



# Optimization of Parameters for Electrochemical Detection: Computer Simulation and Experimental Study

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**Abstract.** It has been shown that for each electrochemical measurement it is necessary first to optimize all the parameters that influence the obtained electrochemical signal. This is a time-consuming process and that is why in this study we focused on the computer simulation of the influence of multiparameters on the electrochemical signal. Herein we used a model system of the electroreduction of ruthenium(III) salt on gold electrode in phosphate buffer solution. Ru<sup>3+</sup> is often used as redox probe for detection and understanding of nucleic acid hybridization on the electrodes. This study should reveal some new aspects of using the computer simulations in determining the optimal measuring conditions for detection of RNA/PNA hybridoms on the gold surface.

**Keywords:** Computer simulation · Electrochemistry · Optimization

## 1 Introduction

Electrochemistry is a discipline widely used in the fields of industry, science, testing of many different small molecules and large biomacromolecules, especially in biosensors [1]. Methods of electrochemical determination are accurate, fast, precise and inexpensive. Defining and establishing of precise and clearly defined parameters of electrochemical determination is of outmost importance. However, practice shows that the establishment and definition of optimal parameters is often very long and the painstaking process which is often far more demanding than later electrochemical analyzes. Determining of individual parameters and their synergy is a time-consuming process. For example, a common electrochemical investigation determines the following parameters: Information about the type of molecule to be tested, type of supporting electrolyte, pH, ionic strength, addition of salts, temperature, type of electrode, voltage range, electrochemical cell geometry, stirring speed, adsorption time and many others. Fortunately, depending on the electrochemical process being monitored and on the applied method of determination, it is often known that a requested electrochemical

signal will behave in relation to the change of the given parameters. For example, in monitoring the process of electrocatalytic elution of hydrogen on the mercury electrode in the determination of polyamino acids the definition of the mentioned parameters is of utmost importance [2, 3]. However, as mentioned above, the synergy of change is also very important. It is necessary to estimate how the change of several parameters at the same time will affect the change in the observed electrochemical signal. Therefore, computer simulation methods allow *in silico* predicting the influence of different parameters on the observed electrochemical signal and optimizing the establishment of the determination conditions. In other words, computer simulation enables us to save time and optimize the process of electrochemical determination. If we know the nature of the examined analyte, the methodology and the basic conditions of electrochemical determination, the computer simulation can offer us the most probable optimal conditions for determining. We firstly described experimental setup and numerical model used for computer simulation. Then some initial results for computer simulation and experimental study are compared. Finally, some discussion and conclusions are given.

## 2 Experimental

### 2.1 Electrochemical Detection of $\text{Ru}^{3+}$ Reduction on Gold Electrode

**Case Presentation.** It has been known that nucleic acids are electroactive [4]. Using the oscillographic polarography method at controlled AC on dropping mercury electrode changes in the DNA structure were followed. Since then, electrochemistry of DNA and RNA has become booming field in electroanalytics, finally with the production of advanced biosensors. One of many possible methods for electrochemical detection of nucleic acids samples onto gold electrodes is using of  $\text{Ru}^{3+}$  salt [5]. Investigation of nucleic acid hybridization on gold electrodes can be monitored using redox probes such as hexamine-ruthenium (III). In this sense, in order to arrive at accurate, precise and reproducible determination, it is necessary to optimize all parameters. In our experiment, we used phosphate buffer of various pHs, ionic strengths, with or without salt addition, in temperature controlled conditions with stirring in various speeds. In this paper, we will try to discuss about fitting of optimal parameters for electrochemical detection of redox probe of  $\text{Ru}^{3+}$  prior to investigate nucleic acid hybridization.

### 2.2 Reagents, Solutions and Apparatus

All reagents were purchased from Sigma-Aldrich and were of analytical grade. The measurements were performed with an EmStat-3 potentiostat (PalmSens BV, The Netherlands) operated with the PSTrace v.4.8 software. We used a standard electrochemical cell equipped with three-electrode system. The working electrode was 2 mm diameter gold electrode, the reference electrode was  $\text{Ag} \mid \text{AgCl} \mid 3 \text{ M KCl}$ , with Pt wire as auxiliary electrode.

### 2.3 Numerical Model

In the modeling of electrochemical deposition on the electrode we used Nernst-Planck definition of species flux through homogeneous media. The flux resulting from the electrochemical potential gradient is typically decoupled into a diffusion term corresponding to activity or concentration gradient driven flow and an electromigration term that accounts for the force of the electric field on charged molecules. The Nernst-Planck flux is defined as:

$$J_i = - \left[ D_i \frac{\partial c_i}{\partial x} + u_i c_i \frac{\partial \varphi}{\partial x} \right] \quad (1)$$

where  $D_i$ ,  $u_i$ ,  $c_i$  and  $\frac{\partial \varphi}{\partial x}$  are diffusion coefficient, electrical mobility, concentration and kinetic potential gradient, respectively.

The diffusion coefficient and electric mobility are proportionality constants between flux and the concentration and potential gradients, respectively. They are related by the Nernst-Einstein equation:

$$u_i = \frac{z_i D_i F}{RT} \quad (2)$$

In our study, we are trying to simulate current versus voltage on the specific electrode with diffusion of investigated substance using finite element method [6], against passive diffusion represented by Fick's law:

$$J_i = -D_i \frac{\partial c_i}{\partial x} \quad (3)$$

Mesh model for finite element method analysis consists of 3D finite element elements that result in good convergence [7].

The governing equations are the following:

Nernst-Planck without electroneutrality

$$\delta_{ts} \frac{\delta c}{\delta t} + \nabla \cdot (-D \nabla c - z u_m F c \nabla V) = 0 \quad (4)$$

where  $\delta_{ts}$  is time scaling coefficient,  $D$  is diffusion coefficient,  $c$  is drug concentration,  $z$  is charge number,  $u_m$  is mobility,  $F$  is Faraday constant, and  $V$  is potential. The Voltage equation which was used is the following:

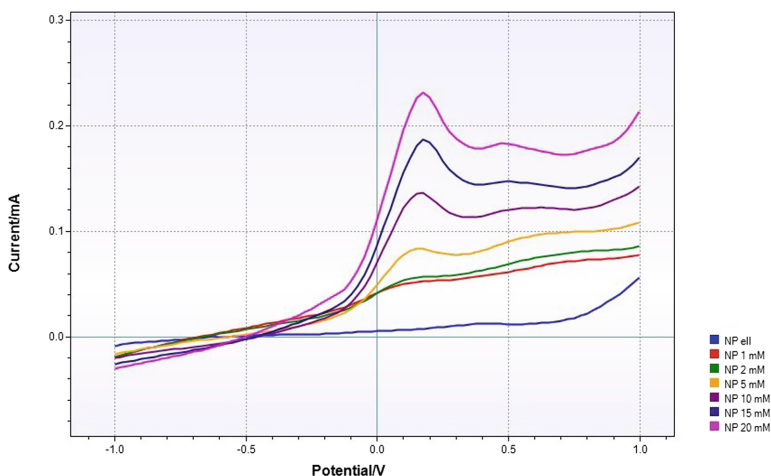
$$-\nabla \cdot (\sigma \nabla V - J^e) = Q_j \quad (5)$$

where  $\sigma$  is electric conductivity,  $V$  is potential,  $J^e$  is external current source and  $Q_j$  is current source.

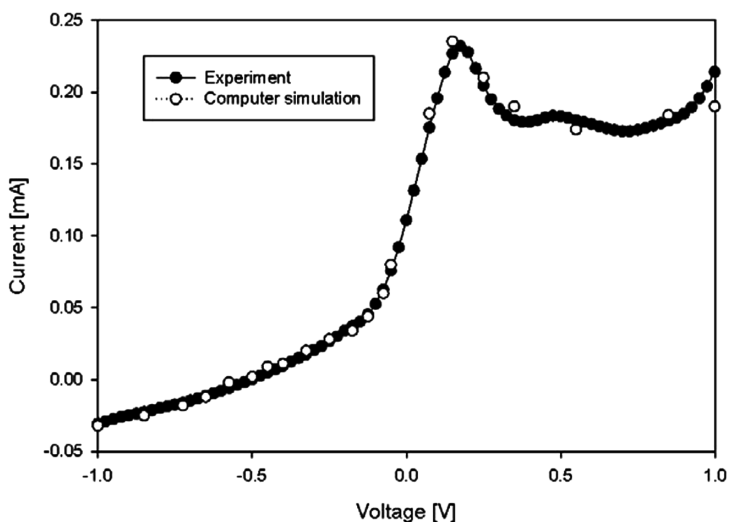
### 3 Results

#### 3.1 Electrochemical Detection

The experimental results of our model obtained with hexaamine-ruthenium(III) in 100 mM phosphate buffer, pH 7.0 are presented in the Fig. 1. Normal pulse voltammograms were obtained at scan rate  $250 \text{ mVs}^{-1}$  in potential range from  $-1.0$  to



**Fig. 1.** Normal pulse voltammograms of hexaamine-ruthenium(III) on gold electrode in 100 mM phosphate buffer, pH 7. Scan rate  $250 \text{ mVs}^{-1}$ .



**Fig. 2.** Comparison of experimental and computer simulation results for normal pulse voltammograms of hexaamine-ruthenium(III) on gold electrode in 100 mM phosphate buffer, pH 7 for NP = 20 mM.

+ 1.0 V,  $E_{\text{step}}$  25 mV, and  $t_{\text{pulse}}$  5 ms. Under these conditions  $\text{Ru}^{3+}$  reduction peak was well distinguished from the background discharge in concentration range from 1 to 20 mM at potential around +0.15 V. The peak height gradually increased with increasing the  $\text{Ru}^{3+}$  concentrations and peak potential,  $E_p$  shifted towards more positive potentials.

Comparison of experimental and computer simulation results for concentration of 20 mM at potential around +0.15 V has been presented in the Fig. 2. It can be observed that good agreement was achieved for this specific parameter optimization procedure with computer simulation.

## 4 Discussion and Conclusions

Computer simulations in this study are useful to do *in-silico* many experiments which cannot be done in real physical world and to better explain the physics behind the process, so it is a high challenge for the future to develop mathematical models that accurately describe effects of electrochemical deposition to the electrode.

**Acknowledgments.** This study was funded by the grants from the Serbian Ministry of Education, Science and Technological Development III41007, ON174028.

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