b \cdot \delta_p}{\alpha_j \cdot \delta_d \cdot D_j^p} \quad (16)$$

and the effectiveness factor defined as the ratio of the effective reaction rate in the polymer to the enzymatic reaction rate without internal diffusion:

$$\eta = \frac{\frac{D_g^b}{\delta_d} (C_g^b - C_g^s)}{r(C_g^s, C_o^s) \cdot \delta_p} \quad (17)$$

The numerical process was carried out by a classic finite difference method. An iterative calculation was necessary because of the non-linearity of the enzymatic kinetics.

## RESULTS AND DISCUSSION

### Model validation

Figure 2 shows the amperometric responses (discrete points) recorded for the oxidation of a  $5 \text{ mmol l}^{-1}$  glucose solution at the rotating disk electrode modified by polypyrrole films of different thicknesses. Glucose oxidase was entrapped in the polymeric matrix and adsorbed at the platinum and at the polymer surfaces as described above. The current increased rapidly in the first part of the curve and then decreased when the thickness of the film went beyond

an optimum value of approximately 250 nm. The evolution of the experimental results showed two different behaviours of the modified electrode: for thin polymeric films, the electrode response depended on the amount of

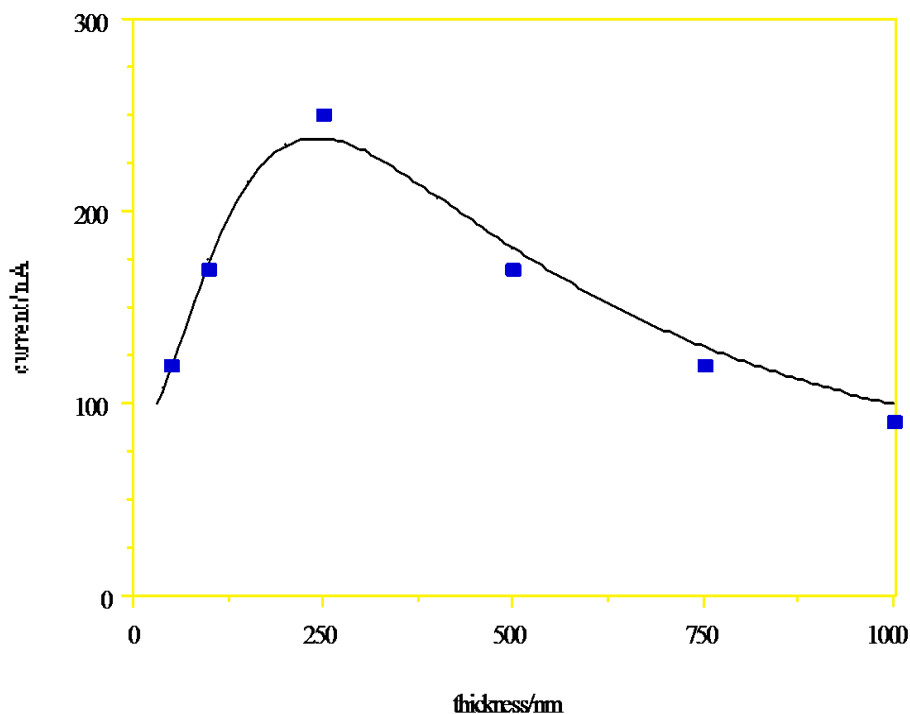


Figure 2. Variation of the amperometric response of the modified electrode as a function of the film thickness. — theoretical curve; ■ experimental results.

enzyme immobilized and was therefore limited by the enzymatic reaction kinetics. The elaboration of thicker films resulted in a greater diffusion barrier for all the species and the current became mass transport controlled. The continuous line represents the best theoretical curve obtained by adjusting a minimum set of parameters. The parameter  $r_{\max}^{\text{ad}}$  was determined by fitting the theoretical result to the experimental amperometric response obtained with a 100 nm thick polypyrrole modified electrode with the enzyme only adsorbed at the platinum surface. A similar experiment performed with the same modified electrode with the glucose oxidase only adsorbed at the polymer surface led to the determination of the value of the parameter  $r_{\max}^{\text{ad,p}}$ . The other parameters adjusted were the maximum activity of the enzyme in the polymeric matrix  $r_{\max}$  and the diffusion coefficient of glucose in the polymer  $D_g^p$ . All other physico-chemical parameters were taken from the literature or determined by independent experiments, as described previously (Gros and Bergel, 1995), and always had the same values for all experiments. Figure 2 shows that the theoretical model succeeded well in fitting the experimental results.

### Efficiency of the catalytic process

The model was further used to predict the evolution of catalyst efficiency as a function of the Thiele modulus and thus to estimate the effect of the electrochemical assistance. The effectiveness factor variation is plotted in Figure 3.a. in the case of a 20 mmol l<sup>-1</sup> glucose solution and where no potential was applied to the modified electrodes. The continuous line shows the theoretical results obtained in the case where only the enzyme entrapped inside the polymeric matrix is involved in the calculation. This curve clearly highlights the two different types of behaviour as a function of the film thickness. For Thiele modulus values less than 0.25 the effectiveness factor is constant and its value is near 1. The reaction rate is limited by the enzymatic kinetics. When the Thiele modulus is more than 0.6

however, the effectiveness factor decreases strongly when the thickness of the polymer increases. The diffusion of glucose inside the film becomes therefore the rate determining factor.

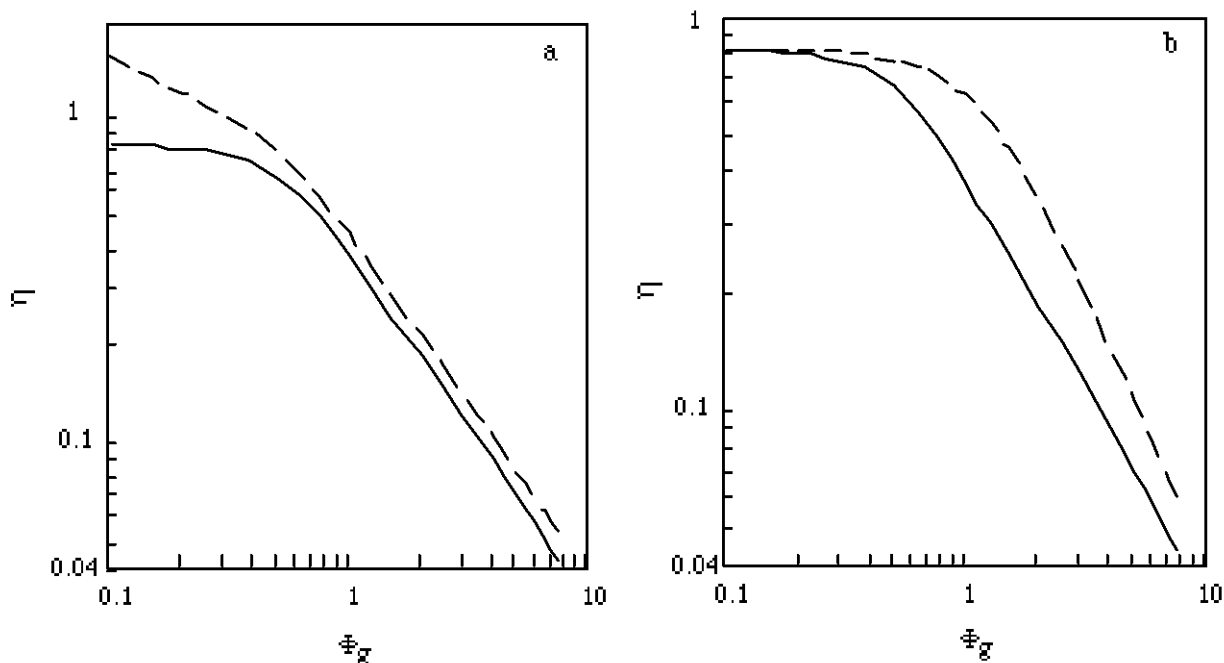


Figure 3. Influence of the Thiele modulus on the effectiveness factor.

Fig. 3.a. Influence of the location of the glucose oxidase. — enzyme only entrapped in the polymeric matrix; - - - enzyme entrapped in the polymer and adsorbed at the electrode and the polymer surfaces.

Fig. 3.b. Influence of the electrochemical step. — without electrochemical assistance; - - - with electrode potential.

The dotted line corresponds to the theoretical calculations taking account of the glucose oxidase both entrapped inside the film and adsorbed at the platinum and at the polymer surfaces. The comparison of the two curves shows that the efficiency of the electrode increases in the presence of the adsorbed glucose oxidase for low Thiele modulus values only. In this case, kinetics is rate determining and the enzyme adsorbed either at the electrode or at the polymer surfaces significantly affected catalyst efficiency. The effectiveness factor reached a value higher than 1 because the maximum enzymatic reaction rate, which was estimated without internal diffusion in the calculation of the effectiveness factor, did not take account of the adsorbed enzyme layers. For Thiele modulus values greater than 0.6 this effect remained secondary. In the case where the potential is applied, experimental results have shown for instance that the adsorbed enzyme layers contributed for less than 10% of the amperometric response due to the oxidation of the glucose at a 250 nm thick polymer modified electrode (Gros and Bergel, 1995).

#### **Influence of the electrochemical assistance**

Figure 3.b. shows the influence of the electrochemical assistance on the efficiency of enzymatic catalysis. The continuous line is the same as that in figure 3.a. whereas the dotted line was obtained in the case where the potential was applied at the electrode. Figure 3.b. clearly shows that the electrochemical step has no influence on the effectiveness factor for low Thiele modulus because the enzymatic kinetics is the rate determining process. For high Thiele modulus, application of the potential, leading to the regeneration of the oxygen, significantly improves the efficiency of the catalysis. In this case the overall rate is limited by the internal diffusion and therefore depends strongly on the concentration of the species in the polymer. This result is confirmed by Figure 4.a. and 4.b. showing the concentration profiles of the oxygen and of the hydrogen peroxide respectively in a 250 nm thick polypyrrole



film with and without electrochemical assistance. Figure 4.a. shows that the average concentration of oxygen, that is the co-substrate of the enzymatic reaction, is greater when the potential is applied whereas the consumption of hydrogen peroxide at the electrode leads to a lower average concentration. These concentration profiles also illustrate the fact that the simplification assumption generally used in previous modellings, e.g. no external diffusion, negligible oxygen and hydrogen peroxide concentration gradient... are not valid for the general case.

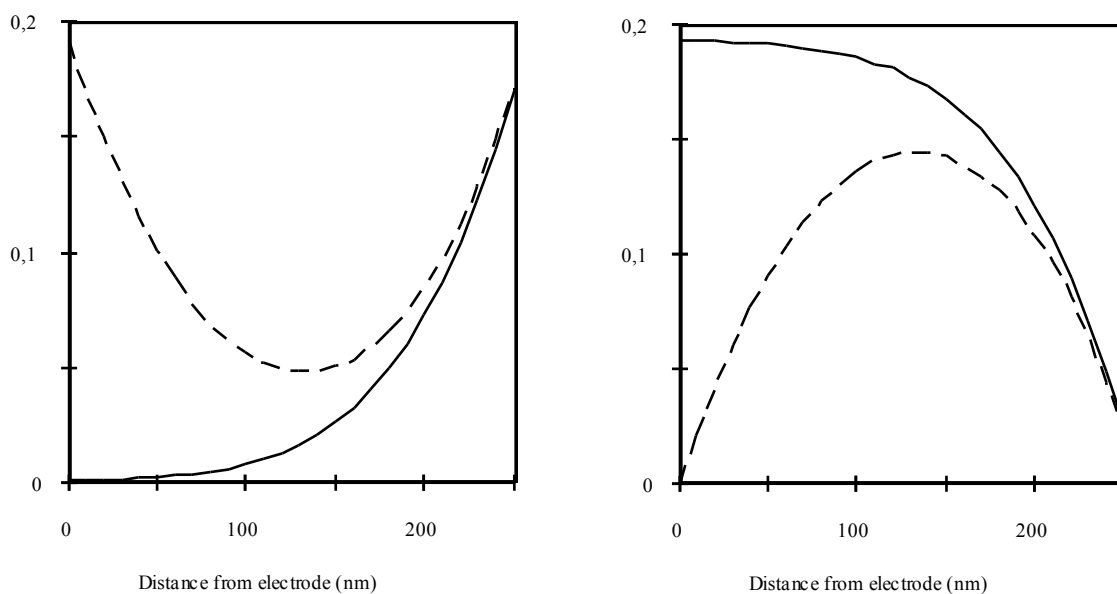


Figure 4. Concentration profile of oxygen (Fig. 4.a) and of hydrogen peroxide (Fig. 4.b) in a 250 nm thick polypyrrole film. — without electrochemical assistance; - - - with electrode potential.

The enzymatic rate expression used in the model only took account of the concentrations of the reactants, oxygen and glucose. As a consequence, the influence of the electrochemical assistance was only estimated by the modifications which affected the oxygen concentration in the polymeric film. It was not possible to predict theoretically the influence of the hydrogen peroxide consumption without using a more complex kinetic expression, which should take account of the enzyme inhibition and/or denaturation by hydrogen peroxide. For this reason, an experimental investigation was carried out with a thin modified electrode. The thickness of the polymer, about 10 nm, corresponded to the Thiele modulus range where no influence of the electrochemical step was expected by the model, because the enzymatic kinetics is the rate determining factor. Nevertheless, the transformation ratios obtained experimentally after a 6 hour electrolysis of a 20 mmol l<sup>-1</sup> glucose solution varied from 25% without the electrochemical step to 50% when the potential was applied. This difference can only be explained by a change in the enzymatic rate. The hydrogen peroxide consumption significantly decreased the enzyme inhibition and/or denaturation, which resulted in greater catalyst efficiency. The kinetics of the enzyme denaturation will have to be determined precisely so that this effect can be quantified by the model.

## CONCLUSION

On the basis of an electrode modification technique, which is commonly used in the field of analytical chemistry, a method for designing catalysts is proposed here. The electrochemical step, which was thus added to the catalytic pathway, allowed the partial monitoring of the concentrations of the electroactive species in the catalyst body. In the example considered, a reactant was generated, while an inhibitory product was consumed. The two actions were demonstrated both theoretically and experimentally. The electrode modification domain, which is growing

continuously, should afford more and more new possibilities for catalyst design. The conducting polymers could for instance help to improve the fine control of the concentrations inside the catalyst. In this case, the electrochemical step could occur throughout the polymeric structure, rather than being limited to the electrode surface.

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## Notation

$A$  = electrode surface area.

$B_{ij}$  = mass transfer Biot number for the species  $j$ .

$C_j$  = concentration of the species  $j$ .

$D_j$  = diffusivity of the species  $j$ .

$F$  = Faraday's constant.

$I$  = current.

$K_j$  = Michaelis-Menten constant relative to the species  $j$ .

$r_{\max}$  = activity of the glucose oxidase in the polymer.

$r_{\max}^{\text{ad}}$  = activity of the glucose oxidase adsorbed on the electrode surface.

$r_{\max}^{\text{ad,p}}$  = activity of the glucose oxidase adsorbed on the polymer surface.

$z$  = distance from the electrode.

$\alpha_j$  = partition coefficient of the species  $j$ .

$\delta_d$  = thickness of the hydrodynamic diffusion layer.

$\delta_p$  = thickness of the polymer film.

$\eta$  = effectiveness factor.

$\Phi_g$  = Thiele modulus.

### Subscripts

$g, o, h$  = indexes for glucose, oxygen and hydrogen peroxide species.

### Superscripts

$b$  = in the bulk of the solution.

$p$  = in the polymer film.

$s$  = at the polymer surface.

## REFERENCES

Bartlett, P.N. and Whitaker, R.G., 1987, *J. Electroanal. Chem.* **224**, 37.

Bartlett, P.N. and Caruana, D.J., 1992, *Analyst* **117**, 1287.

Bauer, R., Friday, D.K. and Kirwan, D.J., 1981, *Ind. Eng. Chem. Fundam.* **20**, 141.

Bergel, A. and Comtat, M., 1986, *Biotechnol. Bioeng.* **28**, 728.

Cantet, J., Bergel, A. and Comtat, M., 1995, *Enzyme and Microb. Technol.* in press.

Coulson, J.M., Richardson, J.E. and Peacock, D.G., 1979, *Chemical Engineering Volume 3*, 2th Edn, pp.115-143. Pergamon Press, Oxford.

Devaux, R., Bergel, A. and Comtat M., 1995, *AIChE J.* **41**, 1944.

Fassouane, A., Laval, J.-M., Moiroux J. and Bourdillon, C., 1990, *Biotechnol. Bioeng.* **35**, 935.

- Fortier, G., Brassard, E. and Bélanger, D., 1990, *Biosens. Bioelectron.* **5**, 473.
- Foulds, N.C. and Lowe, C.R., 1986, *J. Chem. Soc. Faraday Trans. I.* **82**, 1259.
- Grimes, M.T. and Drueckhammer, D.G., 1993, *J. Org. Chem.* **58**, 6148.
- Gros, P. and Bergel, A., 1995, *J. Electroanal. Chem.* **386**, 65.
- Hämmerle, M., Schuhmann, W. and Schmidt, H.L., 1992, *Sensors Actuators* **B6**, 106.
- Holdcroft, S. and Funt, B.L., 1988, *J. Electroanal. Chem.* **240**, 89.
- Imisides, M.D., John, R., Riley, P.J. and Wallace, G.G., 1991, *Electroanalysis* **3**, 879.
- Ivaska, A., 1991, *Electroanalysis* **3**, 247.
- Labrune, P. and Bergel, A., 1992, *Chem. Eng. Sci.* **47**, 1229.
- Leyboldt, J.K. and Gough, D.A., 1984, *Anal. Chem.* **56**, 2896.
- Linford, R.G., 1987, *Electrochemical Science and Technology of Polymers*, Elsevier Applied Science, London.
- Lortie, R., Fassouane, A., Laval J.-M. and Bourdillon C., 1992, *Biotechnol. Bioeng.* **39**, 157.
- Mellor, R.B., Ronnenberg, J., Campbell, W.H. and Diekmann, S., 1992, *Nature* **355**, 717.
- Villiermaux, J., 1993, *Génie de la réaction chimique. Conception et fonctionnement des réacteurs*, 2th Edn, pp. 297-326. Tec & Doc - Lavoisier, Paris.