THE VOLTAMMETRIC PERFORMANCE OF INTERDIGITATED ELECTRODES: COMPUTATION AND EXPERIMENTATION

by

XIAOLING YANG

(Under the Direction of Guigen Zhang)

ABSTRACT

This thesis explores ways to improve the performance of electrochemical affinity sensors by integrating cyclic voltammetric (CV) method and nano or micro interdigitated electrodes (IDEs) in a generator-collector mode.

Affinity-based biosensors are based on the interactions between complementary molecules such as antibody–antigen coupling, aptamer-protein recognition, or DNA hybridization. In these sensors, the functionalization of electrodes will reduce the access for electron-transfer from the electrolyte to the electrode, or vice versa. This, in turn, will decrease the electron transfer rate (or k_0 value) at the surface of the electrode. Hence, for successful applications of IDEs in affinity-based biosensors, it is important to know the effect of changing k_0 on the current performance of IDEs. When it comes to nano-IDEs, the effect of electrical double layer (EDL) will become dominant. Therefore, the k_0 value effect at nano-IDEs needs to be considered along with the EDL effect. In this study, we developed a complete computational model to address the above issues.

To confirm the simulation results, IDEs with 4.25 μ m electrode size and gap spacing are fabricated and characterized by CV method in a generator-collector mode and a single-electrode mode. The electrode surface of IDEs is then successively modified by probe molecules sulfo-

NHS-SS-Biotin (Sulfosuccinimidyl 2-(biotinamido)-ethyl-1, 3-dithiopropionate) self assembled monolayer (SAM) and bond by the target molecules avidin at various concentrations. The CV responses are measured stepwise after each modification of electrodes.

The simulation results shows that the CV responses of micro or submicron IDEs are more sensitive to k_0 value change than the response of single electrodes of microscopic dimension. At micro IDEs the relationship between CV limiting (or peak) current and k_0 value is strongly dependent on the electrode size and spacing. And at nano IDEs, this relationship also varies with charge valence of the redox species. The simulation results are confirmed by the experiment. We thus conclude that the performance of electrochemical affinity sensors can be improved by integrating CV method and micro IDEs. Based one the simulation analysis and experimental results we also speculate that integrating CV method and nano IDEs will further improve the sensing performance of IDEs-based affinity sensors. This study presents some important information for improving the design and development of future electrochemical-based biosensors.

INDEX WORDS: Interdigitated electrodes, Cyclic Voltammetry, Affinity sensor, Finite element analysis, Simulation

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by

XIAOLING YANG

B. M., Taishan Medical University, China, 2001

M.S., Zhejiang University, China, 2004

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by

XIAOLING YANG

Major Professor:

Guigen Zhang

Committee:

James L. Anderson James Kastner Bingqian Xu

Electronic Version Approved:

Maureen Grasso Dean of the Graduate School The University of Georgia August 2008

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LIST OF ABBREVIATION

Abbreviation	Meaning
CV	Cyclic Voltammetry
IDE	Interdigitated Electrodes
FIS	Faradic Impedance Spectroscopy
EB	Electron Beam
Au	Gold
Ti	Titanium
Cr	Chromium
Co	Cobalt
RCA	Radio Corporation of America
EDL	Electrical Double Layer
IHP	Inner Helmholz Plane
OHP	Outer Helmholz Plane
PET	Position of Electron Transfer
FEA	Finite Element Analysis
SAM	Self Assembled Monolayer
MUA	mercaptoundecanoic acid
PBS	Phosphoric buffer solution
MPA	3-mercaptopropionic acid

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1. The history of IDEs

Ultramicroelectrodes (UMEs) have extended electrochemical methodology into previously inaccessible domains of time, space and media. They enable the measurement of kinetics of fast electron transfer reactions by steady-state experiments, which in turn provide improved signal-to-noise behavior (see (Arrigan, 2004) for a detailed review). Of these UMEs, one unique design with an arrangement of interdigitated electrodes (IDE) has received a lot of attention (Bard, Crayston et al., 1986; Aoki, Morita et al., 1988; Aoki and Tanaka, 1989; Niwa, Morita et al., 1990; Paeschke, Wollenberger et al., 1995; Jin, Qian et al., 1996; Morf, 1997; Fulian, Williams et al., 1999; Björefors, Strandman et al., 2000; Strutwolf and Williams, 2005). In this design, the generators are placed side by side with the collectors in an interdigitated manner (see Figure 1.1A)



Figure 1.1. (A) design of IDEs. (B) Redox cycling at IDEs

1.1.1. IDEs in a fluid flow

To elucidate the underlying mass transport mechanism, the performances of microscale IDEs have been investigated over the years with theoretical analyses (Matsuda, 1968; Niwa, Morita et al., 1990; Paeschke, Wollenberger et al., 1995; Morf, 1997; Björefors, Strandman et al., 2000) and numerical simulations (Anderson, Ou et al., 1985; Bard, Crayston et al., 1986; Aoki, Morita et al., 1988; Ou, Moldoveanu et al., 1988; Aoki and Tanaka, 1989; Aoki, 1990; Jin, Qian et al., 1996; Fulian, Williams et al., 1999; Strutwolf and Williams, 2005). In 1968, Matsuda (1968) pointed out that a fundamental factor for the electrolysis technique with two closelyspaced electrodes in flow systems is the collection efficiency. In 1985, Anderson et al. (1985) numerically predicted that a multi-electrode interdigitated electrode array has substantial improvements in signal-to-noise ratio relative to a single generator-detector pair of equal overall area, and the relative enhancement increases significantly with the number of generator-detector pairs. Due to the flow condition, the redox species generated at one electrode are always collected at the other electrodes in the down stream direction, thus the redox process is hardly able to cycle between electrodes. In these studies, the electrode size and gap spacing are at several tens or hundreds of micrometer.

1.1.2. IDEs in a still solution: the study of cyclic voltammetry

With the advance of micro fabrication technology, fabricating arrays of more than one pair of ultramicroelectrodes became possible (Kittlesen, White et al., 1984; White, Kittlesen et al., 1984). These small electrodes can be 50 μ m long and 2 ~ 3 μ m wide with inter-electrode distance as small as 1.2 μ m. To elucidate the profound effect of the ultra small electrode size and inter-electrode gap on the solution amperometric response, Bard et al. (1986) studied the CV

response of redox species at these interdigitated ultramicroelectrode arrays in a still solution experimentally and numerically, and found a good agreement between them. It is also noted that the collection efficiency of a generator with two collector band electrodes (three-microbandelectrode configuration) is strongly dependent on the gap spacing. The collection efficiency, feedback, and shielding at IDEs with different electrode size and gap spacing were systematically studied as well. Because of the still solution, an electroactive species generated by a potential excitation at the generators can first diffuse across the thin-layer gap, due to concentration gradient, and reacts electrochemically at the collectors. The reactant species at the collectors can then diffuse back to the generators, and react there, meaning that the redox species are able to cycle between generators and collectors (see Figure 1.1B). Thus, Niwa et al. (Niwa, Morita et al., 1990) introduced a new parameter, redox cycling number, to characterize the performance of IDEs:

$$RC = N / [N (1 - \Phi_1 \Phi_2)] = 1 / (1 - \Phi_1 \Phi_2)$$
(1.1)

where Φ_1 and Φ_2 are respectively the collection efficiency from generator and collector electrodes. When the sizes of both electrodes are the same, the value of Φ_1 and Φ_2 will also be the same. They (Niwa, Morita et al., 1990) also made it more clear that collection efficiency is dependent on the average diffusion length of $W_e/4 + gap$, where W_e is the band electrode width in IDEs, and found the collection efficiency of IDEs is independent of redox species for a simple reversible reaction. It is noted that a steady state current can be reached at IDEs using CV, and depending on the sweep rate the time needed for reaching the steady state can be less than 1 min.

More detailed work has been done in evaluating the steady state current (Aoki, 1990) and characterizing the relationship between the gap spacing of IDEs and the time to reach the steady state (Paeschke, Wollenberger et al., 1995). Based on these studies in which that IDEs were

mostly coplanar inlaid band electrode arrays, in the late 1990's and early 2000's, elevated IDEs were studied to compare with coplanar inlaid IDEs (Jin, Qian et al., 1996; Strutwolf and Williams, 2005). It was found that redox cycling between the side wall of the generator and collector electrodes contributed a lot more to the total current response.

In summary, the previous studies of IDEs have shed insights into the effect of electrode size, height and spacing of the IDEs on the current collection efficiency in both flow and still solution. Redox cycling is a unique feature of IDEs, and a steady state current can be reached at micro IDEs when the CV method is used.

1.2. Fabrication Methods of IDEs

IDEs with micro or nano dimensions can be fabricated in numerous ways, including photolithography, e-beam lithography, or nano-imprint (Van Gerwen, Laureyn et al., 1998; Beck, Persson et al., 2004; Tamaki, Hashishin et al., 2008), thin film deposition, and wet or dry etching (Giovanni Carlo Fiaccabrino, 1998). To date, various metal films such as gold, platinum, titanium, palladium, and chromium have been widely used in IDEs fabrication because they can be easily deposited by sputtering or vapor deposition on an optically flat and polished substrate, and patterned by lithography technique before or afterwards. By these means, the electrode size and spacing can be as small as several hundreds of nanometers.

The methods of fabricating micro and submicro IDEs through the means of lithography have mainly two types. One is lifting off the metal film by removing the underlying photoresist, and the other is directly etching the metal film. Depending on the methods, the fabrication procedure varies.

1.2.1. Fabrication of IDEs by photolithography and film etching

Table 1.1 shows the sequence of technological steps for IDE fabrication by photolithography and film etching. For electrochemical transducer fabrication, the surface of the substrate has to be chemically and electrically inert. Thus, glass substrates or silicon wafers with silicon oxide on top are frequently used. Electrochemically inert metals such as gold and platinum are deposited after an additional very thin adhesion layer (Ti or Cr) deposited on the substrate surface. Then, photoresist is spin coated on top of the metal thin films and exposed under UV light to form a micro electrode array pattern. After development, the portion of the thin film to be removed is etched by etching solution, ion-beam, or plasma. The latter two dry etching methods usually have better control of the etching speed. The clean metal IDEs are eventually formed by removing the photoresist using acetone.

1.2.2. Fabrication of IDEs by e-beam lithography and lift-off technique

Table 1.2 shows the sequence of technological steps for IDE fabrication by photolithography and liftoff technique. Similarly, glass or silicon wafers with silicon oxide on the top are used as substrates. A negative-type resist is deposited on the substrate by a spinner. The electron beam (EB) is scanned across the resist-coated substrate according to the interdigitated patterning program. The bare substrate is revealed on the EB-scanned trace after removal of the exposed portion of the resist. A uniform Ti or Cr and Au film are deposited on the substrate successively by evaporation under high vacuum environment ($<10^{-6}$ Torr). Then, the Ti or Cr and Au film on the unexposed resist area is removed by immersing into a remover with ultrasonication. By this way, Au IDEs are formed on top of the substrate.

Process No.	Process flow	Cross section	
1	Chemical cleaning substrate by RCA solution		
2	Coat metal thin film on top of substrate		
3	Coat positive photoresist on tope of metal film		
4	UV patterning on pos- resist and developing		
5	Etching metal thin film by solution, or ion beam		
6	Removing photoresist		_

Table 1.1 Fabrication process of gold IDEs by direct etching technique.

Process No.	Process flow	Cross section	
1	Chemical cleaning substrate by RCA solution		
2	Spin coating of PR		
3	E-beam patterning on negative photoresist and developing		
4	Coat Ti/Cr and Au on top of patterned photoresist		
5	Liftoff of metal thin film on developed EB resist		

Table 1.2 Fabrication process of gold IDEs by liftoff technique

1.3. IDEs in biosensing -- applications and challenges

The small dimensions of IDEs can not only help miniaturize the sensing devices but also reduce the requirement for the volume of analytes without sacrificing the sensitivity, selectivity and response time. For example, IDEs have been used to lower the detection limits and make the detection of a small amount of redox species in solution possible (Tomčík and Bustin, 2001). Because of the close distance between the generators and collectors, a very high percentage of the generated species gets collected at the collectors with a very low solution resistance. Such a close distance between the electrodes has enabled the active oxidized or reduced species to be reduced or oxidized rapidly before they became redox-inactive forms (Iwasaki and Morita, 1995). IDEs have found uses in both redox and nonredox based sensing applications. For instance, the mechanism of change in redox potential of $\text{Co}^{2+/3+}$ due to the binding of nitride oxide has been utilized in a reversible nitride oxide sensor (Jin, Yamaguchi et al., 2001; Shioya and Swager, 2002).

Recently, interdigitated electrodes (IDEs) have been used in affinity-based biosensors. With the help of redox mediators, functionalized IDEs are used for the detection of glucose, antigen and DNA (Finot, Bourillot et al., 2003; Koehne, Li et al., 2004; Schlecht, Malav et al., 2006). L. Yang et al. (Yang, Li et al., 2004) used a 15 µm interdigitated array microelectrode to detect Escherichia coli O157:H7, with the lower detection limit of 4.36×10^5 cfu/mL. However, this value is much higher than the maximum level that federal government allows in municipal drinking water -- 500cfu/mL. In this work, the CV method is found not very sensitive when comparing with the Faradic impedance spectroscopy (FIS) method. In 1998, nano sized IDEs were used in monitoring the immobilization of glucose oxidase through measurement of interfacial impedance (Van Gerwen, Laureyn et al., 1998). Later, nano scale IDEs also found uses in DNA hybridization detection, and successfully characterized single strand DNA with a mediator concentration of 10⁻⁴ M by Square-wave voltammograms. Despite a lower signal than macro-electrodes, nanoelectrodes are found to be more sensitive to concentration change than macroelectrodes (Dharuman, Grunwald et al., 2005). Through optimizing the concentration of supporting electrolyte and redox species inside the solution (e.g.10µm K₃[Fe(CN)₆] in 0.01M

phosphoric buffer solution (PBS)), a gold IDEs with 1 μ m finger width and 800nm spacing was developed to reach a lower detection limit of 0.1 μ M (oligonucleotide) and a upper saturation limit of 0.5 mM. One year later, Dharuman et al. (Dharuman, Nebling et al., 2006) reduced the size of gold IDEs (e.g. 800nm finger and 400nm space) and used them to measure DNA hybridization by the coulostatic pulse technique whose response time is much shorter than the FIS method, and the lower detection limit reached 0.1 μ M (Cai, Lee et al., 2006).

The above application of IDEs in the area of affinity sensing is mainly based on the use of the FIS method which requires a long response time and tedious data analysis steps. Even though the CV method is used in some cases, the benefit of IDEs had not been fully realized because the IDEs are not connected in a generator-collector mode. There are reasons that IDEs in a generator-collector mode are supposed to have better performance than other modes when they are used in affinity sensors. Affinity based sensors using CV measurement mainly rely on the electrode surface blockage and the reduction of effective electron transfer rate (k_0 value) of redox species (He, Xie et al., 2005). According to the Butler-Volmer equation the reduced k_0 value will slow down the reaction at both the generators and the collectors, which in turn will reduce the amount of redox cycling and eventually the current level. Due to this redox cycling behavior, the change in CV limiting current for IDEs in a generator-collector mode is expected to be higher than that in single electrodes with the same dimension as the IDEs when the electrode surface is functionalized. Therefore, IDEs used in an affinity sensor could potentially improve the sensitivity when they are connected in a generator-collector mode. Since CV is a rapid and easeof-use method but lacks high sensitivity, employing IDEs in a generator-collector mode using the CV method could be a key to develop a new class of affinity based sensor to achieve easy, rapid and sensitive measurement for clinic application, and it is imperative to understand the

mechanism of the CV performance of IDEs at various k_0 values, in order to optimize the design of IDEs based affinity sensor using CV method.

1.4. Nanometer scale IDEs based biosensors with Electrical Double Layer (EDL) effects – another challenge

The performance of IDEs has been investigated over the years with theoretical analyses. However, these studies are all based on the diffusion theory without considering the effect of the EDL on the CV performance of IDEs. For an electrochemical electrode, once it comes in contact with an electrolytic solution, an EDL structure will form due to the electrostatic interaction between electrons in the metal and ions in the solution. As the electrode size becomes equivalent to that of the EDL (e.g., on a single nanometer scale), the electrical field generated near the electrode surface will influence the transfer of electrons and transport of ions, thus altering the electrode reaction and current response. This is true even in the presence of excess supporting electrolyte. Recently, numerous studies showed that the current responses of nanometer electrodes deviated strongly from the predictions based on the classical diffusion theory that ignored the effect of EDL (Morris, Franta et al., 1987; Seibold, Scott et al., 1989; Conyers and White, 2000; Chen and Kucernak, 2002a; Chen and Kucernak, 2002b; Watkins and White, 2004). In light of the latest advances in nanofabrication (Heller, Kong et al., 2005; Sun, Huang et al., 2005; Anandan, Rao et al., 2006; Tang, Zhang et al., 2006) and the wide-spread application of nano IDEs in the biosensing area, we need to ask if the performance of nanoscale IDEs will be affected by the EDL, and if so, how it will be affected.

The structure of the EDL has been extensively studied over the past century. Based on the classical work by Gouy, Chapman, Stern, Frumkin and Grahame (Grahame, 1947; Bard and

Faulkner, 2001), a converging picture depicting the structure of EDL has emerged. The EDL consists of a surface-localized part (or a compact layer) and a thermally mobile, spatially distributed part (or a diffuse layer). The surface-localized part consists of localized charges, including electrons, solvent molecules, and/or specifically adsorbed ions, and the thermally mobile, spatially distributed part consists of various solvated electroactive and inactive ions. According to Grahame, (Grahame, 1947) some anions have the tendency to specifically adsorb onto the surface of a positively polarized electrode. When this happens, the electronic compact layer is itself a double layer structure with an inner layer filled by these specifically adsorbed anions and an outer layer bounded by the plane of closest approach for the ions in the solution. In this situation, the EDL is actually a triple layer in Grahame's term. When such specific adsorption is absent (e.g., for most cations and some anions such as fluorides and hydroxides), the inner layer will be empty or filled with solvent molecules. While these classical efforts have provided reasonable theoretical insights underlying the many observed metal/electrolyte phenomena, they possess some inherent drawbacks. For example, in most of these studies, the ionic diffuse behavior is studied with the use of Boltzmann distributions for various ions without considering a reactant species, and when a reactant species is considered, however, electroneutrality is often assumed.

The use of Boltzmann equations for describing the distributions of various ions is mainly used to enable solution of the Poisson equation. For instance, Gouy and Chapman utilized Boltzmann equations based on the concept of statistical mechanics for describing the distributions of various ions in their pioneering work (Bard and Faulkner, 2001). Obeying a Boltzmann distribution, however, requires that these ionic species are in a thermodynamic equilibrium state. This requirement turns out to be difficult to meet when electroactive reactant species are present in the solution. When a redox event of the reactant occurs at the electrodes, the net dynamic Faraday current generated by the electron transfer at the metal/electrolyte interface will break up the thermodynamic equilibrium state for the reactant ions.

The assumption of electroneutrality in the electrolytic solution, on the other hand, is useful to simplify the mass transport phenomena encountered in such an electrochemical process. While electroneutrality holds true in the bulk part of the solution, it is questionable whether it is still valid in the vicinity of the electrode surface. When the electrode is electrically polarized, charge separation will occur in the solution close to the electrode to compensate the electrical field generated. Under this condition, it is impossible to maintain electroneutrality. (Smith and White, 1993)

Lately, some efforts have been made to address these problems. Smith and White (Smith and White, 1993) studied the consequence of assuming electroneutrality as well as the effect of EDL on the current response of nanometer electrodes by solving the Poisson equation and the Nernst-Plank equation. In their work, however, the electrode reaction is assumed to be reversible and governed by the Nernst equation. To address this shortcoming, He *et al.* 2006 (He, Chen et al., 2006) investigated the same problem using a similar approach by employing the Butler-Volmer equation for controlling the electrode reaction. This treatment allowed the consideration of both reversible and irreversible systems. While both studies provided useful information about the effect of the EDL on the current performance of nanometer electrodes, they possess some limitations due to the method they used. They are mainly using a finite difference method to seek numerical solutions for the analytically derived closed-form equations. This method is limited in not only the degree of complexity for the geometric shapes of electrodes that can be investigated but also the broader applicability of the method because of the difficulty in extending it to

complex systems of practical significance such as IDEs based biosensors. Therefore, for investigating the performance of nanoscale IDEs, it is imperative that the effects of EDL on the electron transfer and current response be investigated with a method that can overcome these limitations.

1.5. Objectives of this dissertation

Electrochemical based biosensors play a very important role in clinic diagnostics because of their remarkable detection ability, experimental simplicity and low cost. In these sensors, Cyclic Voltammetry (CV) and other label-free methods are used to reduce the required time for sensor preparation. Although the CV method is known for its fast response and ease-of-use, it suffers from a lack of sufficient sensitivity and lower-detection-limit. As a result, the CV method is often used as a supplemental procedure to other measurements.

To make the CV method viable for biosensors application, it is necessary to increase its sensitivity and lower its detection limit. Nano or micro scaled IDEs have been explored to address this issue. One unique feature of IDEs is that the current in both generator and collector can be drastically amplified through redox cycling. However, in previous affinity sensing applications using the CV method, the benefit of IDEs has not been fully utilized because IDEs electrodes are not connected in a generator-collector mode.

Nano or micro scaled IDEs in a generator-collector mode have features like miniaturization and high signal-to-noise ratio. Thus they are able to potentially improve the CV method based affinity sensing. However, some questions need to be answered when they are employed in affinity sensors. One of the questions is how nano or micro IDEs will behave when the surface of the electrodes is functionalized. For biosensing application, the surface of the electrodes usually has to be functionalized, and this functionalization can cause the effective electron transfer rate constant (k_0 value) to change. Thus, it is important to understand the performance of IDEs at various k_0 values so that the design of IDEs based biosensors can be optimized.

The other critical question is whether the redox cycling feature of IDEs will continue when the size of the electrode goes down to the nanometer scale, or whether the traditional electrochemical theory can still be applied to the nanometer IDEs when the size of the electrode is close to the thickness of the electric double layer (EDL). As a matter of fact, a number of studies have shown that the current response of single nanometer-sized electrode deviates strongly from the predictions based on the classical diffusion theory that ignores the effect of EDL. Although many efforts have been made to solve the EDL related problems encountered in various applications, most of them are based on half analytical and half numerical method dealing with a single electrode. Thus they limit not only the geometric shapes of electrodes that can be investigated but also the broader applicability of the method, e.g. IDEs based biosensors.

One approach towards a deeper understanding of the underlying mass transport mechanism for the voltammetric response of IDEs is the use of finite element analysis (FEA) based computational models. FEA models allow us to study the coupled physical chemical problems at more complex 2D and 3D domains, thus making it possible to solve electrochemical problems encountered at IDEs with complex geometries.

The objective of this thesis is to explore ways to improve the performance of electrochemical sensors through integrating cyclic voltammetric (CV) method with nano or micro interdigitated electrodes (IDEs) in a generator-collector mode. To achieve this goal, we develop a complete FEA based computational model to study the voltammetric response of micro or nano IDEs and the relationship between limiting (or peak) current of voltammetric

response and the electron transfer rate constant at various electrode size and gap spacing of IDEs. In addition to the simulation work, this thesis presents an experimental study of IDEs along with a CV method based on affinity sensing for validating that micro IDEs in a generator-collector mode are a better affinity sensor than single electrodes. This study presents some important information for improving the design and development of future electrochemical-based biosensors.

1.6. Thesis Organization

This dissertation is presented as four separate chapters with a preceding introduction (Chapter 1) presenting the previous theoretical and experimental study on the CV performance of micro and nano IDEs, the fabrication method, the application of IDEs in the biosensing field, and the challenges we need to face when the electrode size goes down to nanometer scale. Chapter 2 compares CV performance between macro and micro IDEs, and studies possible application of micro IDEs in affinity sensing by computational simulation. In Chapter 3, the EDL effect on the nanometer IDEs is investigated. Chapter 4 presents the CV performance at micro IDEs in a single-electrode mode and in a generator-collector mode by an experimental means. Chapter 5 summarizes the implications of the results, the primary findings, and recommendations for future work.

CHAPTER 2

SIMULATING THE CV PERFORMANCE OF MICRO AND SUBMICRON IDES

2.1. Introduction

In the case of affinity-based biosensors, where detection is based on the interactions between complementary molecules such as antibody–antigen coupling, aptamer-protein recognition, or DNA hybridization, the functionalization of electrodes with probe molecules and subsequently the coupling of the target and probe molecules will surely reduce the access for electron-transfer from the electrolyte to the electrode, or vice versa. This, in turn, will decrease the k_0 value at the surface of the electrode. Hence, for a successful design of affinity-based biosensors, it is important to know the effect of changing k_0 on the electrical current performance.

Many electrochemical methods have been used in affinity based sensing. Among them, the CV method has features of fast response and ease-of-use, but it suffers from a lack of sufficient sensitivity and lower-detection-limit. To make the CV method viable for affinity biosensors application, it is necessary to increase its sensitivity and lower its detection limit. From the previous chapter we know that affinity based sensors using CV measurement rely on the electrode surface blockage and the reduction of effective electron transfer rate (k_0 value) of redox species (He, Xie et al., 2005). Due to this redox cycling behavior, the change of CV limiting current with the k_0 value for IDEs in a generator-collector mode is expected to be higher than that at single electrodes with the same dimension as the IDEs when the electrode surface is functionalized. However, although IDEs along with the CV method have been used in the

biosensing field, the advantage of IDEs was not fully utilized because in most biosensing cases IDEs were not connected in a generator-collector mode. Moreover, apart from the knowledge on improving the performance of IDEs by changing electrode geometry, not much is known about the electrochemical behavior of IDEs at different the k_0 value.

In this chapter, we developed a finite element analysis (FEA) based computational model to simulate the CV performance of macro, micro and submicron IDEs. By investigating the relationship between k_0 value and CV performance of IDEs with various designs, we explore ways to improve the performance of affinity based biosensors.

2.2. Simulation methods

2.2.1. Governing equations.

This model assure a system in which inlaid IDEs are placed at the bottom of an electrochemical cell containing a supporting electrolyte and a redox couple. The redox couple considered in this study is O (oxidized species) and R (reduced species), and their electrode reaction is governed by equation (2.1):

$$O^{x} + e^{-\frac{k_{T}}{\sum k_{b}}} R^{x-1}$$

$$(2.1)$$

where k_f and k_b are forward (reduction) and backward (oxidation) rate constants, respectively, and they can be expressed as the followings according to Butler-Volmer kinetics (Bard and Faulkner, 2001):

$$k_f = k_0 \cdot \exp[-\alpha F(E - E_{std}) / RT]$$
(2.2)

$$k_b = k_0 \cdot \exp[(1 - \alpha)F(E - E_{std})/RT]$$
(2.3)

where E_{std} is the standard potential of the redox couple, k_0 the electron transfer rate constant, α the charge transfer coefficient, *F* Faraday constant, *E* electrode potential.
In this study, by considering an unstirred solution containing an excess supporting electrolyte, the effects of convection and electromigration in mass transport can be neglected, and the mass transport mechanism of these species is reduced to primarily a diffusion-controlled process (Equation 2.4):

$$\frac{\partial c_i}{\partial t} = D_i \nabla^2 c_i \tag{2.4}$$

In the affinity based sensing method, the preferred concentration of redox species varies from 2 mol/m³ to 10 mol/m³. In this model, there is a linear relationship between the limiting current and the redox species concentration, and in our previous study, a concentration of 5 mol/m³ was used. Thus, to make studies consistent and comparable, in this study the initial surface and bulk concentrations of *O* and *R* (represented by c_o^* and c_R^*) are both set at 5 mol/m³.

2.2.2. Model geometric considerations.

For the 2D models, a unit-cell containing one half of a collector and one half of a generator separated by an insulating gap is considered (Figure 2.1A). The widths of the electrodes and the insulating gap are kept the same (denoted as *w*). In the modeling process, the width of the unit-cell is set at 2w and the height 1000*w*. For comparison purpose, another 2D model (20 µm wide and 20 mm tall) is developed with a single electrode placed at the bottom (Figure 2.1B). To investigate the effect of the electrode width of IDEs on the mass transport of redox species at different k_0 values, three *w* values are considered: 10 µm, 1 µm, and 100 nm at a sweeping rate of 20 mV/s. The reasons for choosing these values include: 1) based on a previous study we found that a significant improvement in the current response is achieved when *w* is reduced from 10 µm to 100 nm, and such a benefit diminished when the size is further reduced; 2) with electrodes

having these sizes, the effect of the electrical double layer on the current response is negligibly small and thereby could be ignored (He et al., 2006).



Figure 2.1 2D models of a unit-cell containing IDEs (A), and of a unit-cell containing a single electrode (B). Note: G represents generator, C represents collector, and W represents working electrode.

For the 3D models, unit-cells with block-elevated IDEs (Figure 2.2A) and nanorod-modified IDEs (Figure 2.2B) placed at the bottom of the cells are considered. For comparisons, another 3D unit-cell model with inlaid IDEs is developed (Figure 2.2C). The width and depth of the unit-cells are set at 200 nm and the height at 100 μ m. In all the 3D cases, the width of the base electrodes is set at 100 nm. For the height of the electrodes, in the case of the block-elevated IDEs the base electrodes are extruded 100 nm upward to form the block electrodes, and in the case of the nanorod-modified IDEs a square array of cylindrical nanorods with 100 nm in diameter, 100 nm in spacing and 264.9 nm in height are added onto the base electrodes the same as that of the block-elevated electrodes, and we did so because it is of practical interest to see how the cost effectiveness varies in these two different cases when the amount of material is the same).



Figure 2.2 3D models of unit-cells containing block-elevated IDEs (A), nanorod-modified IDEs (B) and inlaid IDEs (C) with the width for the base electrode of 100nm.

For all the IDEs, their CV responses are simulated with the potential at the collector electrodes held at 0V and the potential at the generator electrodes swept cyclically between -0.2 V and +0.6 V (up to +1.2 V depending on the k_0 value). For the single electrode case, the electrode is excited with a cyclic potential between -0.2 V and +0.6 V (up to +1 V depending on the k_0 value). The potential sweep followed a simple triangle waveform with a sweep rate of 20 mV/s. For the boundaries, symmetric condition is applied at the two sides of the unit-cells and insulation condition imposed at the gaps. Moreover, inward flux for *R* and outward flux for *O* are applied to the generator (j_g) and the collector (j_c), respectively, with

$$j_g = k_0 \cdot \exp[-\alpha F(E_t - E_{std})/RT] \cdot c_0 - k_0 \cdot \exp[(1 - \alpha)F(E_t - E_{std})/RT] \cdot c_R$$
(2.5)

$$j_c = k_0 \cdot \exp[-\alpha F(0 - E_{std}) / RT] \cdot c_0 - k_0 \cdot \exp[(1 - \alpha)F(0 - E_{std}) / RT] \cdot c_R$$
(2.6)

As discussed in the literature review part, the value of the standard electron-transfer-rate constant (k_0) often varies with both the electrode material and the nature and concentration of the base electrolyte (Horswell, O'Neil et al., 2003). There are several ways to determine k_0 including the fast cyclic voltammetry and FFT-square wave voltammetry (Nicholson, 1965; Baranski and Szulborska, 1994; Winkler, 1995). In all these methods, k_0 is found highly dependent on the diffusivity (Nicholson, 1965; Baranski and Szulborska, 1994; Winkler, 1995). For example, on a bare platinum electrode a value of 1×10^{-3} m/s is measured in a 1.8 M sodium chloride solution

with a diffusivity of 7.8×10^{-10} m²/s, (Baranski and Szulborska, 1994) and in other situations (He, Xie et al., 2005) k_0 can vary from 1×10^{-9} m/s to 1×10^{-5} m/s depending on the degree of functionalization and the level of further complimentary binding at the electrodes. Thus in this study, we assumed a diffusivity of 7.8×10^{-10} m²/s and considered a range of k_0 values from 1.5×10^{-3} m/s, for representing a bare electrode, down to 1.5×10^{-8} m/s for a highly blocked electrode due to either functionalization or complimentary binding. In a real experiment, however, the encountered change in k_0 actually reflects more of the change in the degree of complimentary binding than of the degree of functionalization because the latter is fixed once the sensor is made. The degree of complimentary binding may change when the concentration of the target analyte changes.

D_O	$7.8 \times 10^{-10} \text{ m}^2/\text{s}$	α	0.5
D_R	$7.8 \times 10^{-10} \text{ m}^2/\text{s}$	F	96485.34 C/mol
k_0	$1.5 \times 10^{-3} \text{ m/s} \sim 1.5 \times 10^{-9} \text{ m/s}$	R	8.31 J/K
c_O^*, c_R^*	5 mol/m ³	Т	298 K
E_{std}	0.265 V		

Table 2.1. Material constants and kinetic parameters used in the modeling processes.

The electrochemical process described above is simulated using the time-dependent electrokinetic-flow application mode in COMSOL Multiphysics along with the material constants listed in Table 2.1. To assure the validity of the developed models and the accuracy of the obtained results, prior to all these analyses a mesh refinement convergence study and a verification study are performed. These studies not only showed a good agreement between the

simulated results and the theoretical predictions for three single-band electrode cases, but also helped select a proper mesh density based on the converged results for the rest of analyses.



Figure 2.3 Schematic illustration of a sigmoidal curve along with the parameters of interest: the upper asymptotic response (*a*), the sensitivity (S = a/4b), the center of the responsive range (x_0), the responsive bandwidth (RB = 4*b*), the lower responsive limit (x_0 -RB/2), and the upper responsive limit (x_0 +RB/2).

2.3. The data analysis

CV curves and the relationships between CV limiting (or peak) current and logarithmic k_0 are obtained and analyzed. For quantitative analyses of these relationships, the following 4-parameter sigmoidal function is employed:

$$y = y_0 + \frac{a}{1 + \exp[-(x - x_0)/b]}$$
(2.7)

By letting y be the current response and x be the $log(k_0)$, this function is fit to the obtained data in the respective least-squares regression analyses. From these statistical analyses, the following parameters are determined: the upper asymptotic response (a), the sensitivity (S) of the current response to the change of $log(k_0)$, the center (x_0) of the responsive range, the responsive bandwidth (RB = 4*b*), the lower responsive limit (x_0 -RB/2), and the upper responsive limit (x_0 +RB/2). A schematic illustration of these parameters is depicted in Figure 2.3. Comparisons of these resolved parameters are performed for various related cases.

2.4. Model validation

To assure the validity of the developed models and accuracy of the obtained results, a mesh refinement study is performed to assure the convergence of the results. At each refinement step, an increased mesh density, particularly in the vicinity of the IDEs, is employed. Figure 2.4A shows the result of the mesh refinement study, where it is seen that as the number of elements exceeds 10K a clear convergence of the result emerged. Thus in the 2D modeling, the mesh with 12,413 elements is used for all the analyses.

After this mesh refinement study, three cases of single-electrode are analyzed and the obtained results are compared with the theoretical predictions for single-band electrodes. With a single-band electrode having a hemi-cylindrical diffusion regime, the amperometric current response is predicted to be in quasi steady state with the limiting current (per unit length) of $I_{qss} = 2\pi F D_R C_R^* / \ln(64D_R t/w^2)$, where D_R is the diffusivity of the reaction species, C_R^* the concentration of the reduced species in the bulk solution, *F* the Faraday constant, *w* the electrode width, and *t* the time. Figure 2.4B shows the obtained amperometric current responses for the three single-electrode cases with $w=10 \ \mu\text{m}$, $1 \ \mu\text{m}$, and $100 \ n\text{m}$, along with those based on the theoretical equation. Clearly, the obtained amperometric current responses are indeed in quasi steady state, and the simulated results are in very good agreements with the theoretic predictions. The percent error found at t=30 seconds is only 1.7%, 0.6% and 3.1% for the 100 nm, $1 \ \mu\text{m}$ and 10 $\ \mu\text{m}$ cases, respectively. Note that the current is expressed in units of mA/m due to the 2D modeling nature, and to obtain the actual current values one needs to multiply this current value

by the length of the electrode. So, with such an assurance, we are confident about the validity of the obtained results in this study.



Figure 2.4 (A) Result from a mesh refinement study showing the convergence of the modeling result as the number of elements increases. (B) Amperometric current responses (solid line) for the three single-band electrode cases along with the theoretic predictions (dot lines).

2.5. Results and discussion

2.5.1. CV performance of IDEs with various electrode sizes at $k_0 = 1.5 \times 10^{-3}$ m/s.

In Figure 2.5, the obtained cyclic voltammograms (CV curves) for the IDEs with w_e of 200 µm, 20 µm, 1 µm and 800 nm are shown. Obviously, the CV curve for the case with w_e =200 µm exhibited a peak-shaped voltammogram, thus suggesting that the rate of species-O production at the generator is faster than the speed at which it can diffuse to the collector. However, this behavior changed drastically as the electrode size decreased. With w_e =20 µm, both the peak current and forward/backward hysteresis of the CV curve reduced significantly. When the electrode width was further decreased to w_e =1 µm, the obtained CV curve showed a sigmoidal shape with slight hysteresis. When w_e = 800 nm, the CV curve exhibited a perfect sigmoidal shape with forward and backward branches completely overlapped. This fact indicates that the mass transport of the redox species is enhanced significantly when the width of the electrodes in these IDEs is decreased.

Figure 2.6 shows the CV curves for the IDEs with an electrode width of 400nm or less. Clearly, all the CV curves exhibited a steady-state sigmoidal shape without any hysteresis. Furthermore, of these CV curves the ones associated with narrower electrodes rose slower than those with wider electrodes and the CV curve shifts right as the size of the electrode decreases. This phenomenon can be attributed to the reduced amount of active species produced at the narrower electrodes and the influence of the heterogeneous rate constant caused by the small electrode size calling for a high overpotential to drive the reaction. To quantify the steady-state limiting currents, we measured their values at both the generator (I_{ss}^g) and the collector (I_{ss}^c) and listed them in Table 2.2 for the IDEs with w_e equal to or less than 1µm. Clearly, in each w_{gap}/w_e case the I_{ss}^c value was very close to the I_{ss}^g value, indicating extremely high current collection efficiency. Furthermore, at different w_e the value of either I_{ss}^c or I_{ss}^g was close to its counterpart despite the wide difference in the electrode width. For example, the size of a 50 nm electrode has only 5% of the surface area of a 1 µm electrode, but at $w_{gap}/w_e=1$ the I_{ss}^g reached at the smaller electrode is 92% of that at the larger electrode. This is certainly attributed to the high efficient feedback of the redox species at the IDEs.



Figure 2.5 CV curves obtained for IDEs with (A) $w_e = 200 \ \mu\text{m}$, (B) $w_e = 20 \ \mu\text{m}$, (C) $w_e = 1 \ \mu\text{m}$, and (D) $w_e = 800 \ \text{nm}$ at a sweep rate of 20 mV/s.



Figure 2.6 CV curves for IDEs with w_e of 800 nm, 400 nm, 200 nm and 100 nm at a sweep rate of 20 mV/s.

Table 2.2 Steady-state limiting current at the generator (I_{ss}^{g}) and the collector (I_{ss}^{c}) (mA/m).

Collector/Generator Current: I_{ss}^{g} / I_{ss}^{c} (mA/m)			
	W_{gap} / W_e		
W _e	1⁄2	1	2
100 (nm)	0.411/0.412	0.326/0.327	0.258/0.258
200 (nm)	0.422/0.423	0.334/0.335	0.262/0.263
400 (nm)	0.429/0.431	0.337/0.339	0.264/0.266
800 (nm)	0.432/0.435	0.338/0.341	0.268/0.268
1 (µm)	0.433/0.436	0.339/0.342	0.264/0.269

It should be noted that in the modeling the sigmoidal steady-state CV curves (shown in Figure 2.6) were independent of the voltage sweep rate and the peak level of the applied potential. This finding reveals another benefit of the nanoscale IDEs: the CV response is

independent of the peak level of the potential and the sweep rate. Thus it is advantageous to use nanoscale IDEs for sensing applications.



Figure 2.7 Concentration profiles of the redox species (C_R and C_O) at the electrode surfaces (A) and in the bulk solution (B) in a voltammetric condition. Note that the numbers indicate the time steps in cyclic voltammetry.

Figure 2.7 shows the time transient concentration profile of both the reduced (c_R) and oxidized (c_o) species at the electrode surfaces and in the bulk obtained in cyclic voltammetry. At the generator surface (between -25 s and 25 s, Fig.2.7A), about 99% of species-R was converted to species-O at t = 1 s and 80 s, 60% at t = 10 s and 70 s, 2% at t = 20 s and 60 s, and zero at t = 30 s, 40 s and 50 s. At the collector surface (between -75 s and -62.5 s, or 62.5 s and 75 s, Fig.7A), species-R remained high (>95%) and species-O remained low (<5%) at all time. The change in concentration in the bulk solution over time (Fig.7B) showed a very different phenomenon. At t = 1 s the typical concentration profile with a diffusion depletion layer was seen. This is due to the conversion of species-R to species-O at the generator surface. As the generator potential being swept cathodically (t = 10 s, 20 s and 30 s), species-O at the surface gradually got reduced again. This trend kept going until the anodic sweep reached a sufficient level of potential (t = 60 s). Following that, species-R at the generator surface got oxidized once more (t = 70 s and 80 s). After a full cycle, a small amount of species-O escaped into the bulk, as indicated by the difference between the concentration profiles at t = 1 s and t = 80 s.

Figure 2.8 shows the concentration profile of species-R and species-O at the electrode surfaces and in the bulk solution obtained in step amperometry. Clearly, once the step potential is applied, a time-independent concentration profile is developed at the electrode surfaces between the generator and collector (Fig.2.8A). But the concentration gradient in the bulk follows a time-dependent and diffusion layer limited profile. Such elucidation of the concentration profile of the active species both at the electrode surfaces and in the bulk indicates that while the concentration gradient in the bulk is still time-dependent and diffusion layer limited, a high-profile and steady-state concentration gradient is reached at the electrode surfaces between the generators and collectors. This fact suggests that the localized electrochemical activities can be investigated

with the use of nanoscale IDEs such that the time dependent and diffusion layer limited electrochemical process in the bulk can be overcome.



Figure 2.8 Concentration profiles of the redox species (C_R and C_O) at the electrode surfaces (A) and in the bulk solution (B) in an amperometric condition. Note that the numbers indicate the time steps in amperometry.

2.5.1. CV performance of IDEs with different *k*₀ values.

From the 2D analyses, the CV curves for the inlaid IDEs with $w = 10 \ \mu\text{m}$, 1 μm , 100 nm, and the single electrode at various k_0 values are shown in Figure 2.9A, 2.9B, 2.9C and 2.9D, respectively. Comparing with the single electrode which yielded peak-shaped CV curves (Figure2.9D), the IDEs with $w=10 \ \mu\text{m}$ (Figure 2.9A) produced quasi-steady-state CV curves with only slight peaks, and the IDEs with $w=1 \ \mu\text{m}$ (Figure 2.9B) and $w=100 \ \text{nm}$ (Figure 2.9C) generated steady-state CV curves with sigmoidal shapes. At a higher k_0 value, the limiting (or peak) current densities obtained for the three IDEs are much higher than those for the single electrode (note that the current density is obtained by normalizing the current with respect to the width of the electrode). This increased current response is certainly attributed to the enhanced mass transport near the IDEs. As k_0 decreases, a decrease in the limiting (or peak) current is observed in all four cases, but a more drastic decrease is seen with the IDEs than with the single electrode with which the peak current is already low enough leaving not much a room for further decrease. This fact suggests that the CV performance of the IDEs is more sensitive to the change of k_0 as compared with that of the single electrode.

From Figure 2.9A, 2.9B and 2.9C, it is seen that the narrower the electrode gets, the more sensitive it becomes to the change of k_0 , especially when k_0 is large (>1.5×10⁻⁶ m/s). For instance, a change in k_0 from 1.5×10^{-3} m/s to 1.5×10^{-4} m/s caused almost a 20% reduction in the limiting current for the IDEs with w = 100 nm, whereas a mere 3% reduction is seen for the IDEs with w=1 µm. With each set of IDEs (see Figure 2.9C for example; w=100 nm), as k_0 decreased, not only the limiting current decreased significantly but also the CV curve shifted to the right. These facts indicate that the CV response of the IDEs is indeed sensitive to the change of k_0 , and that a higher overpotential will be needed to drive the electron transfer as k_0 decreases.



Figure 2.9 CV curves obtained for the IDEs with $w = 10 \ \mu m$ (A), 1 μm (B), 100 nm (C), and for the single electrode (D) at various k_0 values (from $1.5 \times 10^{-3} \ m/s$ to $1.5 \times 10^{-9} \ m/s$).

Figure 2.10 shows the relationships between the limiting (or peak) current density and $log(k_0)$ for the IDEs with $w = 10 \mu m$, 1 μm and 100 nm and the flat single electrode. It is seen that the current density at the single electrode is the lowest due to the absence of the redox feedback facilitated by the collector electrodes. For the IDEs, however, a higher current density response is seen with a smaller w due to the much enhanced mass transport activity. This fact implies that when the total surface area of the electrodes is kept the same, the IDEs with a smaller w will produce a higher current response.



Figure 2.10 Variation of the CV limiting current density with $log(k_0)$. Note that the current density is calculated by normalizing the current with respect to the width of electrodes.

Table 2.3 lists the resolved parameters from the regression analyses for the curves shown in Figure 2.3 based on Eq.2.7. Clearly, as *w* decreases the upper asymptote (*a*), the sensitivity (*S*) and the lower and upper responsive limits all increase, while the center of the response range (x_0) shifts right and the responsive bandwidth (RB) decreases. These results suggest that as *w* decreases, there will be significant enhancements in the limiting current response, the sensitivity, and the upper responsive limit, albeit at the expenses of decreased responsive bandwidth and increased lower responsive limit. Of the lower and upper responsive limits, the upper limit is more important than the lower one. In an experiment to determine the concentration of a target analyte, the change in k_0 is likely caused by the change in the degree of complimentary binding due to the change in the concentration of the target analyte. At low concentration, only a small amount of target molecules will bound to the probe molecules on the electrode surface, thus the

electrode surface will only be slightly blocked, and k_0 value will decrease a little bit but still maintain at a relative higher level. When the upper responsive limit is high, even a small amount of complimentary binding induced k_0 value decrease could be detected. But when the upper responsive limit is low, this small decrease will hardly be catch. Therefore, the upper responsive limit may actually reflect the lower-detection-limit for the concentration of the analyte.

Table 2.3 Parameters resolved from the statistical regression analyses with 2D models (note that a $log(k_0)$ value of -3.48 reflects a k_0 value of $10^{-3.48}$ m/s).

Current density $-\log(k_0)$			
	<i>w</i> =10 μm	<i>w</i> =1 μm	<i>w</i> =100 nm
Upper asymptote	6.07	66.64	680.72
$(a: mA/cm^2)$		00.01	
Center of the responsive range	-6.43	-5.36	-4.37
$(x_0: \log(m/s))$			
Sensitivity	2.87	36.71	381.40
$(S: mA/cm^2/log(m/s))$			
Responsive bandwidth	2.12	1.82	1.78
(RB: log(m/s))			
Lower responsive limit	-7.49	-6.27	-5.26
$(x_0 - RB/2: \log(m/s))$			
Upper responsive limit	-5.37	-4.45	-3.48
$(x_0 + RB/2: \log(m/s))$			

The above results show that the CV curves of the IDEs with different electrode widths possess different sensitivity and different responsive range to the change of k_0 . The IDEs with a smaller *w* will generate a higher current density response, a higher sensitivity to the change of k_0 , and an increased upper responsive limit. Because a high upper responsive limit means a low

lower-detection-limit for the concentration of the target analyte, it is thus conceivable that a biosensor using IDEs with nanometer-scale electrodes could lead to high performances including high current response, high sensitivity and low lower-detection-limit. Another benefit of using small nanometer scale IDEs is that detection can be made with a very small volume of the analyte. Moreover, the above results also suggest that different detection ranges can be accommodated by using IDEs with properly selected electrode dimensions.

Figure 2.11 shows the results obtained from the 3D models, where the CV curves for the block-elevated, nanorod-modified and inlaid IDEs at various k_0 values are plotted. The limiting current obtained for the nanorod-modified IDEs is the highest and that for the inlaid IDEs the lowest at any given k_0 value. For instance, at $k_0=1.5\times10^{-3}$ m/s, the highest limiting current reached at the IDEs is 0.31, 0.21 and 0.07 nA for nanorod-modified, block-elevated and inlaid IDEs, respectively. With each set of IDEs the limiting current is found to decrease with decreasing k_0 . By plotting the limiting current density (i.e., the current normalized to the planar area of the 3D IDEs) against $\log(k_0)$ for all three cases, it is found that the current density response (see Figure 2.11D) is the highest, intermediate and the lowest for the nanorod-modified, block-elevated and inlaid IDEs, respectively, at any given k_0 . From the fit of Eq.14 to these curves, the obtained parameters are given in Table 2.4. These results show that both the nanorodmodified and block-elevated IDEs produced increased current responses and enhanced sensitivities as compared with the inlaid IDEs. Such enhancements are thus attributed to the increased surface area of the electrodes due to the taller heights of the 3D electrodes, which enable a heightened redox cycling activity between the vertical walls of the neighboring generator and collector electrodes.



Figure 2.11 CV curves obtained for block-elevated IDEs (A), nanorod-modified IDEs (B) and inlaid IDEs (C) with w=100 nm at different ETR values from 1.5×10^{-3} m/s down to 1.5×10^{-7} m/s. (D) Variation of the CV limiting current density with log(ETR) for the three 3D IDEs cases.

In comparing between the two 3D IDEs, we noted that when the total volume (or the amount of the material) of the 3D electrodes is kept the same, the nanorod-modified IDEs would have taller electrodes than the block-elevated IDEs. As a result, the nanorod-modified IDEs produced a higher limiting current and a higher sensitivity than the block-elevated IDEs. Therefore, it is believed that adding nanorods onto the inlaid IDEs in an affinity-based biosensor will not only improve the CV limiting current response and its sensitivity to the change of k_0 but also help reduce the amount of material needed. From Table 3, it is seen that the values of both the lower and upper responsive limits of nanorod-modified IDEs are very close to their counterparts in the other IDEs. These facts imply that the gain in the increased limiting current and sensitivity in the nanorod-modified IDEs did not come at the expense of sacrificing the upper responsive limit or the lower-detection-limit.

From the 2D modeling, it is found that the CV responses of IDEs are very sensitive to the change of k_0 , especially at a larger k_0 value (or a lower target concentration). As the electrode width (w) decreases, significant improvement in the sensitivity and the upper responsive limit (or the lower-detection-limit) can be achieved. Moreover, different detection ranges can be accommodated by using IDEs with properly selected electrode dimensions. From the 3D modeling, we can see that the CV current responses of IDEs can be further improved by using nanorod-modified IDEs. These findings are important to future design and development of affinity-based biosensors.

Current density – $\log(k_0)$			
	Nanorod Modified IDEs	Block Elevated IDEs	Inlaid IDEs
Upper asymptote $(a: mA/cm^2)$	3062.27	2086.39	682.19
Center of the responsive range $(x_0: \log(m/s))$	-4.41	-4.42	-4.37
Sensitivity (S: mA/cm ² /log(m/s))	343.43	228.98	76.53
Responsive bandwidth (RB: log(m/s))	1.79	1.76	1.80
Lower responsive limit $(x_0 - RB/2: \log(m/s))$	-5.31	-5.30	-5.27
Upper responsive limit $(x_0+RB/2: \log(m/s))$	-3.52	-3.54	-3.48

Table 2.4 Parameters resolved from the statistical regression analyses with 3D models.

2.6. Conclusion

In this chapter, we developed a FEA based computational model to simulate the mass transport activities at micro and submicron IDEs. This model enables us to elucidate the mechanisms governing the mass transport phenomena at micro and submicron IDEs. With micro and submicron IDEs, the close proximity between the generators and collectors at nanoscale facilitated a highly efficient feedback cycle of the redox species, making the steady-state limiting current very high. A high-profile and steady-state concentration gradient can be developed near the electrodes for studying the localized electrochemical activities to defy the time dependent and diffusion layer limited electrochemical process in the bulk.

With this model, we explore ways to improve the performance of affinity-based biosensors by integrating the IDEs and the CV method. From the 2D modeling, it is found that the CV responses of IDEs are very sensitive to the change of k_0 , especially at a larger k_0 value (or a lower target concentration). As the electrode width (w) decreases, significant improvement in the sensitivity and the upper responsive limit (or the lower-detection-limit) can be achieved. Moreover, different detection ranges can be accommodated by using IDEs with properly selected electrode dimensions. The CV current responses of IDEs can be further improved by using 3D nanorod-modified IDEs. These findings are important to future design and development of affinity-based biosensors.

CHAPTER 3

SIMULATING THE CV PERFORMANCE OF NANO IDES

3.1. Introduction

In previous chapter, we numerically studied the relationship between k_0 value and CV performance of IDEs with electrode size and spacing larger than 100 nm by considering the diffusion controlled mass transport theory. It is found that the CV responses of IDEs are more sensitive to the change of k_0 than that of single electrode especially at a larger k_0 value (or a lower target concentration), and IDEs with smaller electrode size and inter-electrode spacing have better sensing performance, such as higher sensitivity and higher upper responsive limit (or the lower-detection-limit).

Upon the above findings, it seems logic to expect much better performance at IDEs with electrode size and spacing on a nanometer scale. However, for a nanometer-sized electrochemical electrode, the thickness of the diffusion layer can be comparable to that of the diffuse layer on a single nanometer scale, therefore, the mass transport of charged ions at nanometer-size electrode is no longer a diffusion-only process but a combined diffusion and electromigration phenomenon. This situation can get even more complicated at nano-IDEs, because the diffuse layers of adjacent electrodes can overlap due to the small nanometer inter-electrode distance. As a result, the redox cycling between the generator and collector electrodes is not only affected by the inter-electrode gap spacing but also by the overlapped electric fields. Therefore, in order to elucidate the performance of nano-IDEs based affinity sensor, it is important and imperative to fully elucidate the effect of EDL on the performance at nano-IDEs

In this chapter, we developed a FEA based computational model to study the potential application of nano-IDEs for affinity based biosensor with considering the EDL effect. The model is validated by simulating the EDL affected voltammetric performance of nanometer single electrodes and comparing the simulation results with theoretical predication. Then the model of nano single electrodes is expanded to simulate the voltammetric performance of nano-IDEs at different charge valence of the redox species, various interelectrode gap spacing, and the presence and absence of the supporting electrolyte. The relationship between the limiting current of the voltammetric response at nano-IDEs and the k_0 value is studied and compared with the results from chapter 2.

3.2. Simulation methods

3.2.1. Model geometric considerations.

We consider nanometer-sized electrodes having a simple spherical shape because with such a shape, direct quantitative comparisons with literature results would be possible for validating our simulation results, and another reason is that based on our simulation results the effect of EDL on the voltammetric performance of nanometer electrodes is somehow independent on the electrode shape while simulating a cylindrical shape is relatively simple. For nanometer single electrodes, a 2D quarter-circle geometric model in a cylindrical coordinate system (u, v) is considered to represent a 3D spherical electrode by taking advantage of the axisymmetry (about the u-axis) and the in-plane symmetry (about the v-axis) (see Figure 3.1A). In this model, an electrode of radius r_0 is placed at the origin of the coordinate system. For nano-IDEs, we consider a system in which nano-IDEs with half cylindrical electrodes were placed at the bottom of an electrochemical cell. One repeating-unit model consisting of a half generator electrode and

a half collector electrode of semi-cylindrical shape was considered (Figure 3.1B). The center of the generator is placed at the origin (0, 0) of a Cartesian coordinate system and the center of the collector at $(2r_0+w_{gap}, 0)$, where r_0 is the radius of the generator and collector electrodes and w_{gap} is the gap spacing between them.

The space surrounding the electrode is divided into two domains: the first one represents the electronic compact layer of EDL located within $r_0 \leq r < r_0 + \mu$ (note: for nanometer single electrodes, $r = \sqrt{u^2 + v^2}$, while for nano-IDEs, *r* takes the expression of $r_1 = \sqrt{x^2 + y^2}$ for the generator and $r_2 = \sqrt{[x - (2r_0 + w_{gop})]^2 + y^2}$ for the collector), and the second one the electrolytic solution located between $r = r_0 + \mu$ and $r = 1000r_0$. Inside the compact layer, there is an inner Helmholtz plane (IHP) and an outer Helmholtz plane (OHP). In this study, it is assumed that there is no specific ionic adsorption at the electrode surface, thus the region inside the IHP is mainly filled with solvent molecules without any ions. Furthermore, it is also assumed that the OHP is the plane of closest approach for all the ions (active and inactive), and the position of electron transfer (PET), which means that electron transfer between the electrode and electrolyte occurs here.

3.2.2. Governing equations.

Since the PET coincides with the plane of closest approach for all the ions in the solution, there will be no ionic flow or transport inside the compact layer. For this reason, only an electrostatic problem governed by the Poisson equation needs to be solved in the compact layer:

$$\nabla(\varepsilon\varepsilon_0\nabla V) = -\rho \tag{3.1}$$

where V is electrical potential, ε_0 the permittivity of vacuum, ε the relative-permittivity of the compact layer, and ρ charge density. Since there is no ionic species inside the compact layer and

all the electronic charges will be distributed at the electrode surface and the PET, the charge density inside the compact layer should be zero: $\rho = 0$. With this condition in place, Eq.1 indicates that the potential drop across the compact layer will vary when the profile of ε changes.



Figure 3.1 (A) A 2D axisymmetric geometric model depicts a spherical nanometer electrode surrounded by an EDL structure in an electrolytic solution. The shaded quarter-circle represents the electrode; (B) A schematic illustration of a 2D repeating-unit model for nano-IDEs. The two shaded quarter circles represent the generator electrode (left) and the collector electrode (right).

It is generally recognized that ε varies smoothly and continuously inside the compact layer. (Levine, Robinson et al., 1974; Levine and Fawcett, 1979) But very often due to the difficulty in dealing with a continuously varying ε , many investigators opted to simplify it as constants. For example, Smith and White (Smith and White, 1993)assumed a single uniform value of ε =78 for the entire compact layer, while He *et al.* (He, Chen et al., 2006) divided the compact layer into two regions and assigned ε '=6 for the inner region bounded by the electrode surface and the IHP and ε ''=40 for the outer region bounded by the IHP and the OHP. In the present study, we take advantage of the completely computational approach to consider a smooth and continuous function for ε . According to Levine and Fawcett, (Levine, Robinson et al., 1974; Levine and Fawcett, 1979) a smooth and continuous profile for the relative-permittivity inside the compact layer can be expressed by joining a hyperbolic curve with a circular-cosine curve:

$$\mathcal{E} = \begin{cases} \mathcal{E}_{1} \cosh^{2}[S_{1}(r-r_{0})], & r_{0} \leq r \leq r_{0} + l_{1} \\ \mathcal{E}_{2} \cos^{2}[S_{2}(l_{1}+l_{2}+r_{0}-r)], & r_{0}+l_{1} \leq r \leq r_{0} + l_{1} + l_{2} \\ \mathcal{E}_{2}, & r_{0}+l_{1}+l_{2} \leq r \end{cases}$$
(3.2)

Here l_1 is where the hyperbolic and circular-cosine segments join, l_1+l_2 is the thickness of the entire compact layer, and ε_1 and ε_2 are the relative-permittivity of the electrode material and the electrolytic solution, respectively. In this equation, S_1 and S_2 are constants for assuring the continuity for the two segments at $r = l_1$ to the first and second orders. A schematic profile for such a smooth and continuous function is shown in Figure 3.2. According to Grahame (Grahame, 1947), different ions have different tendency for specific adsorption to electrode surfaces, and this could lead to different relative-permittivity profiles across the compact layer. The use of Equation 3.2 allows changes of the relative-permittivity profile by simply adjusting the values of l_1 , l_2 , S_1 and S_2 (Levine, Robinson et al., 1974; Levine and Fawcett, 1979). This feature permitts the consideration of situations having different specific ionic adsorption at electrode surfaces.

In the electrolytic domain outside the compact layer, a multi-physics problem of electrostatics and electrokinetic-flow needs to be solved because both electron transfer and ion transport occur here. For the electrostatic part, the same governing equation given in Equation 3.1 will be solved. The only difference is that the charge density in this domain is determined by the concentration and charge valence of the ions in the solution as $\rho = \sum_{i} z_i c_i$, where c_i is the local concentration of ionic species *i*, and z_i is the signed charge valence of the ionic species. In this equation the sum index (*i*) runs over all ionic species including both the electroactive and inactive ions. Instead of using Boltzmann equations to describe the concentrations of various ions as Gouy-Chapman did, we will seek these concentration distributions by solving an electrokinetic-flow problem as described in detail below.



Figure 3.2 A diagram of a smooth and continuous function depicts the relative-permittivity of the compact layer of EDL: a hyperbolic cosine segment (between a and b) joins with a circular cosine segment (between b and c).

For the electrokinetic part, because of the presence of both an electrical filed and concentration gradient, the mass transport of the ionic species will be governed by not only diffusion but also electromigration. Therefore, this electrokinetic-flow problem is solved by using the Nernst-Plank equation (note that convection is ignored):

$$\frac{\partial c_i}{\partial t} = \nabla (D_i \nabla c_i + \frac{z_i F}{RT} D_i c_i \nabla V)$$
(3.3)

Besides the variables noted earlier, D_i is the diffusivity of species *i*, *t* is time, *F* is the Faraday constant, *R* is the gas constant, and *T* is absolute temperature. These governing equations are solved by considering an electrolytic solution containing an electroactive reactant species, a pair of excess electroinactive cation and anion (as the supporting electrolyte), and a counter ion. For the reactant ion, its redox event occurs at the PET according to the following electrode reaction:

$$O^{x} + e^{-\frac{k_{f}}{k_{b}}} R^{x-1}$$
(3.4)

where O^{z} is the reactant species in an oxidized form, R^{z-1} is the reduced species, k_{f} and k_{b} are forward (reduction) and backward (oxidation) rate constants. According to the Bulter-Volmer kinetics (Bard AJ and Faulkner LR 2001), these rate constants can be calculated as:

$$k_{f} = k_{0} \cdot \exp[-\alpha F(E_{t} - V - E^{0}) / RT]$$
(3.5)

$$k_{b} = k_{0} \cdot \exp[(1 - \alpha)F(E_{t} - V - E^{0'})/RT]$$
(3.6)

Here k_0 is the standard electron transfer rate constant, α the charge transfer coefficient, E_t the potential at the electrode, V the potential at PET, and $E^{0'}$ the standard potential of the redox couple (which is assumed to be 0 in this study). Note that the use of E_t -V- $E^{0'}$ in these two equations indicates that the effect of the potential drop across the compact layer on electron transfer has been accounted for. Based on these two equations, a net dynamic electronic flux at the PET is determined:

$$j_{f} = -j_{b} = k_{0} \cdot \exp[-\alpha F(E_{t} - V - E^{0'}) / RT] \cdot c_{0} - k_{0} \cdot \exp[(1 - \alpha)F(E_{t} - V - E^{0'}) / RT] \cdot c_{R}$$
(3.7)

For the supporting electrolyte, an excess amount of the cation/anion pair in the form of A^+B^- with a concentration 100 times that of the reactant (species O^z) is considered. To accompany the reactant initially, a counter ion, Ct, with a charge of -sign(z) (note that $\text{sign}(\pm |z|) = \pm 1$, where |z| is the absolute value of the signed charge valence z) at a concentration |z| times that of the reactant is also considered.

3.2.3. Boundary conditions

To carry out the simulation, following boundary conditions are defined to provide proper constraints for the governing equations. For the electrokinetic-flow problem, a dynamic flux $j = -j_f$ (Eq.7) is applied for species O^z and $j = -j_b$ for species R^{z-1} at $r = r_0 + \mu$, and the known bulk concentrations (see Table 1) for all the ionic species are applied at $r = 1000r_0$ for nanometer single electrodes, and at top boundary for nano-IDEs. For electrostatic problem, in nanometer single electrode model, a known electrode potential (E_t , in the form of a triangle function) is applied at the electrode surface ($r = r_0$), and a zero potential ($E_t=0$) is applied at $r=1000r_0$ due to electroneutrality in the bulk solution, while in nano-IDEs model, a polarization potential E_t from $E_G = -0.4$ V to $E_G = 0.3$ V at a sweeping rate of 20 mV/s is applied at the surface of the generator ($r_1 = r_0$) and a constant potential $E_C = 0.3$ V is applied at the surface of the collector ($r_2 = r_0$). Note that in the cases of varying the electron transfer rate constant k_0 , due to the irreversible heterogeneous nature of the electrode reaction at nanometer-size electrodes,¹⁰ a wider range of polarization potential from -0.7 V to +0.4 V is used. The top boundary of the model is assumed to be electrical neutral with $E_b = 0$. These equations are solved computationally over the geometric domains defined in Figure 3.1 by the commercial FEA package COMSOL Multiphysics (Burlington, MA). For this study, the parameters listed in Table 3.1 are used. Prior to all the analyses, a mesh refinement study is performed to identify a proper mesh for assuring a converged result.

3.3. The model validation

For a simulation analysis, validation of the obtained results is important. Thus, we first took some steps to validate the nanometer single electrode models. Besides the mesh refinement (data not shown), the limiting current density obtained for a nanometer electrode governed by a diffusion process (i.e., the effect of EDL is not considered) is compared with the theoretical prediction (Bard and Faulkner, 2001):

$$i_{dL} = \frac{FD_{O}c_{O}^{b}}{(r_{0} + \mu)}$$
(3.8)

Where D_O is the diffusivity of the reactant and c_O^b is its concentration in the bulk solution. A difference of about 0.1% is found from this comparison, which suggests a very good agreement between the simulated and theoretical results when the effect of EDL is ignored.

After that, the simulated cyclic voltammetric (CV) responses are compared with those by He *et al.* 2006. Figure 3.3A shows two simulated CV curves, normalized to the limiting current obtained under a diffusion process (Eq.3.8), for a nanometer electrode when the effect of EDL is considered. Since a closed-form solution is not available in He *et al.*, we can only match the range and scale of the graphs and compare them visually. From this comparison, we find that the CV curves obtained from our simulation are close to those by He *et al* (He, Chen et al., 2006).

D_O, D_R	$1 \times 10^{-9} (m^2/s)$	$E^{0'}$	0 (V)
D_A	$1.33 \times 10^{-9} (m^2/s)$	Т	298 (K)
D_B	$2.03 \times 10^{-9} (m^2/s)$	80	8.85419×10 ⁻¹² (F/m)
D_{Ct}	$2.0 \times 10^{-9} (m^2/s)$	ε _l	6
$c^b_{\scriptscriptstyle A}, c^b_{\scriptscriptstyle B}$	500 (mol/m ³)	ε_2	78
c^{b}_{Ct}	$ z \cdot c_o^b (\mathrm{mol/m}^3)$	S_1	3.6
c_O^b	5 (mol/m ³)	S_2	3.05
c_R^b	0 (mol/m ³)	l_1	0.4 (nm)
α	0.5	$\mu = l_1 + l_2$	0.7 (nm)
F	9.64853×10 ⁴ (C/mol)	Z	-3, -2, -1, +1, +2, +3
k_0	10 (m/s)	r_0	1~200 (nm)
R	8.31 (J/K)		

Table 3.1 Material constants, and kinetic and geometric parameters used in the simulation. (Bard AJ and Faulkner LR 2001; Levine S et al. 1974)

Note: c_i^b represents the concentration of species *i* in the bulk electrolytic solution.

To further validate the simulation results, the radial concentration distribution of some selected ions at three different electrode potentials: $E - E^{0'} = 0.25V$ (at which $i/i_{dL} = 0$), $E - E^{0'} = 0.05V$ ($i/i_{dL} \approx 0.12$), and $E - E^{0'} = -0.25V$ ($i/i_{dL} \approx 1$) is examined. For the electroinactive ion A⁺ (Figure 3.3B) its concentration follows closely a Boltzmann distribution in all three cases. For the electroactive reactant (species O^z ; Figure 3.3C), however, the concentration follows a Boltzmann distribution only when the net current is zero (at $E - E^{0'} = 0.25V$; note that the solid and dash curves overlap completely), and it deviates significantly from the corresponding Boltzmann distribution when there is a net current flowing (at both $E - E^{0'} = 0.05V$ and $E - E^{0'} = -0.25V$). These results are as expected. In the presence of a net Faraday current, the electron transfer at the electrode will cause redox activities, which in turn will disrupt the thermodynamic equilibrium

state of the electroactive ions. Thus in this situation, a Boltzmann distribution for the reactant will not be maintained. These electroinactive ions and the reactant ion under a zero current condition, however, are able to maintain their thermodynamic equilibrium states, thus their concentrations are expected to follow Boltzmann distributions.



Figure 3.3 (A) Simulated CV curves for a spherical electrode of $r_0 = 1$ nm when $z = \pm 1$. The curve labeled "Diffusion" represents the case in which the effect of EDL is neglected; (B) Concentration distribution of species A⁺ at various electrode potentials of $E - E^{0'} = 0.25$ V ($i/i_{dL} \approx 0$), $E - E^{0'} = 0.05$ V ($i/i_{dL} \approx 0.12$), and $E - E^{0'} = -0.25$ V ($i/i_{dL} \approx 1$); (C) Concentration distribution of the reactant (species O^2) under the same conditions as in (B); (D) The deviation of the simulated concentration for the reactant from its corresponding Boltzmann distribution at a high Faraday current ($E - E^{0'} = -0.25$ V, z = -1). Note that the simulated result matches exactly the closed-form solution given in He *et al.*

As a final step of validation, the deviation of the simulated concentration for the reactant from its corresponding Boltzmann distribution at a high Faraday current ($i/i_{dL} \approx 1$) is calculated and compared with the closed-form solution given in Reference He *et al.* 2006 (see Eq.16). As shown in Figure 3.3D, our simulated result matches exactly that of He *et al* 2006. With these validation steps, we are confident that the modeling procedures are valid and the obtained results are accurate.

3.4. Results and discussions

3.4.1. Simulation results for nano single electrodes

Figure 3.4 shows the CV curves (normalized to their corresponding i_{dL} calculated from Eq.8) for electrodes of various sizes when $z = \pm 1$. All the CV curves exhibit a sigmoidal shape (see Insert-1 in Figure 3.4), and the curves with z = 1 have their normalized limiting current higher than unity while the curves with z = -1 have the normalized limiting current lower than unity. This is so because at a negative (reduction) electrode potential the positive-charged reactant would experience attraction and the negative-charged reactant repulsion, thus causing the current to be either enhanced or suppressed in the respective conditions. Insert-2 in Figure 3.4 shows how the size of the electrodes affects the limiting current: the smaller the electrode becomes the more the normalized limiting current deviates from unity, and such an EDL induced current change becomes negligible when the size of the electrode is sufficiently large (>100nm).

To elucidate the reasons for such a size effect of EDL, the radial distributions of the electrical potential and reactant concentration inside as well as outside the compact layer are examined for the two electrodes with radii of 1nm and 100nm at $E-E^{0'} = -0.25$ when z = -1. As shown in Figure 3.5A, the potential drop inside the compact layer is indeed electrode-size

dependent. Although the overall potential drop is more for the 1nm electrode than for the 100nm electrode, the rate of drop for the larger electrode exceeds that for the smaller one as it gets closer to the PET. The potential drop outside the compact layer is shown in Figure 3.5B (solid curves), where the scale of the horizontal axis has been normalized to δ , which measures the radial distance from the PET to a point where the concentration of the O^z ion reaches 90% of its bulk value. Clearly the potential drops to zero in a distance that is much shorter for the larger electrode than for the smaller electrode. Here the region of the electrolytic solution having a non-zero electrical potential, in which cations (or anions) will be in excess and anions (or cations) will be in deficiency, is defined as the ionic diffuse layer.



Figure 3.4 A zoom-in view of the reduction end of the simulated CV curves for electrodes of various sizes. Insert-1: the entire CV curves; Insert-2: variation of the normalized limiting current with the electrode size (from 1nm to 200nm).

Also plotted in Figure 3.5B is the concentration distribution (dash curves) for the reactant. In both cases, the reactant is completely depleted at the PET, creating a region of concentration

gradient. Here we term the region of concentration gradient with a thickness of δ (defined above) as the diffusion layer of the reactant. The thickness of the diffusion layer is measure to be $\delta_{r0=1}$ =14.15nm for the smaller electrode and $\delta_{r0=100}$ =819.77nm for the larger electrode. It is thus clear that the diffuse layer for the smaller electrode has a thickness about 13% of its diffusion layer, while the thickness of the diffuse layer for the larger for the larger electrode is only 0.55% of its diffusion layer. Thus the EDL structure at the smaller electrode has caused its diffuse layer to expand deeper into the diffusion layer as compared with the larger electrode. As a result, the current response of the 1nm electrode is significantly altered, while the change in the current response for the 100nm electrode is negligibly small.

In an electrochemical system a supporting electrolyte plays an important role in minimizing solution resistance, reducing the contribution of electromigration, and compensating the potential drop.13 Thus it is important to know how a supporting electrolyte influences the effect of EDL. Figure 3.6 shows the CV curves for two cases: one is with an excess amount of (500mM) and the other is without (0mM) the supporting electrolyte of A+B. Clearly, the effect of EDL on the current response is significantly magnified when the supporting electrolyte is absent in the solution. By examining the thickness of the diffuse layer, it is clear that (see insert; solid curves) the diffuse layer for the case without the supporting electrolyte expands over almost the entire diffusion layer, while the diffuse layer for the case with the supporting electrolyte expands only a very shorter distance into the diffusion layer. These results confirm that the expansion of the diffuse layer deep into the diffusion layer is responsible for the change in the CV current response. The distribution of the reactant concentration (see insert; dash curves) for the two cases differs slightly. In the case having a wider diffuse layer (i.e., the case without the supporting electrolyte) the negatively charged reactant is expected to experience more repulsion in the

diffuse layer, thus more of the reactant species gets pushed away from the electrode surface, resulting in a lower reactant concentration in the vicinity of the electrode surface as compared with the case with the supporting electrolyte.



Figure 3.5 (A) Potential drop across the compact layer for two electrodes with radii of 1nm and 100nm; (B) Potential drop (solid curves) and reactant concentration (species O^z ; dash curves) outside the compact layer. In both cases, $E - E^{0'} = -0.25$ and z = -1. Note that $\delta_{r0=1} = 14.15$ nm and $\delta_{r0=100} = 819.77$ nm.
From this study we can draw the following conclusions. As the size of the electrode decreases to single nanometers, the EDL structure will cause altered current response at the electrode due to the expansion of the diffuse layer into the diffusion layer, and this effect can be neglected when the size of the electrode becomes sufficiently large (e.g., >100nm).



Figure 3.6 Simulated CV curves for an electrode of $r_0=1$ nm when an excess of (500mM) and no (0mM) supporting electrolyte is present in the solution. Insert: potential drop and reactant concentration (species O^z) outside the compact layer at $E - E^{0'} = -0.25$ and z = -1. Note that $\delta_{500mM} = 14.15$ nm and $\delta_{0mM} = 11.84$ nm.

3.4.2. Simulation results for nano IDEs

Figure 3.7 shows the effect of varying w_{gap} on the voltammetric limiting current and the potential distribution between the generator and collector at r = 1 nm, $\mu = 0.7$ nm, and $k_0 = 0.1$ m/s. The limiting current (Figure 3.7A) at both the generator and collector of nano-IDEs decreases as w_{gap} increases, as is the case with micro-IDEs (Bard, Crayston et al., 1986; Niwa, Morita et al., 1990). This, however, is different from that of micro-IDEs where the limiting

current of nano-IDEs is significantly dependent on the charge valence -- the limiting current of a positive active ion is enhanced and that of a negative active ion is slightly suppressed as compared with the diffusion controlled case in which the effect of the EDL is ignored. Moreover, the deviation of the EDL-influenced limiting current from the diffusion limited current increases as w_{gap} decreases. The potential distribution between the generator and collector electrodes (along the dotted line in Figure 3.1B) at w_{gap} = 4 nm and 16 nm explains why this happens (Figure 3.7B). When w_{gap} is large (16 nm), the electrical potential (dotted line) drops quickly to zero in the inter-electrode space in both $i/i_{dL}\approx 0$ and $i/i_{dL}\approx 1$ conditions when the concentration of the supporting electrolyte is in excess (500 mM), where i_{dL} represents the limiting current in a diffusion-only case in which the EDL is not considered (Note that $i/i_{dL}\approx 0$ is reached at $E_G = 0.3$ V and $E_C = 0.3$ V, and $i/i_{dL} \approx 1$ at $E_G = -0.4$ V and $E_C = 0.3$ V). In this case the electrical fields of the generator and collector hardly overlap, while when w_{gap} is small (4 nm), the electric fields (solid lines) between the generator and collector overlap with each other causing the potential in the inter-electrode space to barely approach zero only in the center region, suggesting a higher intensity of electric field occurring at nano-IDEs with smaller w_{gap} . As a result, the voltammetric limiting current deviates significantly from the diffusion limited case when w_{gap} is small as is observed in Figure 3.6A. These results suggest that deviation of the limiting current for a charged species from that for a diffusion controlled case is affected by the degree of EDL overlap.



Figure 3.7 (A) Variation of the voltammetric limiting current with w_{gap} along with the result from the diffusion-only case as a reference. (B) Distribution of the electrical potential in the inter-electrode space at two conditions: $i/i_{dL} \approx 0$ and $i/i_{dL} \approx 1$. Note that the concentration of the supporting electrolyte is 500mM for all cases shown here and $z=\pm 1$ represents the charge valence of the oxidized species. $k_0 = 0.1$ m/s.

The significant impact of the presence or absence of the supporting electrolyte on the voltammetric current response of the nano-IDEs when the EDL is considered further supports the

above argument. As shown in Figure 3.8A, when $i/i_{dL} \approx 0$ the potential drops to zero in the center region when the supporting electrolyte is in excess, and it drops to about 80 mV when the supporting electrolyte is absent; when $i/i_{dL}\approx 1$ the potential reaches zero in the center region when the supporting electrolyte is in excess, but it varies almost linearly passing through zero when the supporting electrolyte is absent. These results suggest that when the supporting electrolyte is absent, the diffuse layers of the generator and the collector will overlap with each other significantly resulting significantly increased electric field intensity. Figure 3.8B shows the consequence of this severe diffuse layer overlap: when the supporting electrolyte is in excess (500 mM) the voltammetric current of the nano-IDEs differs slightly from that of the diffusiononly case for both the negative and positive redox ions, however, when the supporting electrolyte is absent (0 mM), the voltammetric current for the z = -1 case takes a peak shape rather than a sigmoid, and that for the z = +1 case (Figure 3.8B) varies almost monotonically. Moreover, the observed difference of the voltammetric curve shape between charged species and diffusion controlled case is much larger at nano-IDEs than at a single nanometer electrode,¹⁰ indicating that due to the severe diffuse layer overlap in the absence of supporting electrolyte the effect of the EDL on the voltammetric current response drastically amplified for the nano-IDEs when compared with that for single nanometer electrodes.



Figure 3.8 (A) Distribution of the electrical potential in the inter-electrode space at two conditions: $i/i_{dL} \approx 0$ and $i/i_{dL} \approx 1$ when an excess (500mM) amount of or no (0mM) supporting electrolyte is present in the solution. (B)Voltammetric current responses for the nano-IDEs when an excess (500mM) amount of or no (0mM) supporting electrolyte is present in the solution for a positively charged redox species z = -1 and a negatively charged redox species z = +1 (inset). In all cases, $w_{gap} = 4$ nm. $z = \pm 1$ is the charge valence of the oxidized species.

According to our study on single nano electrodes, the thinner the compact layer thickness is, the thicker the diffuse layer and the thinner the diffusion layer become. At nano IDEs, a similar phenomenon is expected and observed. In Figure 3.9A, the potential and concentration profiles at the generator electrode are plotted at different compact layer thickness 0.7nm and 0.35nm, where scale of the horizontal axis has been normalized to δ , which measures the vertical distance from the PET to a point where the concentration of the O^{z} ion reaches 90% of its bulk value (Note that the thickness of the diffusion layer is measured as $\delta_{\mu=0.35} = 2.50 \ \mu m$ and $\delta_{\mu=0.7} = 2.92 \ \mu m$, respectively). Because a thicker diffuse layer is observed for the case with μ =0.35 nm than for $\mu=0.7$ nm, we expect a more severe EDL effect at $\mu=0.35$ nm than at $\mu=0.7$ nm due to higher intensity of electric field. However, at nano-IDEs, another factor has to be considered -- when the compact layer thickness decreases, the distance between PETs of both generator and collector electrodes (d_{PET}) increases, which imposes a negative impact on the collection efficiency. The net outcome of the compact layer thickness change is therefore dependent on the fact that which of the two effects dominates: the value of d_{PET} or the intensity of electric field. Figure 3.9B and 3.9C show that the thinner the compact layer, the lower the limiting current response for both z =-1 and z = +1 cases regardless the higher intensity of electric field at nano IDEs with smaller μ_{i} suggesting that in this case the enhanced electric field is not large enough to dominate the effect of d_{PET} change.



Figure 3.9 Voltammetric current responses of the nano-IDEs at $r_0 = 1$ nm with different thickness of the compact layer for a negatively charged redox species (A: z =-1) and a positively charged redox species (B: z =+1). (C) Potential drop and reactant (species O^z) concentration outside the compact layer of the generator electrode (along the dashed line in Figure 3.1) at $E_G = -0.4$ and z = -1. Note that $\delta_{\mu=0.35} = 2.5 \ \mu\text{m}$ and $\delta_{\mu=0.7} = 2.92 \ \mu\text{m}$.

Figure 3.10A and 3.10B shows the effect of the electrode size on the voltammetric performance of nano-IDEs with the influence of the EDL. It is seen that the limiting current

density decreases with increasing electrode size as what was seen for micro electrodes(Yang and Zhang, 2007). Also, difference of the limiting currents between the z = -1 case and the z = +1 case decreases with increasing of the electrode size at a constant gap spacing as what we saw in nano single electrode. Figure 3.10C plots the electrical potential and reactant concentration outside the compact layer for the nano-IDEs with electrode radii of 1 and 50 nm at when z = -1 when $i/i_{dL} \approx 1$ ($E_G = -0.4$ V), where the scale of the horizontal axis has been normalized with respect to the diffusion layer thickness δ , which measures the vertical distance from the PET to a point where the concentration of the O^r ion reaches 90% of its bulk value. As is the case in nano single electrode, the EDL structure at the smaller electrode has caused its diffuse layer to expand deeper into the diffusion layer as compared with the larger electrode. As a result, the EDL effect is less severe at r = 50 nm than at r = 1 nm, and the limiting currents for the z = -1 case and the z = +1 case are more close to that for diffusion controlled case at the 50 nm IDEs than at 1 nm IDEs (Figure 3.10D).

We then studied the relationship between the voltammetric limiting current of the nano-IDEs and the electron transfer rate constant (k_0) with considering the effect of EDL when the concentration of the supporting electrolyte is in excess (500 mM) (see Figure 3.11). As the value of k_0 decreases the limiting current decreases, but the decrease is slower for the z = -1 case than for the z = +1 case. This phenomenon is attributed to the combined influence of the electrical force experienced by the ionic species and the electron transfer rate constant. At a negatively polarized generator electrode, a negatively charged oxidized ion (O^{-1}) experiences a repulsive force and a positively charged oxidized ion (O^{+1}) an attractive force, thus more positively charged oxidized ions than negatively charged ones will be reduced at the generator. But at a positively biased collector ($E_C = 0.3$ V), the reduced ion R^0 (for z = +1) is electrically neutral thus experiencing no electrical force, while the reduced ion R^{-2} (for z = -1) is negatively charged thus experiencing a higher attractive force. When the rate constant k_0 is high ($k_0 = 0.1 \text{ m/s}$) the reaction at the generator dominates the one at the collector, thus making the voltammetric limiting current higher for the z = +1 case than for the z = -1 case. When k_0 is low ($k_0 = 0.0001$ m/s), the electron transfer is significantly slowed at both the generator and the collector, thus both species O^z and R^{z-1} are tending to escape to the bulk solution. However, due to the experience attractive force for R^{-2} ion (z = -1), the collection R^{-2} ion is heightened, which makes the reaction at the collector electrode more dominating. As shown in Figure 3.11B, when k = 0.001m/s the concentration of R species is much higher at z = -1 than at z = +1, but when k = 0.1m/s the difference of R species concentration is diminishing. As a result, the increase in collection efficiency from the z = 1 case to the z = -1 case is much higher at $k_0 = 0.0001$ m/s (from 97.52% to 99.54%) than at $k_0 = 0.1$ m/s (from 99.92% to 99.94%), and the limiting current for the z = -1 case becomes higher than that for the z = +1 case when $k_0 = 0.0001$ m/s.

To quantify the sensing performance of the nano-IDEs under the influence of the EDL, we performed regression analyses using the four-parameter sigmoidal curve (Equation 2.7). The resolved parameters from the regression analyses are listed in Table 3.2. It can be seen that the z = -1 case has a lower upper asymptote (*a*) and lower sensitivity (*S*) with a broader responsive band width (*RB*), whereas the z=+1 case has a higher upper asymptote (*a*) and higher sensitivity (*S*) with a narrower responsive bandwidth (*RB*). These results suggest that when the generator is negatively polarized, the limiting current response, the sensitivity, and the upper responsive limit can be significant enhanced by using redox species with positive charges.



Figure 3.10 Voltammetric current responses for the nano-IDEs of various electrode size for a negatively charged redox species (A: z = -1) and a positively charged redox species (B: z = +1). (C) Potential drop and reactant (species O^z) concentration outside the compact layer of the generator electrode (along the dashed line in Figure 3.1) at $E_G = -0.4$ and z = -1. Note that $\delta_{r0=1} = 2.92 \ \mu m$ and $\delta_{r0=50} = 159.3 \ \mu m$. (D) Variation of the normalized limiting current with the electrode size. In all cases, the concentration of the supporting electrolyte is 500 mM, and $w_{gap} = 4 \ nm$. $z = \pm 1$ is the charge valence of the oxidized species.

By comparing the current results with that of micro-IDEs, we can see that at nano-IDEs the upper asymptote (a), the sensitivity (S) and the lower and upper responsive limits all increase, and the center of the response range (x_0) shifts right and the responsive bandwidth (RB) decreases. These results suggest that there will be significant enhancements in the limiting

current response, the sensitivity, and the upper responsive limit at nano-IDEs compared with micro-IDEs. Moreover, according to the simulation results in Chapter 3 without considering the EDL effect, the responsive bandwidth decreases with electrode size, while at nano-IDEs with considering the EDL effect, we noticed that the responsive bandwidth for negatively charged species is even higher than that at 10µm IDEs, and the responsive bandwidth for positively charged species is lower than that at 100nm IDEs. These results suggest that when a generator is swept towards negative potential, a wider bandwidth can be achieved at nano-IDEs by using a negatively charged redox species.

Table 3.2 Parameters resolved from the statistical regression analyses (note that a $log(k_0)$ value of -3.04 reflects a k_0 value of $10^{-3.04}$ m/s; G=generator, and C= Collector)

	z = -1		z = +1	
	G	С	G	С
Upper asymptote ($a: A/cm^2$)	46.22	46.00	52.72	52.27
Center of the responsive range ($x0$: log(m/s))	-3.47	-3.48	-2.84	-2.85
Sensitivity (S: A/cm ² /log(m/s))	21.14	-21.27	32.22	-32.62
Responsive bandwidth (RB: log(m/s))	2.19	-2.16	1.64	-1.60
Lower responsive limit ($x0 - RB/2$: log(m/s))	-4.57	-2.40	-3.66	-2.04
Upper responsive limit (x0 + RB/2: log(m/s))	-2.38	-4.68	-2.02	-3.87

The analysis presented above has shed some important insight into the effect of the EDL on the electrochemical processes of nano-IDEs. It should be pointed out that the present model possesses some limitations. All ionic species are treated as volumeless point charges. In this case, ionic density adjacent to the electrode would be considerably higher than in reality where the size of ions is finite. Moreover, the influence of the hydrodynamic interactions among ionic



Figure 3.11 (A) Variation of voltammetric limiting current with electron transfer rate constant in logarithm, log (k_0), for cases with $w_{gap} = 4$ nm. (B) Reactant (species Rz-1) concentration outside the compact layer of the collector electrode (along the dashed line in Figure 3.1) for both z = +1 and z = -1 cases at $k_0 = 0.001$ m/s. Inset: Reactant (species Rz-1) concentration outside the compact layer of the collector electrode (along the dashed line in Figure 3.1) at $k_0 = 0.1$ m/s. In all cases shown here, the concentration of the supporting electrolyte is 500 mM and $z = \pm 1$ is the charge valence of the oxidized species.

species near the electrodes is ignored. Both these treatments could lead to an overestimate for the limiting current, especially when the reactant concentration is high.(Krapf, Quinn et al., 2006) This model is also limited by the assumption of perfectly smooth surfaces for the electrodes. In reality solid surfaces are rarely smooth, especially on a nanoscopic scale. Another limitation is that the chemical heterogeneities induced by specific adsorption at the electrode surface is neglected.(Duval, Leermakers et al., 2004; Lyklema and Duval, 2005) Future efforts should be devoted to address these issues.

3.5. Conclusion

In this chapter, we presented a completely computational method for investigating the effect of the EDL on the voltammetric performance of nano-IDEs in the presence of a redox electrode reaction. It is found that for nano-IDEs, the EDL structure will alter their voltammetric current response due to the overlap of the diffuse layers of the generator and collector electrodes. This effect is dependent on the gap spacing between the generator and collector, the size of the electrode, the charge valence of the redox species, the electron transfer rate constant, the presence of the supporting electrolyte, and the thickness of the compact layer.

Compared with micro- and submicro- IDEs, we see significant enhancements in the limiting current response, the sensitivity, and the upper responsive limit at nano-IDEs. Moreover, we observe a unique feature at nano-IDEs that the responsive bandwidth differs with redox charge valence. When redox species are positively charged, the responsive bandwidth of nano-IDEs is much smaller than that of 100nm IDEs, while when redox species are negatively charged, the responsive bandwidth of nano-IDEs is even larger than that of 10µm IDEs. These results suggest that a different sensing performance can be achieved by using redox species with different charge valences.

CHAPTER 4

CV MEASUREMNT AT IDES

4.1. Introduction

In previous two chapters, we simulated the CV performance for micro and nano IDEs, and studied the relationship between CV response of IDEs and electron transfer rate in order to explore the ways to improve the performance of affinity based sensor. The simulation results shows that the CV responses of micro or submicron IDEs are more sensitive to k_0 value change than that of single electrodes. For micro IDEs the relationship between CV limiting (or peak) current and k_0 value is strongly dependent on the electrode size and spacing, and for at nano IDEs, this relationship also varies with charge valence of the redox species.

In order to confirm the simulation results that micro IDEs have better performance than single electrode when used as affinity sensor, in this chapter, we will first discuss the measured CV performance of IDEs with 4.25 µm electrode size and gap spacing in a generator-collector mode and a single-electrode mode. Then, we will present some results from successively modifying the electrode surface of IDEs using probe molecules of sulfo-NHS-SS-Biotin SAM and detecting the target molecules avidin at various concentrations. The CV responses are measured stepwise after each modification of electrodes.

The selection of a biomolecule immobilization method for a specific redox species is critical, and the following are reasons that we choose sulfo-NHS-SS-Biotin SAM and avidin as probe and target molecules in the present study. The most often used method for biomolecule immobilization is to first functionalize electrode surface by 11-mercaptoundecanoic acid (MUA)

or 3-mercaptopropionic acid (MPA) self-assembled monolayer (SAM) (Cui, Pei et al., 2003; Yam, Deluge et al., 2006), and then add a layer of biomolecules on top of the SAM. By this approach, the surface of the electrodes will be severely blocked because the size of MUA or MPA molecules is so small that the density of the immobilized molecules will be very high and the CV response of the functionalized electrode extremely low (see Figure 4.1). To avoid this



Figure 4.1 (A) The procedure of immobilizing biotin on top of gold disc electrode through link molecules. (B) The CV response at bare gold electrode (a), MPA SAM functionalized gold electrode (b), biotin immobilized gold electrode (c).

severe blockage, in this study, we functionalize IDEs directly by using sulfo-NHS-SS-Biotin without using any linker molecules (see Figure 4.2). Once sulfo-NHS-SS-Biotin is in the water, biotinylated thiol species and o-mercapto propionic acid will be modified on the gold surface because the N-hydroxysulfosuccinimide moiety in the Sulfo-NHS-SS-Biotin is easily hydrolyzed by water. Due to the large size of sulfo-NHS-SS-Biotin compared with MUA/MPA SAM, the density of immobilized molecules will not be high. This will lead to a higher electron transfer rate (Hideki Kuramitz, 2000). From the simulation results in Chapter 3, we can see that the CV responses of IDEs are more sensitive to the change of k_0 at a larger k_0 value. Thus for this reason we chose to use sulfo-NHS-SS-Biotin SAM as our probe molecules.

Besides the biomolecule immobilization method, the k_0 value is also affected by the type of redox species. The experimentally measured electron transfer rate of $Fe(CN)_6^{3-/4-}$ at bare gold is about $10^{-3} \sim 10^{-5}$ m/s. After MUA functionalization, this value could drop to around 10^{-9} . By comparing Figure 4.1 with Figure 4.2, we estimate that after biotin-SAM functionalization the electron transfer rate is much higher than 10^{-9} . According to our simulation results, the linear range of biosensing is from $10^{-5.37}$ m/s to $10^{-7.49}$ m/s for IDEs with 10 µm electrode size and spacing, and $10^{-4.45}$ m/s to $10^{-6.27}$ m/s for IDE with 1 µm electrode size and spacing. This means that the linear range is about 10^{-5} m/s to 10^{-7} m/s for IDEs with 4.25 µm electrode size and spacing. Therefore, we decide to use $Fe(CN)_6^{3-/4-}$ as our redox species. In this case, the range of electron transfer rate will be from 10^{-3-5} down to 10^{-7-8} when we detect avidin by biotin-SAM modified gold electrode.



Figure 4.2 (A) The Scheme of Sulfo-NHS-SS-Biotin molecule. (B) The procedure of directly immobilizing biotin on top of gold disc electrode. (C) The CV response at bare gold electrode (a), biotin-SAM functionalized gold electrode (b), Avidin immobilized gold electrode (c).

4.2. CV measurement at micro IDEs

4.2.1. Apparatus and experiment set-up

For the fabrication procedure for IDEs, please refer to the appendix. After fabrication, wiring of the proper electrodes of the IDEs along with copper tapes (Cinta aisladora) is made using a wire bonder (Model 4524, Kulicke & Soffa Industries, Inc.) (Figure 4.3). The contact pads of the IDEs are covered by Micro Shield (Tolber Division, Pyramid plastics, Inc.) to insure the insulation. All voltammetric measurements were carried out using a Multistat 1480 analyzer



(Advanced Measurement Technology, Inc, United Kindom). A three-electrode electrochemical

Figure 4.3 The contact pad on the gold electrode (A) and on the copper tape (B).

system is used in all experiments. A platinum wire is used as a counter electrode, and a calomel reference electrode (Thermo Fisher Scientific Inc.) as a reference electrode (see Figure 4.4).

When IDEs are connected in a generator-collector mode, two channels of Multistat 1480 analyzer are used simultaneously. The working electrode from one channel is connected to the generator and swept cyclically from 0V to 0.6V, and the working electrode from the other channel is connected to the collector and held at -0.1V. All potentials were measured against the reference electrode. The pH value of the PBS solutions is measured with a Horiba pH meter M-13.





Figure 4.4 (A) Experimental setup. (B) Schematic expression of experimental setup.

4.2.2. Reagents

PBS of pH 7.0 is prepared with 0.1 M HCl and 0.1 M NaOH. High purified nitrogen gas is used for deaerating the solution during electrochemical measurement. All reagents are analytical grade and diluted, if necessary, with deionized water.

4.2.3. Results and discussions

Figure 4.5 shows the CV response of IDEs in a single-electrode mode (Figure 4.5A) and that of Au/Ti micro IDEs in a generator-collector mode (Figure 4.5B) at scan rate of 50mV/s and 20mV/s. It can be seen that CV response of micro IDEs in a generator-collector mode is almost 2 times higher than that of IDEs in a single-electrode mode. At different scan rates, the CV curves of IDEs in a generator-collector mode are very close to each other while the CV curves of IDEs

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in a single-electrode mode have much higher peak current at a higher scan rate. It is also noticed that the charging current at collector electrodes is much smaller than that at generator electrodes.



Figure 4.5 The CV response of Au/Ti micro IDEs with $4.25\mu m$ electrode size and spacing in a single-electrode mode (A), and that in a generator-collector mode (B) at scan rate of 50mV/s and 20mV/s

4.3 Avidin Detection by Biotin-SAM functionalized micro IDEs using the CV method

4.3.1. Materials and Methods

Sulfo-NHS-SS-Biotin was obtained from Pierce Rockford (IL, USA). Avidin, potassium ferrocyanide, potassium ferricyanide are purchased from Sigma-Aldrich, Inc. Prior to running the experiments, the gold IDEs were cleaned by an electrochemical cleaning procedure: applying the potential between -0.2V and 0.6mV at IDEs until a reproducible voltammogram was obtained. The cleaned electrodes were then immersed overnight at 4°C in 0.1 M phosphate buffer solution (pH = 7.0) including 2.5 mM Sulfo-NHS-SS-Biotin. In this case, biotinylated thiol species and omercapto propionic acid were immobilized onto the gold electrode surface due to the hydrolyzation of the because the N-hydroxysulfosuccinimide moiety in the Sulfo-NHS-SS-Biotin. During incubation, extra DI water was placed in Petri dish surrounding the IDEs, and the Petri dish was sealed by Parafilm "M" laboratory film (Pechiney Plastic Packaging) to avoid drying of the biotin solution (see Figure 4.6). To detect avidin, the biotin-SAM modified IDEs are incubated in 0.1 M PBS (pH = 7.0) containing various concentration of avidin for at least 20 min. And then 0.1 M phosphate (pH = 5.5) containing 10mM $Fe(CN)_6^{3-1/4-}$ was used to measure the CV response. At this pH value, the Avidin is positively charged, thus increasing the local concentration of negatively charged redox species $Fe(CN)_6^{3-/4-}$ by electrical attraction (see Figure 4.2). Prior to each voltammetric measurement, the solution is deaerated for 15 min with nitrogen gas. The potential scan for CV was conducted for a potential range from 0 to 600mV at a sweep rate of 50mV (Hideki Kuramitz, 2000).



Figure 4.6 Functionalizing IDEs with biotin-SAM.

4.3.2. Results and discussions

Figure 4.7 shows the CV curves measured during the stepwise surface modification of the micro IDEs in a single-electrode mode and in response to avidin at different concentrations. Clearly, a bare gold IDEs in a single-electrode mode exhibits a peak-shaped CV curve for the oxidation and reduction of $[Fe(CN)_6]^{3-/4-}$. After the biotin-SAM layer is adsorbed to the electrode surface, the peak-shaped CV curve exhibits much reduced peak currents and an increased peak-to-peak separation, suggesting blockage for electron transfer due to biotin adsorption. After Avidin is absorbed on the biotin functionalized IDEs, the redox peak slightly increases with the concentration of avidin, suggesting that the steric hindrance of the biotin-SAM against the redox species was canceled by the entrapment of $[Fe(CN)_6]^{3-/4-}$ ions in the avidin

membrane because of the electrostatic attraction between negatively charged ferrocyanide ions and the positively charged avidin on the electrode surface.



Figure 4.7 Cyclic voltammograms of 10mM $[Fe(CN)_6]^{3-/4-}$ bare Au IDEs in a single-electrode mode, biotin-SAM electrode, and avidin modified electrode in 0.1M phosphate buffer solution (pH 5.5). Scan rate: 50mV=s.



Figure 4.8 (A) CV of 10mM $[Fe(CN)_6]^{3^{-/4^-}}$ at bare Au, biotin-SAM functionalized , and avidin modified IDEs in a generator-collector mode in 0.1M phosphate buffer solution (pH 5.5). (B) CV of 10mM $[Fe(CN)_6]^{3^{-/4^-}}$ at avidin modified IDE. The concentration of avidin varies from 1µg/mL to 5mg/mL. Scan rate: 50mV=s.

Figure 4.8 and 4.9 respectively shows the CV measurements at generators and collectors during the stepwise surface modification of the micro IDEs in a generator-collector mode and in response to avidin at different concentrations. At both generator and collector electrodes, the bare gold IDEs exhibits a sigmoidal shaped CV curve for the oxidation and reduction of $[Fe(CN)_6]^{3-/4-}$, and the limiting current value is almost 2 times higher than when IDEs is used in a single-electrode mode, suggesting enhanced mass transport by redox cycling between generator and collector. After the biotin-SAM layer is adsorbed to the electrode surface, the limiting current value is still higher than that of IDEs in a single-electrode mode (see Table 4.1). After avidin is absorbed on the biotin functionalized IDEs, similar to the case when IDEs are in a single-electrode mode, the redox peak slightly increases with the concentration of avidin.



Figure 4.9 (A) CV of 10mM $[Fe(CN)_6]^{3-/4-}$ at bare Au, biotin-SAM functionalized , and avidin modified IDEs in a generator-collector mode in 0.1M phosphate buffer solution (pH 5.5). (B) CV of 10mM $[Fe(CN)_6]^{3-/4-}$ at avidin modified IDE. The concentration of avidin varies from 1µg/mL to 5mg/mL. Scan rate: 50mV=s.

To further quantify the sensing performance of IDEs in a single-electrode mode and in a generator-collector mode, we analyzed the relationship between the limiting (or peak) current of CV curve and avidin concentration by a logarithmic regression analysis (Equation 4.1).

$$y = y_0 + \frac{a}{1 + \exp[-(x - x_0)/b]}$$
(4.1)

where x represents the concentration of avidin, y represents CV limiting (or peak) current, y0 is the intercept when x = 1, and *a* is the slop or sensitivity. Figure 4.10A shows the variation of limiting (or peak) current of CV curve with the concentration of avidin (from 1µg to 1mg/mL) along with the corresponding regression lines. By taking the slope of the regression lines, we obtained sensitivity values 6.27 µA/ (mg/mL) vs. 22.18 µA/ (mg/mL) for the IDEs in a singleelectrode mode vs. that in a generator-collector mode. Clearly, the IDEs in a generator-collector mode shows sensitivity about 3.53 times higher than the IDEs in a single-electrode mode.

Table 4.1 Limiting or peak current of IDEs in a generator-collector mode or in a single-electrode mode.

	Limiting (peak) current (µA)		
	Generator-collector mode	Single-electrode mode	
Bare electrodes	17.796	8.4	
Biotin-SAM modified	8.495	3.22	
Avidin (1µg)	12.96	4.32	
Avidin (10µg)	14.025	4.785	
Avidin (100µg)	15.315	5.271	
Avidin (500µg)	16.56	6.166	
Avidin (1mg)	18.911	7.49	



Figure 4.10 (A) Variation of the CV limiting (or peak) current with concentration of avidin (in logarithmic scale from 1µg to 1mg). The regression analysis is based on Equation 4.1. (B) Variation of the CV peak current density at single electrode with $log(k_0)$ at 20mV/s and at 50mV/s (Simulation results).

4.3.3. Conclusion

In this study, we confirmed, by an experimental means, that the CV response of IDEs in a generator-collector mode is more sensitive to the surface modification than in a single-electrode mode. At IDEs with 10 pairs of electrodes, 4.25 µm electrode width and 4.25 µm gap spacing, the sensitivity of CV response is 3.54 times higher in a generator-collector mode than in a single-electrode mode. These results seem slightly lower than the simulation estimation, because in the simulation, we consider a unit cell instead of a whole device. This unit cell model represents an infinity number of electrode pairs, thus the edge effect can not be taken into be taken into account in the simulation. Additionally, in the simulation, the potential scanning rate is 20 mV/s while in the experiment it is 50 mV/s. From the experimental results we see that a higher scanning rate will lead to a much higher peak current for the IDEs in a single-electrode mode but

has less effect on the IDEs in a generator-collector mode, and from Figure 4.10B we can see that both the peak current and sensitivity of CV response at single electrode are higher when scanning rate is 50 mV/s than when scanning rate is 20 mV/s. Thus the different scanning rate may also attribute to a less sensitivity increase in the experiment when compared with the simulation results.

CHAPTER 5

CONCLUTION AND FUTURE WORK

5.1 Conclusion

This thesis has contributed to advance our understanding of the underlying mechanism of the voltammetric performance at micro and nano IDEs in order to explore the potential application of IDEs in affinity based biosensors.

Through simulation study, we found that the CV responses of micro and submicro IDEs in a generator-collector mode are very sensitive to the change of k_0 , especially at a larger k_0 value (or a lower target concentration). As the electrode width (w) decreases, significant improvement in the sensitivity and the upper responsive limit (or the lower-detection-limit) can be achieved despite a smaller bandwidth at IDEs with smaller electrode size and spacing. Different detection ranges can be accommodated by using IDEs with properly selected electrode dimensions. Compared with micro- and submicro- IDEs, we see significant enhancements in the limiting current response, the sensitivity, and the upper responsive limit for nano-IDEs when EDL effect is considered. One unique feature of nano-IDEs is that the responsive bandwidth differs when the redox charge valence is different. When the redox species is positively charged, the responsive bandwidth of 1 nm IDEs is much smaller than that of 100 nm IDEs, while when the redox species is negatively charged, the responsive bandwidth of 1 nm IDEs is even larger than that of 10µm IDEs. These results suggest that a different sensing performance can be achieved by using redox species with different charge valences. Furthermore, the CV current responses of IDEs can be further improved by using 3D nanorod-modified IDEs. Our experimental results confirm the

simulation estimation that IDEs in a generator-collector mode have better performance than IDEs in a single-electrode mode when they are used in an affinity sensor.

5.2. Future Work

Many interesting projects can be developed based on the present work in terms of improving the performance of IDEs based affinity biosensors.

5.2.1. Using IDEs with different electrode size

From the simulation results, we see that IDEs based affinity biosensors have different performances when the electrode size of IDEs is different, and a smaller electrode size and spacing may lead to better sensitivity. In the experimental part of this thesis, we confirm that IDEs with 4.25 µm electrode size and spacing exhibit better sensing performance in a generator-collector mode than in a single-electrode mode. It is thus of practical interest to see how the sensing performance of IDEs can be further improved with even smaller sizes for electrodes, for example, using IDEs with 1 µm or even 100 nm electrode size.

The fabrication of IDEs with 1 μ m or even 100 nm electrode size requires different procedures. Instead of the photolithography method used in this thesis, the electron-beam lithography method is necessary to eliminate the diffraction limit of the light and make the electrode features down to nanometer range.

5.2.2. Using nano structure modified IDEs

From the simulation results, we also see that the CV current responses of IDEs can be further improved by using nanorod-modified IDEs. Thus, it would be interesting to experimentally demonstrate how nanostructure modified IDEs can improve the sensing performance of a CV method.



Figure 5.1 The top view and side view of porous anodized aluminum



Figure 5.2 The top view of gold nano-rod and nano-tube fabricated by electrodeposition through porous anodized aluminum

One way to make nanostructures is by electrochemical deposition through porous anodized alumina, and these nanostructures can be nanorod or nanotube with diameter varying from several hundreds of nanometers to several tens of nanometers depending on the pore size of anodized aluminum (see Figure 5.1 and 5.2). By depositing aluminum thin film on chemical and electrochemical inert substrate (e.g. glass or silicon wafer with silicon oxide on the surface) with a conductive metal layer at the interface, anodizing aluminum, making the through pores at nanometer size, and electrodepositing metals through these nanometer pores, we are able to fabricate nanostructures on the hard substrate, and by patterning these nanostructures eventually integrate nanostructures with IDEs using lithography technique.

5.2.3. Using different electrode material, redox species and method of functionalization.

The present study is mainly based on the theory that the electron transfer rate (k_0 value) changes after electrode surface is functionalized. Practically, there are three issues affecting the k_0 value at electrode surface, the material of the electrode (Horswell, O'Neil et al., 2003; Muzikar and Fawcett, 2006), the type of the redox species (Nicholson, 1965; Baranski and Szulborska, 1994; Winkler, 1995), and the method of functionalization (Hideki Kuramitz, 2000; He, Xie et al., 2005).

The electron transfer rate is highly influenced by the atomic nature and structure of the electrode material. Platinum is known to be catalytic for the oxidation and reduction of all kinds of redox species, thus may have higher k_0 value to these redox species than any other material does. For certain material, the crystal structure also affects the k_0 value. For example, it has been reported that the value of the heterogeneous rate constant of $[Ru(NH_3)_6]^{3+}$ at Au (111) was significantly larger than that at Au (100). Therefore, a different sensing performance can be achieved by using different materials for electrodes.

As discussed in chapter 2, the k_0 value is found highly dependent on the diffusivity of the redox species (Nicholson, 1965; Baranski and Szulborska, 1994; Winkler, 1995). With different molecular size of redox species in different solvent, the diffusivity of redox species will be

different, thus leading to a different k_0 value. Therefore, by manipulating the type of redox species and solvents, a different sensing performance for affinity sensing may be achieved.

Also as discussed in chapter 5, the change of k_0 value differs when different surface functionalization methods are used. Usually, a direct functionalization method using large molecules will result in larger k_0 values. Currently, the direct immobilization method has been found in molecules, such as antibody (specifically on ITO IDEs) (Yang, Li et al., 2004) and thiolated single strand DNA or aptamer. Thus, future IDEs based affinity sensors may be used to target these molecules and their complimentary molecules.

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APPENDIX

I. Micro IDEs fabrication

I.I. Methods

A mask of IDEs with electrode finger and spacing width of $4.25\mu m$ is fabricated for pattern transfer (see Figure I.1). The IDEs fabrication process is shown in Figure I.2.



Figure I.1 (A) AutoCAD drawing of the mask for IDEs device; (B) Schematic representation of IDEs designed in AutoCAD, where W_e represent electrode width 4.25µm, W_{gap} represents electrode gap 4.25µm, and L represents the overlapped generator and collector length;



Figure I.2 Process flow diagram for fabrication of micro IDEs

I.I.I. Substrate Cleaning

First, the glass substrate is cleaned by standard RCA solution. The first step is performed with a 1:1:5 solution of $NH_4OH + H_2O_2 + H_2O$ at 75 or 80 degrees (Celsius) for 10 minutes. In this step the organic contaminants is removed (Organic Clean). The second step is a short immersion in a 1:50 solution of HF + H₂O at 25 degrees Celsius for 10 seconds, in order to remove the thin oxide layer and some fraction of ionic contaminants. The third and last step (called SC-2) is performed with a 1:1:6 solution of HCl + H₂O₂ + H₂O at 75 or 80 degrees Celsius for 10 minutes. This treatment effectively removes the remaining traces of metallic (ionic) contaminants. Finally, the sample is rinsed thoroughly in DI water and dried with N₂.

I.I.II. Metallization

After substrate cleaning, two approaches are employed to coat gold thin film on top of substrate. One approach is to successively sputtering coat 7nm Cr and 150nm Au thin film on top of glass substrate by ion beam sputtering at a coating rate ~ 2 Å/sec with coating pressure 10^{-4} torr. The other approach is to successively deposit 15nm Ti and 150nm Au on top of glass substrate by e-beam vapor deposition at a evaporation rate ~ 2 Å/sec with deposition pressure 10^{-6} torr.

I.I.III. IDEs patterning

The cleaned metallized sample is spin coated with HDMS adhesion promoter layer at 6000 rpm followed with Shipley 1818 positive photoresist at 6000 rpm. This should yield a photoresist (PR) thickness of ~1.8 μ m. The sample is then baked at 95°C for 1 minute on a hot plate. Then, the PR coated metallized sample is exposed to UV light (365nm) using an MJB3

mask aligner system for 10 seconds. The UV exposed sample is developed using Developer 319 solution for ~2 minutes followed by a DI rinse. After development, the sample is again baked at 95° C for 5min prior to etching. H₂O : KI : I₂=40ml : 4g : 1g and H₂O:HF:H₂O₂ = 20 : 1 : 1 or Chromium Mask Etchant are used for gold and titanium or chromium etching respectively, and the etching rates at room temperature are 800 Å/min, 25 Å/sec, and 800 Å/min, respectively. All these etchants are compatible to the photoresist.

I.II. Results and Discussion

I.II.I. Au/Cr micro IDEs on glass substrate

Chromium has very good adhesion compared with Titanium, and mechanically sputtering coated film is very strong. Thus, after micro IDEs are patterned, cleaning procedure such as sonication won't damage the sample at all (see Figure I.3). However, electrochemically, chromium is very sensitive to the chloride ion which accelerates corrosion of chromium (see Figure I.4) (Kittlesen, White et al., 1984). Thus, sodium perchlorate (NaClO₄) is usually a suggested supporting electrolyte for Au/Cr IDEs. However, for biosensing application, the biomolecules preferred environment is buffer solution which usually contains chloride ions. Thus, Au/Cr IDEs may not be a choice for biosensing purpose.



Figure I.3 Au/Cr micro IDEs before applying potential.



Figure I.4 Au/Cr micro IDEs were dissolved after potential was applied

I.II.II. Au/Ti micro IDEs on glass substrate

The patterned Au/Ti micro IDEs are shown in Figure I.5. We can see that all the electrodes are clearly patterned without any unexpected joint or broken lines. The adhesion of titanium seems not as good as chromium, because after applying potential, the gold layer is completely peeled off (see Figure I.6). This phenomenon also partially attributed to the etching method we used, because the wet etching step could bring a lot of tension on the thin film interface thereby cause failure. There are three ways to solve this problem. The first is to use dry etching method which has better control of the etching speed. The second is to use liftoff technique in order to avoid wet etching step. The third is to anneal the Au/Ti micro IDEs after wet etching.

Both the first and the second solutions are not available on campus. So, the only choice we have is the third solution. We thus annealed Au/Ti micro IDEs at 350~400 °C for 20~30min.

The resulted film is much stronger, and gold film won't peel off after applying potential (see Figure I.7). Thus, the annealed Au/Ti IDEs are used in this thesis.



Figure I.5 Micro interdigitated electrodes (IDEs) before applying potential



Figure I.6 Au/Ti interdigitated electrodes (IDEs) after applying potential without annealing step. The gold layer peeled off completely, and the titanium layer is left on the glass substrate.



Figure I.7 Au/Ti interdigitated electrodes (IDEs) after applying potential with annealing step. The gold layer peeled off completely, and the titanium layer is left on the glass substrate.