Electrochemically Enhanced Biosynthesis of Gluconic Acid

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DOI 10.1002/aic.10411
Published online in Wiley InterScience (www.interscience.wiley.com).

A bioelectrochemical interface was designed by entrapping glucose oxidase (GOx) in a polypyrrole film electrosynthesized on a platinum electrode. The efficiency of the modified electrodes for synthesis was evaluated with the reaction of glucose oxidation into gluconic acid by oxygen. Glucose transformation was carried out with or without the electrochemical step. The electrochemical step led to the regeneration of oxygen, which also ensured the oxidation of the hydrogen peroxide produced as a by-product, thus protecting GOx against denaturing. The experimental results were first analyzed according to the "effectiveness factor/Thiele modulus" classic approach. They were confirmed theoretically by a model based on the resolution of glucose mass balance equation. Second, the simulation of the concentration profiles of oxygen and hydrogen peroxide inside the polymer film explained the experimental data, highlighting the protection of the enzyme by the electrochemical step. The maximum conversion yield was obtained with a 500-nm-thick polymer. As a consequence of the combined effects of oxygen regeneration and enzyme protection, the electrochemical step increased by a factor of 2 the transformation ratios obtained with immobilized GOx. Furthermore, the electrochemically enhanced process was 50% more efficient than the equivalent homogeneous process carried out for 24 h with the same amount of enzyme. © 2005 American Institute of Chemical Engineers *AIChE J,* 51: 989–997, 2005

Introduction

In the field of large-scale biotransformations, replacing the usual fermentation techniques with processes based on isolated enzymes would represent a major improvement. Using only the right enzyme(s) instead of whole cells limits the number of side reactions and consequently reduces the quantity of by-products and the number of downstream extraction, separation, and purification steps. Oxidoreductases, which catalyze electron-transfer reactions, offer real opportunities for very selective syntheses in many industrial fields such as chemistry, pharmacy, cosmetology, or food.[1] However the use of this class of enzymes is still regarded as uneconomical for industrial processes.[2] The main drawback is that oxidoreductases require an end-electron donor (to reduce the substrate) or an end-electron acceptor (to oxidize the substrate), which must be added at least in stoichiometric amounts. An elegant alternative consists in replacing the end-electron donor or acceptor by an electrode. In this way, the electrochemistry appears as "an intrinsically environmentally friendly technique"[3]: electrons can be taken from or given to the catalytic mechanism without using an electron acceptor or donor in stoichiometric amount at the end of the electron-transfer chain.

Designing efficient and low-cost electroenzymatic processes requires the successful coupling of the enzyme-catalyzed reaction and the electrochemical reaction. Only the part of the enzyme that is close to the electrode surface is really involved in the biochemical synthesis. Enzyme immobilization is thus strongly desirable for industrial purposes because it reduces the amount of the enzyme required, it allows the enzyme to be reused, and minimizes its loss in solution. One way is to physically retain a thin film of the biocatalyst solution by

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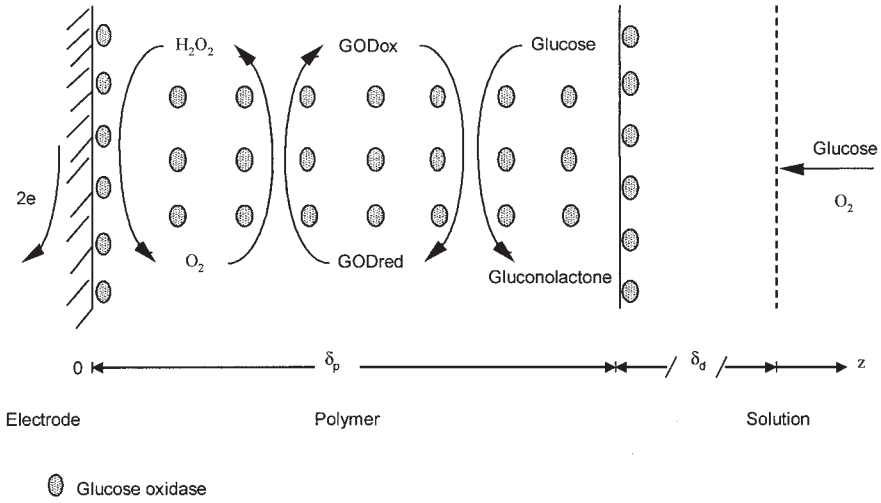

Figure 1. General kinetic scheme for the polypyrrole glucose oxidase modified electrode.

means of a filtration membrane.[4] A few articles have been devoted to the development of a membrane electrochemical reactor for the synthesis of lactate[5] or the reduction of cyclohexanone into cyclohexanol.[6] Nevertheless, previous studies concluded that this method of confinement did not allow a close contact between the enzyme and the electrode surface, which was particularly critical when a direct electron transfer had to be promoted.[7]

Numerous techniques have been attempted to immobilize oxidoreductases on electrode surfaces, such as crosslinking with glutaraldehyde with glucose dehydrogenase[8] or films grown layer upon layer and cast surfactant films, to carry out heme-catalyzed synthesis.[9] Among them, immobilization with electrogenerated conducting polymers has been the subject of an increasing number of research articles and reviews.[10,11] The method is simple and attractive: the polymer films can be prepared in one-step electrolysis from either aqueous[12] or organic solvent.[13] The characteristics of the films obtained—homogeneous, chemically stable, strongly adherent to the electrode surface—make them really suitable for large-scale synthesis. Several parameters, such as the thickness,[14] and the amount of enzyme immobilized[15] can be finely controlled by the choice of the electropolymerization conditions, such as the concentration of the monomer,[16] the electrolysis time, and the electrolysis current or potential.[17] Despite all these advantages, articles dealing with the use of an enzyme–polymer-based electrochemical interface for preparative purposes are very scarce compared to those focused on analytical applications. Amounas et al.[18] used a glass column reactor with glucose oxidase (GOx) attached on a polymer membrane formed by electropolymerization of biotinylated pyrrole on a carbon felt. In this study the biochemical transformation of glucose into gluconic acid was not electrochemically supported. De Benedetto et al.[19] designed a continuous flow-through tyrosinase reactor for removing phenol in low concentration from aqueous solution; the electrochemical interface was based on a reticulated vitreous carbon functionalized by a polytyramine film. Bartlett et al.[20] used polyaniline for the adsorption of horseradish peroxidase, which catalyzed the model reaction of hydrogen peroxide reduction. These experimental approaches demonstrated the feasibility of such processes, but no theoretical investigations, with the aim of optimizing the system, have yet been realized.

A laboratory-scale batch electrochemical reactor was developed in this article for the transformation of glucose into gluconic acid. The bioelectrochemical interface was based on an electrogenerated polypyrrole film–modified electrode immobilizing glucose oxidase. Oxidation of glucose consumed the dissolved oxygen, which gave hydrogen peroxide. The electrochemical step oxidized hydrogen peroxide back to oxygen. Here, the electrode played the role of end-electron acceptor through the regeneration of oxygen, which was continuously consumed by the GOx-catalyzed synthesis of gluconic acid. Nevertheless, the essential effect of the electrochemical step was to destroy hydrogen peroxide, which strongly inactivates glucose oxidase.[21] Three configurations were compared: the whole bioelectrosynthesis, the biosynthesis with immobilized GOx but without electrochemical step, and the homogeneous catalysis with the same quantity of GOx dissolved in solution. The experimental work was coupled to theoretical modeling to quantify the improvement in catalytic efficiency given by the electrochemical step.

Theory

Physical description

Figure 1 represents the structure of the polypyrrole glucose oxidase–modified electrode. The enzyme was assumed to be uniformly distributed inside the matrix as a result of the galvanostatic electropolymerization, which ensured a constant growth rate of the polypyrrole film. Previous works have shown that glucose oxidase was also adsorbed on the electrode surface and on the external polymer surface.[22,23] Dipping the clean platinum electrode into the solution 1 min before the electropolymerization started, and extracting the polypyrrole–GOx electrode 1 min after it ended ensured reproducible adsorption on both surfaces. Glucose and oxygen diffused from the well-stirred bulk solution through the hydrodynamic diffusion layer (δ_d) and the polymer film (thickness δ_p), where they simultaneously underwent the GOx-catalyzed reaction:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \quad (1)$$

In the case where the electrochemical step was implemented, the hydrogen peroxide produced was electrochemically oxidized and oxygen was thus regenerated on the electrode

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

Mass balance equations

Neglecting the volume of the hydrodynamic diffusion layer vs. the volume of the bulk solution, the glucose mass balance equation becomes

$$V\frac{dC_g^b(t)}{dt} = -AD_g^b\frac{C_g^b - C_g^s}{\delta_d} \quad (3)$$

where $[dC_g^b(t)]/dt$ is the evolution of the glucose concentration in the bulk solution, whereas the second term corresponds to the substrate flux at the polymer–solution interface. Calculation of the mass flux of glucose at the polymer–solution interface required the concentration profile of glucose inside the polymer. The pseudo-steady-state approach was used, assuming that mass-transfer rates and enzyme kinetics in the polymer film were fast with respect to the evolution rate of the bulk concentrations. In this condition, the glucose, oxygen, and hydrogen peroxide concentration profiles in the film can be described by steady-state mass balance equations. Assuming "ping-pong"-type enzyme kinetics,[24] the steady-state mass balance equations in the film were as follows

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

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

$$D_h^p\frac{d^2C_h^p}{dz^2} + \frac{r_{\max}}{1 + \frac{K_g}{C_g^p} + \frac{K_o}{C_o^p}} = 0 \quad (6)$$

The boundary conditions at the platinum surface took account of both the electrochemical conditions and the adsorption of glucose oxidase. In the case where no potential was applied at the electrode, only the enzymatic catalysis arising from the adsorbed GOx on the platinum surface was considered

$$D_g^p\left(\frac{dC_g^p}{dz}\right)_{z=0} = \frac{r_{\max}^{ad}}{1 + \frac{K_g}{(C_g^p)_{z=0}} + \frac{K_o}{(C_o^p)_{z=0}}} \quad (7)$$

$$D_o^p\left(\frac{dC_o^p}{dz}\right)_{z=0} = D_g^p\left(\frac{dC_g^p}{dz}\right)_{z=0} \quad (8)$$

$$D_h^p\left(\frac{dC_h^p}{dz}\right)_{z=0} = -D_g^p\left(\frac{dC_g^p}{dz}\right)_{z=0} \quad (9)$$

In the case of electrochemically supported enzymatic catalysis, experiments showed that applying an electrode potential of 0.4 V/SCE (saturated calomel electrode) allowed the electrochemical oxidation of hydrogen peroxide to be mass transport controlled. Its concentration at the electrode surface was then zero

$$(C_h^p)_{z=0} = 0 \quad (10)$$

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

$$D_h^p\left(\frac{dC_h^p}{dz}\right)_{z=0} = -D_o^p\left(\frac{dC_o^p}{dz}\right)_{z=0} \quad (11)$$

The adsorption of the enzyme at the polypyrrole film surface was considered in the corresponding boundary conditions. Assuming a linear concentration profile in the hydrodynamic diffusion layer, the boundary conditions at the polymer–solution interface were

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

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

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

Using dimensionless concentrations,[25] the steady-state mass balance differential equations in the polymer film were transformed into a system of algebraic equations and solved numerically by a classic finite-difference method.[26] An iterative calculation was necessary because of the nonlinearity of the enzymatic kinetics. Resolution of the equations required the determination of the parameters listed in Table 1. Diffusion coefficients of oxygen and hydrogen peroxide in the bulk solution and in the polypyrrole film were obtained by means of independent experiments consisting in measuring the limiting current corresponding to the reduction of oxygen and the oxidation of hydrogen peroxide on a rotating disk of bare platinum or on a 0.1-μm-thick polypyrrole-modified electrode, respectively.[22] The other parameters were obtained by independent theoretical fitting (see below). Finally, the glucose concentration profile in the polymer was integrated as a subroutine in a Runge–Kutta order 2 algorithm to solve the pseudo-steady-state mass balance equation of glucose in the bulk (Eq. 3).

Table 1. Values of the Parameters Used in the Modeling*

Parameter	Value	Reference	$\Delta I^{\dagger}$ (%)
K_g	33 mol m^{-3}	[34]	
K_o	0.2 mol m^{-3}	[34]	
α_g	0.8	[35]	±9
α_o	0.8	[35]	±0.5
α_h	0.8	[35]	±0.5
D_g^b	6.7×10^{-10} m^2 s^{-1}	[36]	
D_o^b	2.0×10^{-9} m^2 s^{-1} **	[22]	
D_h^b	2.0×10^{-9} m^2 s^{-1} **	[22]	
D_o^p	5.0×10^{-13} m^2 s^{-1} **	[22]	
D_h^p	5.0×10^{-13} m^2 s^{-1} **	[22]	
D_g^p	4.0×10^{-14} m^2 s^{-1} *	[22]	±5
r_{max}	60 mol m^{-3} s^{-1} *	[22]	±5
r_{max}^{ad}	8.0×10^{-8} mol m^{-2} s^{-1} *	[22]	±10‡
$r_{max}^{ad,p}$	1.0×10^{-6} mol m^{-2} s^{-1} *	[22]	±10‡

*One asterisk indicates that the value has been adjusted by theoretically fitting the experimental values of the current; two asterisks indicate that the value has been determined by independent experiments.
†Variation of the theoretical initial current obtained with a 0.25-μm-thick film for a variation of ±10% in the corresponding parameter.
‡For a 0.05-μm-thick film without enzyme entrapped inside the polymer.

Materials and Methods

Glucose oxidase [220 U mg^{-1} type VII from *Aspergillus niger* (E.C. 1.1.3.4)], β-D-glucose, potassium perchlorate, polyethylene glycol (PEG) 1000 g mol^{-1}, pyrrole, and hydrogen hexachloroplatinate were purchased from Sigma and used as received. The lyophilized enzyme powder contained catalase < 2 U mg^{-1}. All solutions were prepared in phosphate buffer 0.1 mol L^{-1}, pH 7.0.

All the electrochemical experiments were carried out at room temperature with a Radiometer model PGP 201 potentiostat-galvanostat connected to a Sefram x–y recorder. A cylindrical one-compartment batch reactor was used, with a total volume of 10 cm^3. The working electrode was a 2-cm-diameter platinum disk placed at the bottom of the reactor. A platinized platinum electrode was also used; it was obtained by immersing the previous working electrode in sulfuric acid solution (50 mmol (L^{-1}) containing H_2PtCl_6 (30 mmol (L^{-1})) and by applying a potential of 0.1 V/SCE for 1 h. A large-area platinum grid was used as counterelectrode and placed opposite to the working electrode to obtain homogeneous polymeric films covering the whole working electrode surface. The distance between working and auxiliary electrodes was about 2 cm. All potentials were measured vs. a saturated calomel electrode (SCE) connected to the reactor by a Luggin capillary. Glucose concentrations were determined by means of an enzymatic glucose assay purchased from Sigma based on a spectrophotometric measurement at $\lambda = 340$ nm.

The polypyrrole–GOx-modified electrodes were prepared as previously described[22] in deoxygenated phosphate buffer solution containing pyrrole (0.1 mol L^{-1}), glucose oxidase (1.5 mg mL^{-1}), and perchlorate ions (0.1 mol L^{-1}). When necessary, PEG (1 mmol L^{-1}) was added in solution. The working electrode was immersed in the solution 1 min before starting the electropolymerization to obtain reproducible adsorption of GOx on the clean electrode surface. The galvanostatic electropolymerization was performed with a current density of 600 μA cm^{-2}. The polypyrrole–GOx-modified electrode was extracted from the solution 1 min after the end of electropolymerization to ensure the adsorption of glucose oxidase on the polymer surfaces. The thickness of the polymeric films was varied between 50 nm and 1.5 μm by changing the electrolysis time and was evaluated by means of the charge passed.[27] When PEG was used, the modified electrode was then immersed for 30 min in phosphate buffer solution to remove PEG molecules entrapped inside the polymeric matrix. To obtain an inert and nonconducting matrix, the polypyrrole films were in all cases overoxidized by cycling the potential of the modified electrode twice between the open-circuit potential and 1.2 V at 5 mV s^{-1} in phosphate buffer.

Gluconic acid production was performed with the polypyrrole–GOx-modified electrodes immersed in 2.5 mL phosphate stirred solution containing 20 mmol L^{-1} glucose. This initial concentration was sufficiently high to detect a significant evolution during the experience, whereas it restricted as much as possible the enzyme autoinactivation by the substrate.[28] The concentration of dissolved oxygen (that is, 0.24 mmol L^{-1}) was maintained constant by continuous air flux into the reactor. When the electrochemical mediation was implemented, the working electrode was maintained at 0.4 V/SCE.

Results and Discussion

Model validation

Several electrolyses were performed with polypyrrole–GOx-modified electrodes immersed in a 2.5 mL solution containing 20 mmol L^{-1} glucose. The thickness of the polymer film was varied from 50 nm to 1.5 μm according to the electropolymerization time. For all the experiments the potential of the working electrode was held at 0.4 V/SCE.

Control experiments were carried out with polypyrrole-modified electrodes that did not contain GOx. The 20 mmol L^{-1} initial glucose concentration was not changed after 24 h electrolysis. It was also verified that gluconic acid did not react on the electrode surface in such operational conditions.

The bioelectrocatalytic properties of the different interfaces were first evaluated by means of the initial reaction rate. This was derived from the initial slope of the curve recording glucose concentration as a function of time $[dC_g^b(t)/dt]_{t=0}$. This value took advantage of considering the maximum activity of glucose oxidase entrapped in the film without being dependent on its further inhibition/degradation by hydrogen peroxide (see below). Experimental results (discrete points) are reported in Figure 2 by means of the effectiveness factor η, defined as the ratio of the effective initial reaction rate in the polymer to the enzymatic reaction rate without internal diffusion[29]

$$\eta = \frac{\int_0^{\delta_p} r[C_g^p(x),\, C_o^p(x)]dx}{r(C_g^s,\, C_o^s)\delta_p} = \frac{-\dfrac{V}{A}\left[\dfrac{dC_g^b(t)}{dt}\right]_{t=0}}{r(C_g^s,\, C_o^s)\delta_p} \qquad (15)$$

They are plotted as a function of the Thiele modulus Φ_g relating to glucose species, which is defined as the ratio of the enzyme-catalyzed reaction rate without internal diffusion limitation to the maximum diffusion rate in the polymer[30]

$$\Phi_g^2 = \frac{r(C_g^s,\, C_o^s)\delta_p^2}{\alpha_g D_g^p C_g^s} \qquad (16)$$

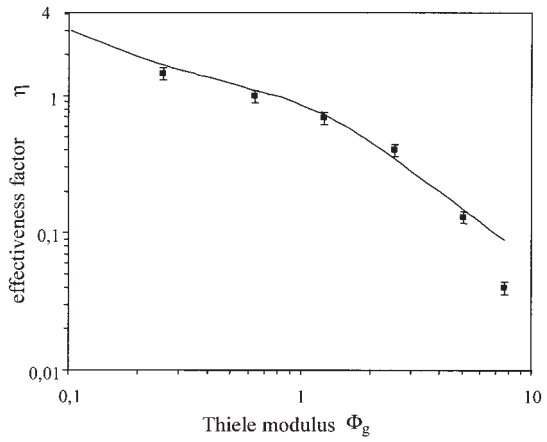

Figure 2. Influence of the Thiele modulus on the effectiveness factor.

Electrolysis of a 20 mmol L^{-1} glucose solution using a polypyrrole glucose oxidase modified electrode held at 0.4 V: ■ experimental results ; — theoretical curve.

Values of the Thiele modulus varied by means of the different polymer thicknesses. The other physicochemical parameters had the values reported in Table 1 for all experiments. Results show three different types of behavior of the modified electrode. For films thinner than 125 nm ($\Phi_g < 0.6$), the effectiveness factor was >1. Therefore the reaction was not limited by the internal diffusion and depended only on the enzymatic kinetics. The amount of glucose oxidase was in this case the determining parameter. It is not surprising that the effectiveness factor exceeded 1, given that Eq. 15 did not take account of the enzyme adsorbed on the platinum and on the polymer surfaces in the calculation of the reaction rate without internal diffusion.[31] For films thicker than 300 nm ($\Phi_g > 1.5$), the effectiveness factor decreased substantially. The formation of thick films resulted in a large diffusion barrier for glucose and oxygen and the reaction became mass transport controlled. For intermediate values of Φ_g, enzymatic kinetics and mass transfer rates were of the same order of magnitude. The increasing diffusion barrier imposed by thicker films was in this case balanced by the additional amount of biocatalyst entrapped in the polymer.

The electrolysis performed with the 250-nm-thick polymer-modified electrode was repeated three times. Reproducibility of the experiments was then evaluated by comparing the different values of the initial slope of the curve giving the concentration of glucose with time as well as the conversion yield for an 8-h electrolysis (see below). The standard deviation was 10%.

The solid curve in Figure 2 represents the theoretical results obtained with the adjusted parameters listed in Table 1, that is, the diffusion coefficient of glucose in the polymer and the biochemical reaction rates attributed to glucose oxidase entrapped in the film and adsorbed at the electrode and at the polymer surfaces. The theoretical effectiveness factor was calculated from the mass flux of glucose at the polymer–solution interface

$$\eta = \frac{\int_0^{\delta_p} r[C_g^p(x),\, C_o^p(x)]dx}{r(C_g^s,\, C_o^s)\delta_p} = \frac{\dfrac{D_g^b}{\delta_d}(C_g^b - C_g^s)}{r(C_g^s,\, C_o^s)\delta_p} \qquad (17)$$

Figure 2 shows that experimental results were satisfactorily fitted by the theoretical curve, excepted that recorded with a 1.5-μm-thick polypyrrole matrix ($\Phi_g = 7.8$). In this latter case the polymer film was very thick and rough. It has to be noted that the calculation of the enzymatic reaction rate in the polymer implies the values of the adjusted parameters r_{max}, r_{max}^{ad}, and $r_{max}^{ad,p}$. These parameters represent the specific activity of glucose oxidase in the different locations of the polymer. These values are quite different from the enzymatic activity in the monomer solution that was used to form the polymer. Determining the parameters numerically, by fitting experimental results with theoretical data, allowed determination of the actual immobilized enzyme activity, taking into account the mass transfer through the polymer and the exact concentrations of the different species in the film.

Sensitivity of the model was evaluated by measuring the variation of the theoretical initial current obtained with a 250-nm-thick polypyrrole film for a variation of ±10% in the different adjusted parameters. Results indicated in Table 1 show that the current sensitivity was at most 10%, whatever the parameter.

Evidence of the inhibition of glucose oxidase by hydrogen peroxide

Figure 3 represents the glucose concentration in the bulk (discrete points) as a function of time in the case of electrochemically supported transformations performed for 8 h with a 50-nm, a 500-nm, and a 1.5-μm-thick polypyrrole glucose oxidase–modified electrode (Figures 3a, b and c, respectively). The solid curves represent the theoretical results obtained with the model, keeping the same values of the parameters that were determined previously. The experimental results and theoretical data were in excellent agreement for 50-nm-thick films, and rather good for 500-nm-thick films. A discrepancy was observed for the 1.5-μm-thick films. The relative difference between experimental and theoretical glucose concentrations at the end of the experiment was only 3% for a 50-nm-thick film, 20% for a 500-nm-thick film, but >55% for a 1.5-μm-thick polymer. In each case, the experimental glucose concentration was higher than that theoretically predicted: the effective glucose consumption rate therefore decreased more rapidly than the theoretical one. This result can be explained considering the inhibition of glucose oxidase by hydrogen peroxide.[32] In the case of a 50-nm-thick polymer film the reaction took place very close to the platinum electrode surface and most of the hydrogen peroxide produced by the enzymatic reaction was consumed electrochemically. As a result inhibition of the enzyme was substantially avoided. No change in the actual activity of GOx occurred, and the experimental results were well predicted by the model, which worked with a constant r_{max} value. In the case of thicker polymer films, a greater amount of enzyme was immobilized, inducing a more important production of hydrogen peroxide. Because the reaction was located farther from the platinum electrode surface, only a part of hydrogen peroxide was consumed electrochemically. The major portion stayed in the polymer or in solution, inducing a significant inhibition of glucose oxidase along the transformation. The actual value of r_{max} decreased as a function of time, but this effect was not taken into account in the model.

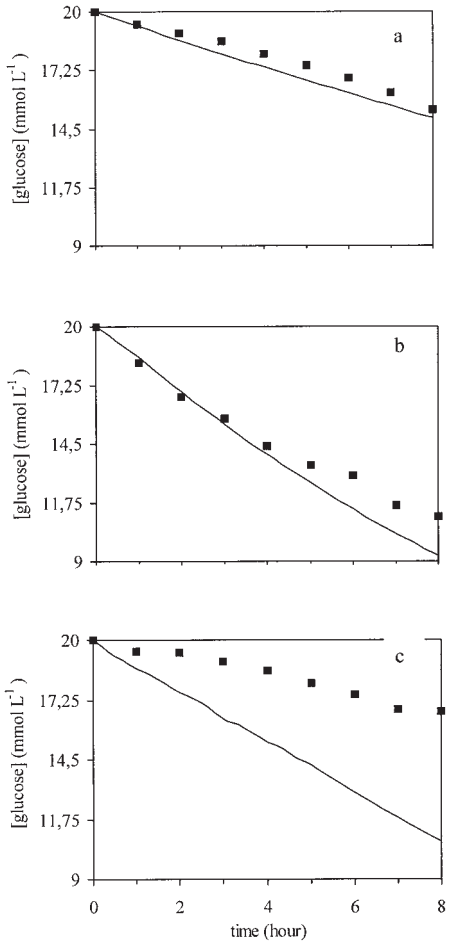

Figure 3. Variation of the glucose concentration with time.

Electrolysis of a 20 mmol L^{-1} glucose solution with a 50-nm (a), a 500-nm (b), and a 1.5-μm (c) thick polypyrrole glucose oxidase–modified electrode held at 0.4 V. ■ experimental results ; — theoretical curve.

Intensification of the transformation by electrochemical mediation

Figures 4a and b show the initial theoretical concentration profile of oxygen and hydrogen peroxide, respectively, inside a 500-nm-thick polypyrrole–GOx-modified electrode immersed in a 2.5 mL solution containing 20 mmol L^{-1} glucose. The calculation was realized in the case of an electrochemically supported reaction (dashed curve), and in the case where no potential was applied (solid curve). In the latter case the concentration of oxygen (Figure 4a, solid line) was nil in half of the film closed to the platinum electrode surface because of its slow diffusion rate inside the polymer compared to the consumption rate by the reaction. Consequently, only the enzyme entrapped in the zone between 375 and 500 nm, that is, the external polymer surface, was satisfactory provided with oxygen. Only around 25% of GOx entrapped in the film can therefore actually be effective. On the contrary, when the electrochemical step was implemented, oxygen was effectively regenerated inside the polymer (Figure 4, dashed line). This clearly multiplied by a factor of 2 the amount of enzyme that is satisfactorily provided with oxygen. In Figure 5 the theoretical effectiveness factor, calculated from the initial reaction rate, is plotted as a function of the Thiele modulus Φ_g for the same experimental case with (dashed curve) or without (solid curve) the electrochemical step. For $\Phi_g < 0.4$, corresponding to a film thickness of <80 nm, both curves were identical. The electrochemical step had no influence on the catalytic effi-

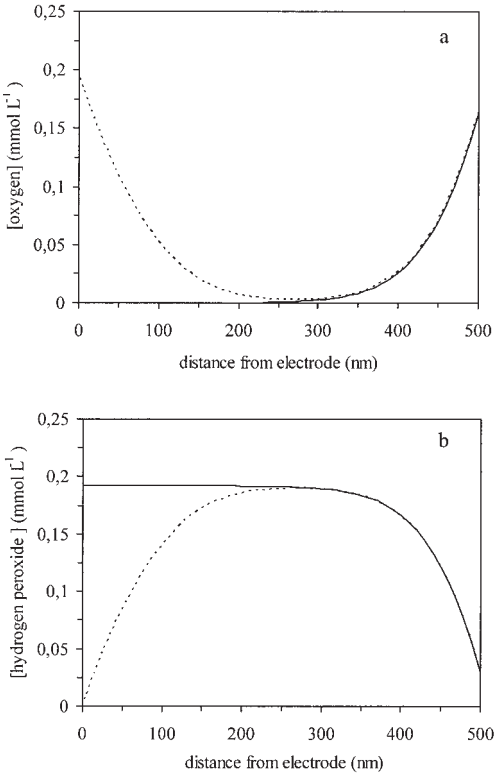

Figure 4. Concentration profile of oxygen (a) and of hydrogen peroxide (b) in a 500-nm-thick polypyrrole glucose oxidase–modified electrode immersed in a 20 mmol L^{-1} glucose solution.

— without electrochemical step ; - - - electrode potential held at 0.4 V.

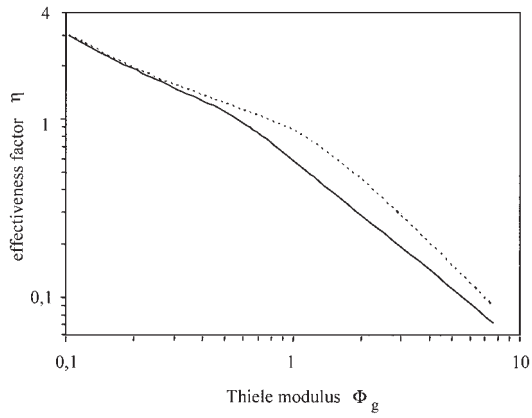

Figure 5. Influence of the Thiele modulus on the effectiveness factor.

Oxidation of a 20 mmol L^{-1} glucose solution with a polypyrrole glucose oxidase–modified electrode: — without electrochemical step; - - - electrode potential held at 0.4 V.

Table 2. Influence of the Polypyrrole Film Thickness on the Initial Reaction Rate and on the Conversion Yield of a 20 mmol L^{-1} Glucose Solution Oxidized for 8 h with or without Electrochemical Step

	δ_p (nm)		
	50	250	500
Initial rate (mmol L^{-1} h^{-1})			
With electrochemical step	0.54	1.30	1.60
Without electrochemical step	0.55	1.00	1.20
Conversion yield (%)			
With electrochemical step	23	40.7	44.7
Without electrochemical step	11.5	20	25

ciency of the process: the reaction rate was limited by the enzymatic kinetics and thus did not depend on the concentration of oxygen in the polymer. For higher Φ_g, the reaction rate was limited by the internal diffusion and therefore strongly depended on the concentration of the species in the film. In this case application of the potential, leading to the regeneration of oxygen, significantly improved the efficiency of the catalysis.

No evidence of the influence of the electrochemical consumption of hydrogen peroxide on the process efficiency could be theoretically shown because no enzyme inhibition by hydrogen peroxide was introduced into the model. However, Figure 4b shows that holding the potential of the modified electrode at 0.4 V/SCE led to a lower average concentration of hydrogen peroxide in the polymer than in the absence of electrochemical step. To completely evaluate the influence of the electrochemical step, a set of syntheses were performed for 8 h involving electrodes modified with a 50-, a 250-, and a 500-nm-thick polypyrrole film, with or without potential applied. The initial reaction rate and the conversion yield of glucose at the end of the experiments are reported in Table 2. Results concerning the initial reaction rate confirm the previous theoretical analysis: for a 50-nm-thick polymer, the initial reaction rate was the same with or without electrochemical mediation. For thicker films, holding the modified electrode at 0.4 V/SCE allowed the initial reaction rate to be increased by 30%. As theoretically predicted, the electrochemical regeneration of oxygen in the environment of the enzyme made it possible to improve the efficiency of the process since the first minutes of the electrolysis. The intensification of the enzymatic catalysis was even more pronounced after 8 h: without the electrochemical step the conversion yield of glucose was roughly half the conversion yield obtained when the process was electrochemically enhanced. This relative difference was observed even for a 50-nm-thick polypyrrole-modified electrode. However, the reaction rate was in this case not mass transfer controlled because the effectiveness factor of such interface, corresponding to a Thiele modulus of 0.25, was >1 (Figure 5). The difference thus cannot be explained by a possible modification of the oxygen concentration in the film but better by the consumption of hydrogen peroxide, thus protecting the enzyme against inhibition.

Reactor performances

Table 3 shows the conversion yield of glucose for electrolyses performed for 8 h with polypyrrole–GOx-modified electrodes whose thicknesses varied from 50 nm to 1.5 μm. In all these experiments the potential of the working electrode was held at 0.4 V/SCE. The best transformation yield was obtained with a 500-nm-thick polymer. Results exhibited the two different types of behavior of the electrochemical interface as previously described. For films thinner than 500 nm, the conversion yield increased with film thickness; the process efficiency depended essentially on the amount of enzyme entrapped in the polymer. Beyond 500 nm, the transformation yield decreased when the film thickness increased because of the low diffusivity and the accumulation of hydrogen peroxide in the polymer. The same electrochemical interface has been previously used to design a glucose biosensor. Both experimental and theoretical approaches showed that the optimum film thickness that induced the highest current, that is, the maximum amperometric response, was 250 nm.[22] The discrepancy between the two values highlights the difference of behavior between a biosensor and a reactor. In the former, the parameter to be optimized was the amperometric response. This response depended on the hydrogen peroxide flux at the electrode surface. In the latter, the goal was to maximize the substrate consumption rate. The parameter to be optimized was consequently the flux of glucose at the polymer surface, which depended on the catalytic efficiency of the whole interface.

The performances of the bioelectrochemical synthesis were compared to those of the homogeneous catalysis. On one hand, a 500-nm-thick polypyrrole–GOx-modified electrode held at 0.4 V/SCE was used for the oxidation of 2.5 mL of a 20 mmol L^{-1} glucose solution. It was theoretically expected that such a 500-nm-thick film entrapped 0.565 U GOx, according to the value of r_{max}. On the other hand, the same amount of enzyme was dissolved in solution and the biosynthesis was not electrically mediated. The variation of the glucose concentration as a function of time for both operational conditions was recorded over 24 h (Figure 6). In the case of the homogeneous catalysis, the glucose concentration sharply decreased in the first part of the experiment; the initial reaction rate was equal to 3 mmol L^{-1} h^{-1}. Nevertheless hydrogen peroxide was accumulated in solution. This induced the inhibition of the dissolved enzyme, which completely lost its catalytic properties after 6 h. No supplementary transformation occurred from 6 to 24 h in homogeneous conditions, and the conversion yield was limited to 45%. On the other hand, the initial reaction rate of the electrochemically enhanced process was 45% lower than the previous one, because of the limiting internal mass transfer. In return the inhibition was in part avoided because of the electrochemical consumption of hydrogen peroxide. Then the transformation yield was the same as for the homogeneous catalysis after 7 h, and reached 68% after 24 h. Furthermore, the glucose consumption rate, initially at 1.6 mmol L^{-1} h^{-1}, decreased to more than 35% after 3 h, but still represented 35% of its initial value after 8 h and 15% after 24 h. That means that the catalytic properties of the enzyme were lost, particularly in the first part of the electrolysis where hydrogen peroxide was

Table 3. Influence of the Polypyrrole Film Thickness on the Conversion Yield of a 20 mmol L^{-1} Glucose Solution Oxidized Bioelectrochemically for 8 h

	δ_p (nm)					
	50	125	250	500	1000	1500
Conversion yield (%)	23	29.5	40.7	44.7	38.7	16.7

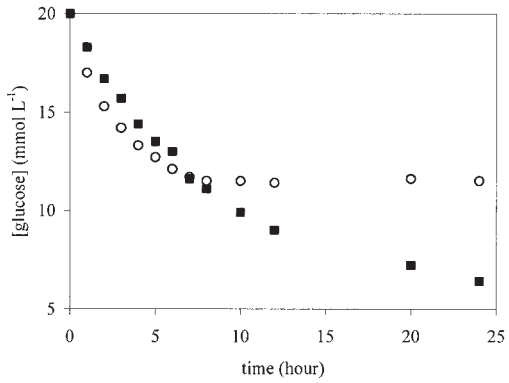

Figure 6. Variation of the glucose concentration with time.

Oxidation of a 20 mmol L^{-1} glucose solution: ■ with a 500-nm-thick polypyrrole glucose oxidase modified electrode held at 0.4 V; ○ homogeneous catalysis (0.565 U) without electrochemical mediation.

rapidly produced. In the second part of the experiment, the reaction rate was lower and the production of hydrogen peroxide less important. The electrochemical step then allowed the consumption of the major part of hydrogen peroxide produced and maintained for a longer period the biocatalytic properties of the modified electrode. Nonetheless, results demonstrated that the electrochemically enhanced process was more efficient than the homogeneous catalysis when using the same amount of enzyme, despite the unavoidable mass transfer limitations inside the polymer.

To increase the process efficiency, the protocol of electrode fabrication was modified. First, a platinized platinum electrode was used to increase its specific surface and, consequently, the quantity of glucose oxidase adsorbed on this surface, where the protection against inhibition by hydrogen peroxide is optimal. Second, PEG was introduced in the pyrrole solution before electropolymerization. This electroinactive water-soluble molecule has been found to change the polymeric matrix morphology in such a way that the permeability of the resulting film was greatly enhanced.[33] The electrolysis of a 20 mmol L^{-1} glucose solution realized with a 500-nm-thick polypyrrole–GOx-modified electrode fabricated with this new protocol was performed, and its performances were compared to that of the previous experiment. The experimental effectiveness factor deduced from the initial reaction rate increased from 0.44 with the nonplatinized modified electrode to 1.33 with these new operational conditions. This improvement resulted from both the supplementary amount of enzyme adsorbed on the electrode surface, and the enhanced permeability of the polymer. The conversion yield of glucose increased significantly from 45 to 62% after 8 h. Consequently, this improved operational conditions allowed the production of gluconic acid at an average rate of 0.2 g L^{-1} h^{-1} for 8 h.

Conclusion

A polypyrrole glucose oxidase–modified electrode, commonly used in the field of analytical chemistry, was used here in a laboratory-scale batch reactor for the transformation of glucose into gluconic acid. The bioelectrochemical interface was efficient for at least 24 h, provided that the enzymatic catalysis was electrochemically supported. This electrical mediation allowed the regeneration of oxygen and the consumption of the inhibitor hydrogen peroxide inside the polymer. The analysis, through the effectiveness factor and Thiele modulus, provided useful information to optimize the modified electrode with the aim of designing a tool for synthesis, but failed in quantifying the inhibition of the enzyme by hydrogen peroxide. Modeling the concentration profiles of oxygen and hydrogen peroxide inside the polymer film usefully completed the theoretical analysis, and explained how the catalytic efficiency could be significantly improved by electrochemically monitoring the concentration of both species inside the polymer. As a result of the combined experimental and theoretical approaches, the optimized bioelectrochemical process was 50% more effective than the homogeneous process performed with the same quantity of enzyme. Moreover, this quantity of enzyme may be reused without any intermediate separation in the bioelectrochemical process. The mass-transfer hindrances, which are unavoidably linked to any heterogeneous processes, were here largely compensated by the electrochemical step.

Notation

A = electrode surface area
C_j = concentration of the species j, mol m^{-3}
D_j = diffusivity of the species j, m^2 s^{-1}
GOx = glucose oxidase
K_j = Michaelis–Menten constant relative to the species j, mol m^{-3}
r_{max} = activity of glucose oxidase in the polymer, mol m^{-3} s^{-1}
r_{max}^{ad} = activity of glucose oxidase adsorbed at the electrode surface, mol m^{-2} s^{-1}
$r_{max}^{ad,p}$ = activity of glucose oxidase adsorbed at the polymer surface, mol m^{-2} s^{-1}
V = volume of the solution, m^3
z = distance from the electrode, m

Greek letters

α_j = partition coefficient of the species j
δ_d = thickness of the hydrodynamic diffusion layer, m
δ_p = thickness of the polymer film, m
η = effectiveness factor
Φ_g = Thiele modulus

Subscripts

g, o, h = index for glucose, oxygen, and hydrogen peroxide, respectively

Superscripts

b = relative to the bulk of the solution
p = relative to the polymer film
s = relative to the polymer surface

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Manuscript received Oct. 31, 2003, revision received July 12, 2004, and final revision received Sept. 22, 2004.