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Impedimetric Biosensors and Immunosensors

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Abstract

The development of methods targeting the direct monitoring of antibody-antigen interactions is particularly attractive. The design of label-free affinity-based probing concepts is the objective of much current research, at both academic and industrial levels, towards establishing alternative methods to the already existing ELISA-based immunoassays. Among these, Electrochemical Impedance Spectroscopy (EIS) represents one of the most powerful methods, due to the ability of EIS-based sensors to be more easily integrated into multi-array or microprocessor- controlled diagnostic tools. During the last decade, EIS and the concept of biochemical capacitors have been widely used for probing various types of biomolecular interactions (immunosensors, DNA hybridization, protein-protein interactions). So far, impedimetric or capacitive immunosensors have been successfully applied at the academic level. However, no prototypes have been released into the market, since major fundamental issues still exist. Even though this fact has brought the reliability of impedimetric immunosensors into question, features associated with electrochemical approaches, namely the ability to be miniaturized, remote control of implanted sensors, low cost of electrode mass production, and cost effective instrumentation (without need of high-energy sources) keep impedimetric sensors particularly attractive as compared to other approaches based on microbalances, surface plasmon resonance or ellipsometry. This lecture outlines the theoretical background of impedimetric immunosensors and presents different types of impedimetric biosensors as well as the instrumental approaches that have been so far proposed in the literature.

Introduction

Since pioneering works of Newman [1] and Martelet [2] on the concept of capacitive, or impedimetric based immunosensors, a lot of work has been done in this specific area. During the last decade, impedance spectroscopy has been widely used for probing various types of biomolecular interactions (immunosensors, DNA hybridization, protein-protein interactions) and relevant literature has been comprehensively reviewed [3-6]. Features associated with electrochemical approaches, namely the ability to be miniaturized, remote control of implanted sensors, low cost of electrode mass production, and cost effective instrumentation have made impedimetric sensors particularly attractive as compared to other approaches based on microbalances [7], surface plasmon resonance [8] or ellipsometry [9].

Theoretical background

EIS is an ac method that describes the response of an electrochemical cell to a small amplitude sinusoidal voltage signal as a function of frequency. The resulting current sine wave differs in time (phase shift) with respect to the perturbing (voltage) wave, and the ratio V(t)/I(t) is defined as the impedance (Z), and accounts for the combined opposition of all the components within the electrochemical cell (resistors, capacitors, inductors) to the flow of electrons. In an electrochemical cell, electrode kinetics, redox reactions, diffusion phenomena and molecular interactions at the electrode surface can be considered analogous to the above components that impede the flow of electrons in an ac circuit [10].

Impedance is usually expressed as a complex number, where the ohmic reactance is the real component and the capacitive reactance is the imaginary one. The most popular formats for evaluating electrochemical impedance data are the Nyquist and Bode plots. In the former format, the imaginary impedance component (Z", out-of-phase) is plotted

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against the real impedance component (Z', in-phase) at each excitation frequency, whereas in the latter format, both the logarithm of the absolute impedance, |Z| and the phase shift, θ, of the impedance, are plotted against the logarithm of the excitation frequency.

Capacitive immunosensors exploit the change in dielectric properties and/or thickness of the dielectric layer at the electrolyte-electrode interfaces, due to the antibody-antigen (Ab-Ag) interaction, for monitoring this process. An electrolytic capacitor allows the detection of an analyte specific to the receptor that has been immobilized on the insulating dielectric layer, which has previously been deposited on the surface of the working electrode [1-6]**.** Ideally, this configuration resembles a capacitor in its ability to store charge and thus, the electric capacitance between the working electrode and the electrolyte is given by the basic equation 1:

$$C = \frac{\varepsilon_o \varepsilon A}{d} \qquad (1)$$

A decrease of the total capacitance, due to the increase of the distance between the plates is thus expected upon the binding of the analyte to its specific receptor. Plausibly, this phenomenon can be represented by two capacitors in series; the inner one corresponds to the dielectric (C_{dl}) layer and the outer one corresponds to the biomolecule layer (C_{bm}) and consequently to the interactions of this layer with its specific ligand. Since the current must pass through the uncompensated resistance of the electrolyte solution, its resistance is inserted as a series element in the circuit (Fig.1A). In this case, the total capacitance C_t, can be described by Equation 2

$$\frac{1}{C_t} = \frac{1}{C_{dl}} + \frac{1}{C_{bm}} \qquad (2)$$

As can be seen from equation 2, in order to design a sensitive sensor with a wide dynamic range, the insulating layer should be thin enough and/or have a high dielectric constant. Otherwise, capacitance changes originating in the binding of the analyte to the receptor might not dominate the total capacitance. In addition, the insulating layer should be complete (pin-holes free), stable with the time, and provide functional groups for the immobilization of the receptor [11].

While no equivalent model can be guaranteed to be unique, simulation of the recorded impedimetric data to an equivalent electric circuit is a common strategy for understanding the physical origin of the observed response. The simplest, and in fact the most frequently used equivalent circuit for modelling of EIS experimental data is the so called Randles circuit (Figure 1B), which comprises the uncompensated resistance of the electrolyte (R_s), in series with the capacitance of the dielectric layer (C_{dl}) and the charge-transfer resistance (R_{ct}), if a redox probe is present in the electrochemical cell. The latter two components are connected in parallel. An additional component, connected in series with R_{ct}, the Warburg impedance (Z_w) accounts for the diffusion of ions from bulk electrolyte to the electrode interface. A typical shape of the impedance spectrum of this circuit presented in a Nyquist plot (*see* Figure 1b) includes a semicircle region lying on the real axis followed by a straight line. The linear part ($\varphi=\pi/4$), observed at the low frequency range, implies a mass-transfer limited process [3,10], whereas the semicircle portion, observed at high frequency range, implies a charge-transfer limited process.

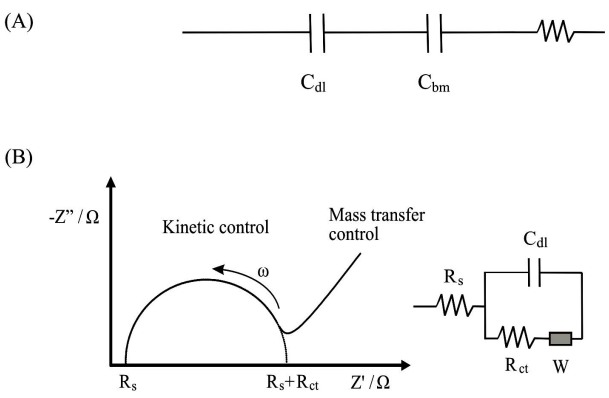

Figure 1. **(A) Electrical circuit model used to represent an electrolytic capacitor coupled to a biolayer. (B) Nyquist plot arising from a Randles' circuit (showing in the side panel).**

Based on the nature of the measuring signal, impedance immunosensors can be classified into two main categories:

(a) *Capacitive*, where the surface of the electrode is completely covered by a dielectric layer and the whole electrode assembly behaves as an insulator. In this type of sensor, no redox probe is present in the measuring solution and the capacitive current is measured under a small amplitude sinusoidal voltage signal, at low excitation frequencies (typically 10-1000 Hz). Ab-Ag interactions are expected to cause a decrease of the measuring capacitance since less polar protein molecules replace water molecules from the electrode surface.

(b) *Faradaic*, where the surface of the electrode, which is partially or wholly covered by a non insulating layer, or partially covered by an insulating layer is able to catalyse a redox probe, which exists in the measuring solution. In this case, the measured parameter is the charge transfer resistance (the real component of impedance at low frequency values, typically 0.1-1.0 Hz), and Ab-Ag interactions are expected to cause an increase in its value as the faradaic reaction becomes increasingly hindered. In general, faradaic impedimetric immunosensors exhibit a higher sensitivity to Ab-Ag interactions. However, the redox species may have an effect on both the stability and the activity of the electrode assembly.

Instrumentation and quantification parameters

Several instruments based on small-signal ac admittance measurements such as LCR-meters, impedance analyzers, lock-in amplifiers and frequency response analyzers (FRA) [3] have been used to monitor interactions between biomolecules. The last two approaches are the most widely used and bring both inherent advantages and disadvantages. Impedance systems based on lock-in amplifiers are very sensitive, can effectively remove background noise, minimize harmonic distortions and are relatively cost effective. On the other hand, it is difficult to use them for stand-alone measurements which are somewhat slow, and they cannot be used over a wide frequency range. Impedance systems based on FRA, provide fast analysis over a wide frequency range, remove harmonic distortions and dc components and can be easily fully automated. Limited sensitivity and background removal as well as their relatively high cost are disadvantages associated with FRA based measuring systems. Recently, Efstathiou and colleagues [12] reported on the construction of a stand-alone, low-cost electronic device (Multipulser), for monitoring interactions between biomolecules that may change the capacitance of an electrode. The operation of the Multipulser is based on the repetitive charging of the electrochemical cell capacitance by applying a predetermined number of short-duration, low-amplitude voltage pulses (perturbation pulses). All packets of charge are accumulated in an analog integrator whose output voltage is proportional to the cell capacitance. The Multipulser features three user-selectable operating modes, each one characterized by its own particular shape of the applied perturbation pulses. The physical parameters that have been used so far, as a measure of the analyte concentration include capacitance, charge-transfer resistance and impedance. Capacitance and impedance (Z', Z'' or the magnitude $|Z|$) measurements provide fast measurements and the ability to monitor the kinetics of the immunocoupling. Charge transfer resistance measurements are rather time consuming process, as they require first a Z'' = f(Z)' spectra over a wide frequency range to be obtained. Another option for the calculation of the above parameters is the fitting of equations associated with an equivalent circuit to the experimental data using commercial software based on the Boukamp algorithm [13].

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