Sensors and Actuators B 144 (2010) 349–353

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

# Mass transport effect of mesoscopic domains in the amperometric response of an electroactive species: Modeling for its applications in biomolecule detection

Graciela González, Graciela Priano, Mauricio Günther, Fernando Battaglini*

*INQUIMAE - DQIAQF, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428, Buenos Aires, Argentina*

ARTICLE INFO

*Article history:*
Available online 19 November 2008

*Keywords:*
Mesoporous membrane
Digital simulation
Amperometry
Label-free sensing

ABSTRACT

We report the numerical simulation of an electrochemical system compromising a mesoporous material placed at a close distance of a working electrode. The effect of mesoscopic domains to the amperometric response of an electroactive species by applying a cyclic voltammetry is simulated to establish the influence of different parameters on the sensitivity of this system to detect molecules able to block the pores. Alumina membranes were chosen as mesoporous material; they were modified with anti-horseradish peroxidase as model system to test the behavior predicted by the simulation. The label-free assembled electrochemical system shows a reproducible behavior and it is able to detect a 10 nM protein concentration.

© 2008 Elsevier B.V. All rights reserved.

## 1. Introduction

In the last 15 years, important advances in the synthesis of mesoporous materials have made possible the development of very sensitive systems for molecule detection or ion channel investigations. Several systems have been reported in the literature, among them porous silicon [1–5], multipore membrane [6–10], single pore membrane [10–12] and more recently the glass nanopore electrode introduced by White et al., which combines, in the same device, a glass nanopore with a platinum or gold microelectrode [13]. These are label-free devices, since they can detect an analyte by simply using a common property to all molecules, its size. This fact, combined with the selective interaction through a recognition agent, can be useful to detect practically any molecule, providing the adequate size pore. Among the proposed solutions, single pore systems show a great sensitivity, potentially they can achieve single molecule detection; their response can also be easily modeled, however their experimental implementation is not so simple. On the other hand, even though multipore systems would not be able to carry out single molecule detection, they can achieve detection limits useful for a myriad of determinations. Among the multiporous membranes, alumina membranes present some advantages, they can be easily modified through a covalent link [6] or by a self-assembled process [14,15], pores in the size of biomolecules can be easily obtained and they are commercially available. On the other hand, they are fragile and models describing its possible behavior were not developed.

This work presents an electrochemical system that can be easily assembled, maintaining the integrity of the membrane and the reproducibility of results; at the same time a numerical tool was developed allowing the study of the system (electrochemical cell–porous membrane) identifying optimal parameters of operation. Finally, an experimental system compromising the modification of the alumina membrane with anti-horseradish peroxidase (anti-HRP) allows the detection of HRP as low as 10 nM.

## 2. Experimental and numerical system

### 2.1. Reagents and materials

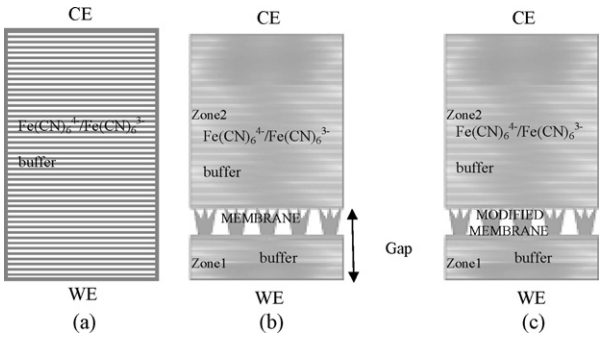
Horseradish peroxidase (HRP) was provided by Biozyme, anti-horseradish peroxidase (anti-HRP), 3-aminopropyl(triethoxy) silane (APTES), 2-(N-morpholino)ethanesulfonic acid (MES), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were provided by Sigma; alumina membranes, Anodisc 25, were provided by Whatman. All other reagents were of analytical grade.

### 2.2. Electrochemical cell

A two-part electrochemical cell made in Teflon was used. In the lower part, the working electrode is placed leaving a shallow cavity where a solution is introduced (Zone 1 in Scheme 1b). On the

* Corresponding author. Tel.: +54 11 45763380; fax: +54 11 45763341.
*E-mail address:* battagli@qi.fcen.uba.ar (F. Battaglini).

0925-4005/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2008.11.006




**Scheme 1.** Schematics (not on scale) of the cell configurations used in this work.

top of this part, an alumina membrane is placed. Over the alumina membrane the upper part of the cell is adjusted and filled with a solution (Zone 2 in Scheme 1b); then, the counter and Ag/AgCl reference electrodes are introduced.

### 2.3. Membrane modification

Alumina membranes were modified by two different ways: by partial insulation with an electrical insulating varnish (Electroquímica Delta, Argentina) and by covalent linking of anti-HRP. In this case, an alumina membrane was immersed in a 5% APTES solution in dry toluene under stirring for 1 h. The membrane is rinsed with toluene and placed in an oven at 120 °C for 20 min. Then, the membrane is placed in a 300 mM succinic anhydride solution in dimethyl sulfoxide (DMSO) under stirring overnight. The membrane is rinsed with DMSO, acetone and water. The surface is activated with a 100 mM EDC and 100 mM NHS in 50 mM MES buffer (pH 5.5) for 30 min under stirring, thereafter the surface is rinsed with water and incubated with a 1.8 mg mL$^{-1}$ anti-horseradish peroxidase in 50 mM HEPES (pH 8.0) for 3 h under stirring. Then, the membrane is rinsed with water and the non-modified carboxylic groups were quenched with a 0.1 M ethanolamine solution (pH 8.5) for 15 min and then rinsed with water. The membrane is left in 0.1 M phosphate buffer (pH 7.4) for 10 min and rinsed. HRP is incubated at different concentrations in a 0.1 M phosphate buffer (pH 7.4) for 40 min, then, the membrane is rinsed with water.

### 2.4. Electrochemical experiments

Cyclic voltammetry experiments were carried out in a three electrode cell, using gold as working electrode at the base of the cell, in three different configurations (Scheme 1): (a) A standard three electrodes immersed in 2 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ solution containing 0.1 M supporting electrolyte in buffer pH 7; (b) In the presence of the porous alumina membrane placed at specific distance from the working electrode. The gap between the working electrode and the membrane is filled with buffer, while over the membrane, the cell is filled with the same solution than (a); and (c) the same as (b), with the membrane modified with an antibody (anti-HRP) or blocked with an insulating varnish.

### 2.5. Numerical model

This experimental system was modeled solving Poisson and Nernst–Plank without electroneutrality equations [16] using a finite-element software (Comsol Multiphysics 3.4) to obtain the voltammetric response and the concentration profiles, as previous works [13,17,18]. The space dimension was set to 2D and the boundary conditions were as an infinite plane electrode and semi-infinite diffusion. We employed a physical constant referred to the 2 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox couple in a solution containing 0.1 M supporting electrolyte. The flux of electroactive species at the electrode is given by

$$\frac{J_{\text{reac}}}{F} = \frac{J_0}{F}\left[\frac{C_R}{C_R^*}\exp\left(\frac{F}{2RT}\eta\right) - \frac{C_O}{C_O^*}\exp\left(-\frac{F}{2RT}\eta\right)\right] \quad (1)$$

where $C_R^*$ and $C_O^*$ are the concentration in the bulk solution, of the reduced and the oxidized species, respectively; $C_R$ and $C_O$ are the concentration on the working electrode surface of the reduced and the oxidized species, respectively; $\eta = E - E_{eq}$ is the applied overpotential and $E_{eq}$ is the equilibrium electrode potential.

**Table 1**
Predicted peak current densities.

| Sweep rate (mV s$^{-1}$) | Theoretical | Numerical |
|---|---|---|
| 50 | 563 | 549 |
| 100 | 796 | 757 |

Current densities in μA cm$^{-2}$.

### 2.6. Micrographs

Micrographs were taken with a field emission scanning electron microscope (FESEM) Zeiss DSM 982 Gemini at the Advanced Center for Microscopies (CMA, Universidad de Buenos Aires).

## 3. Results and discussion

Initially, the proposed simulation model was evaluated using a configuration without membrane (Scheme 1a) considering that the ferro/ferricyanide couple presents a quasireversible behavior. In this context, it is possible to compare the results obtained by the proposed simulation for the peak current density ($j$) with those predicted by the well established model published elsewhere [19]:

$$j_{p,qrev} = 6.02 \times 10^5 n^{3/2} D^{1/2} C^* \nu^{1/2} \Psi(E) \quad (2)$$

where $\Psi(E)$ is the quasireversible current function and can be considered 0.4 for a system like ferro/ferricyanide [19]; $n$ is the number of electrons involved in the reaction; $C^*$ is the bulk concentration of the electroactive species, $D$ is the diffusion coefficient of the electroactive species and $\nu$ is the potential scan rate. Table 1 summarizes the peak current densities obtained using Eq. (2) and by the simulation procedure developed in this work, showing that it is able to generate an adequate solution.

Once, the simulation codes were validated, a model for the description of the experiments involving the introduction of a porous membrane was written (Scheme 1, cell configuration b). In this configuration, the electrochemical cell is divided in two by the introduction of the porous membrane. In the lower part of the cell is the working electrode, between the membrane and the working electrode is a shallow cavity filled with buffer, its effect was simulated considering a gap between 0 and 600 μm. On the other hand, in the upper part of the cell a solution of an electroactive species was considered. As the membrane is blocked (Scheme 1, configuration c), the passage of the electroactive species is more difficult, therefore the peak current is smaller.

In the simulation, the effect of the introduction of the membrane was taken into account using the information given by the membrane supplier (thickness: 60 μm, hole diameters: 20 and 200 nm, and porosity 25–50%) plus the structure information given by FESEM (Fig. 1). As it can be observed from the micrographs, the membranes have a very characteristic shape, the smaller pore size, at one side of the membrane (Fig. 1a), only extends for a few micrometers (Fig. 1c). Different geometries for the pore shape were considered, the one depicted in Scheme 1b shows the best fit with

Download English Version:

https://daneshyari.com/en/article/744917

Download Persian Version:

https://daneshyari.com/article/744917

Daneshyari.com