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Chapter 8

Impedimetric Biosensors for Label-Free and Enzymless Detection

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Additional information is available at the end of the chapter

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1. Introduction

Currently, Biosensor technology has provided a number of benefits to detect both biological and chemical molecules. Abiosensor is a promising device, which is combination of sensitivity of electrochemistry and specificity of biological recognition, enables to detect any kind of molecules in a short time with selectively and sensitively. Likewise many analytical methods, it has also limitations, such as high oxidation potentials lead to detection of non-target molecules, furthermore non-electroactive species cannot show electroactive signal for measurement or some biomolecules cannot be transformed by enzymes, even if they can be transformed, they require secondary molecules such as mediators, coenzymes or labels. In order to detect molecules without electrochemical reaction, electrochemical impedance spectroscopy (EIS) can be employed as a measurement technique "to see electrode surface modifications just by looking impedance curves". As it is known, electrochemical impedance spectroscopy is an electrochemical technique that provides the examination of electrical properties of electrode surface and binding kinetics of molecules between electrolyte and electrode surface. Therefore it can be used for biomolecular recognition, biomolecular bindings and biomolecular interactions between molecules such as DNA-DNA, DNA-protein, Receptor-Ligand, Protein-Ligand, Antibody-Antigen, and Ion Channels-Ligands. As a consequence of this affinity provides label-free detection without chemical transformation and this binding property can be monitored by electrochemical impedance spectroscopy expeditiously. In this chapter, the information will answer a number of questions about the development of impedimetric biosensors. In fact that is focused on the usage of impedance for biosensor technology, and to demonstrate impedance curves and the meaning of electrical elements for obtained Nyquist Plots especially faradaic impedance. Employed biorecognition receptors and not yet employed biorecognition receptors, which have different chemical residues, are discussed.

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2. Theory of electrochemical impedance spectroscopy for biosensors

Electrochemical impedance spectroscopy and the method of impedance are widely used for corrosion, batteries, bioelectrochemistry and electrochemistry. EIS provide electrochemical examination of electrical properties of electrode surface; on the other hand it can be called as electrochemical surface characterization. Therefore it is possible to realize the differentiation of electrode surface alterations easily. In biosensor technology it is used for monitoring biosensor modifications, layer formation on electrode surface and binding kinetics between molecules such as DNAs, receptors, antibodies, antigens, proteins, ions etc. This advantage provides examination binding kinetics of molecules, just by using obtained impedance spectrums for binding kinetics of molecules leads to label-free detection. As it is known, for enzyme based biosensors, a molecule needs to be transformed into another molecule by enzyme for obtain electroactive molecules or electrons for gain an electroactive signal electroactive signal can be disturbed by other molecules, which oxidation reduction potentials are same as analyte molecule. Electrochemical impedance spectroscopy overcomes this problem and provides non-electroactive detection of molecules. There is only one condition, which is the most important handicap, is to find a most specific biorecognition receptor for analyte. Likewise all electrochemical measurement techniques, by employing EIS for biosensor technology has the same fundamentals, which are composed of electrical circuits in order to determine electrochemical measurement. This electrical circuit is affected by AC current, which is generally used for impedimetric experiments. Alternative current (AC) is a wave shaped and has a frequency; therefore both potential and current oscillate (Fig. 1). This oscillation causes differentiation in time because AC excitation signal and sinusoidal current response are both based on Ohm law. As it is known the Ohm law includes; a potential, a current and a resistance for ideal DC circuits. However, for AC, some mathematical units must be added because of the frequency of AC.

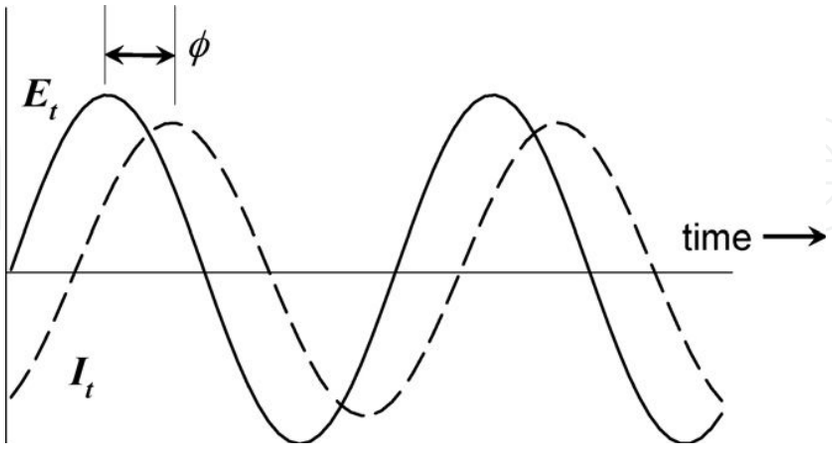


Figure 1. Alternative current; E_t and I_t.

As it can be seen in figure 1, the sinusoidal fluctuation of both current and potential show a difference, this difference, Φ, is determined as impedance which is an alternative current

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system resistance. Mathematical equationof this system is transformed into this equation 1 (Z; impedance, E_t; potential in a time, I_t; current in a time, E_0; potential at zero point, I_0; current at zero point, ω; frequency, t; time)

$$Z = \frac{E_t}{I_t} = \frac{E_0 Sin(\omega t)}{I_0 (Sin(\omega t + \Phi)} = Z_0 \frac{Sin(\omega t)}{Sin(\omega t + \Phi)} \quad (1)$$

In this equation, impedance is represented as Z, and Z is a phase shift of AC, furthermore this phase shift is angle of impedance curve of Nyquist plot.

This theory has been performed for biosensor technology for a long time, its aim is examination of electrical characteristics of electrode surface for every layer formation and every interaction between molecules, and the obtained signal variations. In fact that charged groups of molecules have effect on impedance curves, layer has influence on electrical characteristic of electrode, this causes distribution of electrode surface charge, subsequently capacitive current varies, hence electrical circuit of the system keeps it balance and impedance increases or decreases [1].

EIS has an advantage over the other electrical measurement technique, which is an opportunity to design electrical circuit, according to obtained Nyquist plot curve. For figure 2, there is an electrical circuit model for obtained impedance curve. As you can see there are resistances and capacitance, in figure 2 there are both parallel capacitance and resistance, which they represent electrode surface, and a resistance is serial over this circuit, capacitance represents electrical double layer of electrode, R_2 represents resistance of the electrode, and R1 represents resistance of the solution in cell which is located electrodes inside of it. The Nyquist plot of this electrode starts not zero point which means that the solution in the cell shows a resistance (If it started at zero point, that means the resistance of solution(R_1) does not exist); therefore a resistance element(R_1) is added in circuit model. The rest of the curve shows a characteristic sinusoidal impedance curve, which means only a resistance occurs, and R_2 is added on circuit, and capacitance always occurs as a function of capacitive current, which represents in homogenity on electrode surface, because electrical double layer occurs.

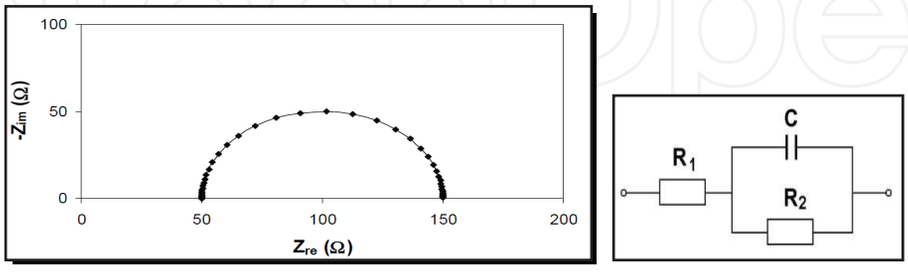


Figure 2. Non-faradaic impedance curve, R_2 electrode surface resistance and capacitance, and R_1cell surface resistance.

Variation on impedance curve changes the electrical circuit model, an alteration especially on Nyquist curve an circuit element is added after R_2 circuit element as serial [2].

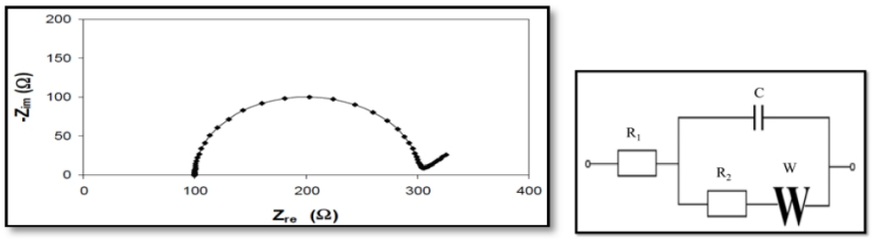


Figure 3. Faradaic impedance curve, R_2; electrode surface resistance, W; Warburg impedance and C; capacitance, and R_1; cell solution resistance.

For figure 3, there is an additional circuit element, Warburg impedance(W) which represents mass transfer to electrode surface. This resistance occurs when an interaction formation, which is formed by electrical interaction, adsorption e.g., between electrode surface and solution a mass transfer occurs towards electrode surface, this transfer cannot be calculated as diffusion because there is an accelerated mass transfer by affection. Therefore Nyquist curve varies and becomes linear; this linearity represents Warburg impedance, which means mass transfer resistance. The interaction between electrode surface and solution, keep the balance between Warburg impedance and electrode surface resistance(R_1), this balance can be unbalanced by mass transfer and after any increase on mass transfer the Warburg impedance shows dominancy on electrode surface resistance (R_1) [3]. This domination shields resistance and sometimes resistance doesn't occur. A study was performed by Uygun and Sezgintürk, in this study gold film modified glassy carbon electrode was modified by SAM of Cysteamine layer and positively charged Cysteamine attracted negatively charged redox probe($Fe(CN)_6^{-3}/Fe(CN)_6^{-4}$), this mass transfer and this attraction shadowed no resistance and Warburg impedance showed dominancy on circuit [4]. On the other hand a redox probe as $[Ru(NH_3)_6]^{3+}$ will be repelled by positively charged modification. As you can see there are a number of conditions for impedance to design a circuit model, which surface of electrode, content of solution, characteristics of redox probe are important on electrical circuit modelling.

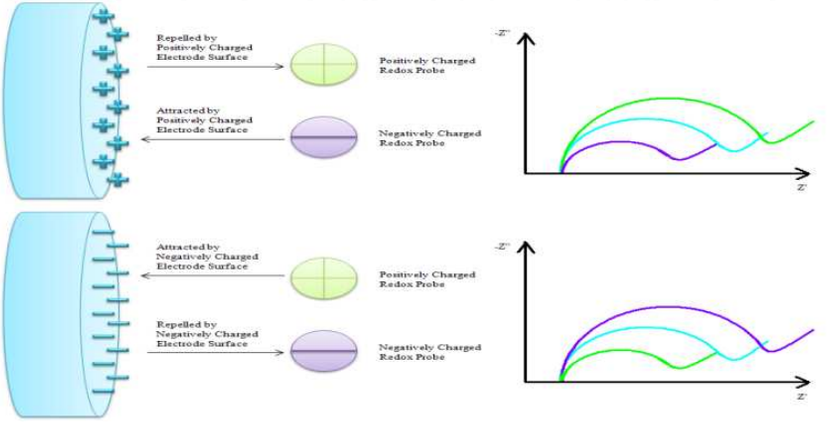


Figure 4. A schematic representation of electrode surface and redox probe interaction and their impedance spectrums.

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For electrochemical impedance spectroscopy based biosensor systems, frequency scanning between two frequencies were chosen according to the solution, on the other hand electrical conductivity of solution is very important for choosing frequencies, in higher electrical conduction ability of solution, which means the solution is highly concentrated by ions, lowest frequencies can be chosen especially in the presence of redox probe (lower than 0.1 Hz). A potential must be applied to gain a proper signal, this potential is called as AC excitation signal [2]. Its magnitude depends on the solution of measurement system's cell. When the solution includes redox probe such as ferricyanide, osmium complexes or ferrocene, according to the oxidation or reduction potentials of these materials, the beginning of the electrochemical transformation potential is chosen. An unknown solution or unknown potential can be measured by cyclic voltammetry to find out the beginning oxidation/reduction potential of the electrolyte solution.

3. The importance of label-free detection

Most affinity biosensor systems require secondary molecule for amperometric or voltammetric experiments, which is attached to analyte or biorecognition receptor molecule to obtain an electroactive signal for measurement. It is necessary that labeling process, because some biomolecules or molecules cannot be transformed or cannot give electroactive signal for measurement; therefore another electroactive molecule must be used for electroactive signal. These labels must be easily applicable to gain proper signal such as fluorophores, nanoparticles, enzymes, quantum particles. This labeling process may need an extra molecule and extra preparation process to detect the real analyte. Moreover, this process can change properties of biorecognition receptor or analyte molecule and their affinity to each other and the most important thing these processes increase the expenses. Because of all these problems, electrochemical impedance spectroscopy becomes a phenomenon technique for label-free detection. In order to obtain electrochemical impedance signal, only interaction between two molecules suffices for measurement. By using enzymes some molecules can be detected easily, however instead of enzymes, transporters such as glucose transporters, ion channels, receptors, antibodies or aptamers can be used for impedimetric measurement because when these biomaterials attach their target molecules, their structural composition changes, this changing leads to rearrangement of the capacitive double layer of the electrode and the electron transfer resistance will alter. Thus they can be used as biorecognition receptors. Traditional affinity biosensors, antibody-antigen and receptor-ligand couples can be used for label-free detection.

4. Construction of impedance based biosensors

Our chapter mainly focuses on development of non-faradaic and mostly faradaic electrochemical impedance based biosensors, in other words how to construct them, which biore-

cognition receptor can be employed. This heading gives development of impedimetric biosensors based on different biorecognition receptor to detect different molecules.

4.1. Impedimetric receptor based biosensors

As it is known that, receptors are located on cell surface area for sensing molecules to receive and transfer signal into cell. They are usually found on the outer surface of cells, extending through the plasma membrane.Their sensing mechanism is based on weak interaction between their ligand and receptor. Therefore they can show regeneration potential to sense and induced their roles for biological form, when they are used as biosensor recognition element. This would be an advantage for reusable for biosensor. For extracellular membrane, receptors take the signals such as hormones, growth factors, ions, neurotransmitters etc. Hormones and other factors are very useful, their detection reveal a number dysfunctions, cancer and sicknesses. Excessive and deficient biomolecule concentration in the organism can be a signal of any diseases. Hormones and growth factors are peptides or proteins and their measurement is hard, because they cannot give any electroactive signals by transformation. In this problem, impedimetric biosensor systems are prominence, to immobilize a receptor on electrode surface provides to detect its ligand by biosensor technology. Before we mentioned that the most receptors are located on cell surfaces, their structure integral proteins, this proteins have two domains, which are hydrophobic and hydrophilic. For hydrophobic domains that may be a problem when immobilization process because forming a proper hydrophobic environment is very hard and may harm hydrophobic domain. This complications lead to find a proper immobilization technique, which is low cost for material and less complicated.

Uygun and Sezgintürk have developed an ultrasensitive impedimetric vascular endothelial growth factor receptor 1(VEGFR-1) based biosensor system for vascular endothelial growth factor, which is a protein produced by cells to stimulate cellular growth. It's important that is a biomarker for metastasis and lower and higher levels have disaster meanings for organism. In this study, a protein, VEGF was analyzed by electrochemical impedance spectroscopy, VEGFR-1 used as a biorecognition receptor and this affinity biosensor proved its ability that provide measurement lower concentrations, which 100 femtogram in per milliliter. Gold electrode was coated layer-by-layer; a gold film layer, SAM of Cysteamine layer, Avidin, Biotin and VEGFR-1, respectively. This long immobilization layer provides electrode ultra sensitivity. Because of using a redox probe, $[Fe(CN)_6]^{3-/4-}$, faradic impedance was in progress and Randles circuit model was applied to this impedimetric biosensor. Calibration curve was constructed 100-600 fg/ml. By calculating this calibration curve alteration of electron transfer resistance (ΔR_{et}) was used for calibration curve. In addition, this biosensor was acknowledged by Kramer-Kronig transforms to correlate of biosensors repeatability, stability and linearity [4]. This study was combination of EIS and biological recognition receptor, VEGFR-1, which shows high affinity to its ligand, therefore the study provides lower concentrations, and good selectivity without any electrochemical transformation of analyte.

Kim et al. were constructed an estrogen biosensors, which is based on impedance spectroscopy as well. For this biosensor estrogen receptors were used as biorecognition receptors of

17β-estradiol. As it is known that estrogen shows sexual characteristics of females [5], however estrogen is a carcinogen for tumor initiation and promotion [6,7]. For this reason its measurement, in a good sensitivity, plays a crucial role for female health. In this study, Gold electrode was coated a SAM layer, which is 3-mercaptopropionic acid to bind estrogen receptors via –COOH groups. LOD was $1.0x10^{-13}$M. calibration curve was constructed in a linear range between 1.0x10-$1.0x10^{-11}$M [8]. This study is another receptor based study, it can be seen that its immobilization method is very easy, and estrogen hormone is a steroid, which is insoluble in water, is detected easily by EIS.

4.2. Impedimetric antibody-antigen based biosensors

Antibodies (Ab), or immunoglobulin, are elements of immune system to take an action in case of contamination of body. Antibodies are most widely used affinity element of biosensor technology, because of their sensitive and selective properties against antigens. Antibody based biosensor technology has a great advantage of affinity biosensor technology, because antibodies have a wide range affinity from low molecular weight molecules to high molecular weight proteins. High affinity is the major factor for an antibody to use as biorecognition receptors for biosensors. As a consequence of Ab characteristic, when an immobilization is on process then it must not be forgotten that stability, matrix effect and susceptibility [9]. In addition antibody production is very useful or any antigen, because it is widely used for antibody production in vitro. Ab and its antigen play a key role on development of an Ab based biosensor, therefore it must be known that the interaction between Ab and antigen, which they are hydrogen bonding, hydrophobic, van der Waals and coulomb interactions [10]. These forces are called as "weak interactions", therefore it is easy to break apart Ab-Ag binds, and moreover this provides regeneration for biosensor technology. Another advantages of Ab usage for biosensor technology,Ab production is very effective for obtaining proper Ab, experimental animals can produce antibody when they counter any xenobiotic, thus antibody production is stimulated for unknown molecule and new antibodies are purified. For further production recombinant technology can be used to obtain antibody. Antibodies can be divided into two main groups, which are monoclonal and polyclonal. In short, monoclonal antibodies show higher affinity to their antigen in comparison with polyclonal antibodies and it is very useful for selective measurements. Using antibody for biosensor technology has another advantage, which is capable of signal amplifying. It is known a common name as "sandwich method"; this method is combination of antigen and a secondary antibody(Figure 5). This secondary antibody can be labeled such as an enzyme, nanoparticles, quantum dots, etc. However, for impedimetric biosensors it is not necessary to label secondary antibody, because binding of antigen and antibody will give impedimetric signal, additional secondary antibody label-free increases the measurement signal that is impedance.

Pichetsurnthorn et al. have proposed a study, which is about impedimetric biosensor for trace atrazine detection from water samples. Atrazine is a small xenobiotic, a pesticide, and it is very harmful any organism when it is in taken. Therefore pesticide analysis plays a crucial role for food and environmental industry. As it is known that a number of biosensor for

pesticide detection have been published and continuing, but enzymatic biosensors show less selectivity, because they depend on enzymatic inhibition, which an enzyme can be affected by ionic strength, pH, heavy metals etc. and this enzymatic inhibition is not reliable for pesticide detection. In this manner, electrochemical impedance spectroscopy has become prominence for label-free and enzymless detection. In this study, nanoporous alumina membranes were used just as ELISA method without using enzyme to achieve non-faradaic measurement, in spiked samples, the biosensor gives as low as femtogram/ml. On a gold metal electrode alumina membrane, for biomolecular confinement, was used because of nanomolecular space. For antibody immobilization *thio bis succinimidyl propionate* (DSP), a linker, was used. This provides thiol links with gold surface, and its amine groups were used immobilization of anti-atrazine. Calibration curve was constructed 10fg/ml to 1ng/ml in wide range. This sensor was designed in three ways to increase selectivity, which one of them is used planar sensor, second is 200nm alumina pores and three is 20nm alumina pores. Decreasing in alumina pore increased the selectivity of atrazine molecule. This study provided label-free, selective and sensitive atrazine measurement in ultra low concentrations [11]. Based on antibody efficacy, a cross-reactivity was observed, which was the result of antibody specification against other molecule, malathion.

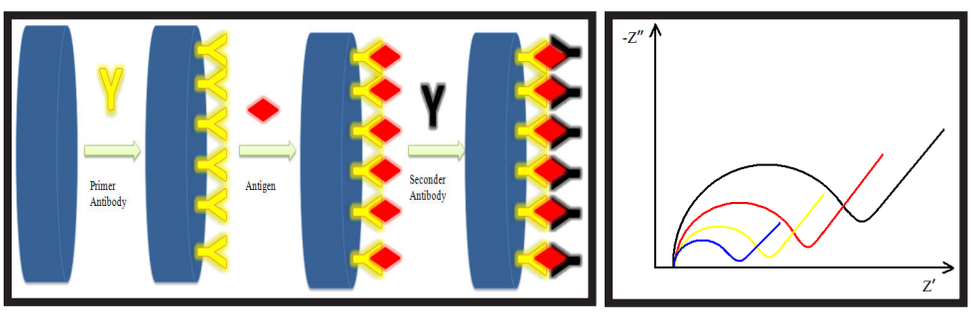


Figure 5. Schematic representation of Sandwich Method and its Impedance Spectrums

Huang et al. have developed allergen biosensors, which is based on mite allergen Der f2 and its antibody by using electrochemical impedance spectroscopy. Type I allergic reaction is a reaction of immune dysfunction and represents a problem of health of organism. Development of industry, especially on home industry, increases the allergic reactions of bodies because the natural environment of body has been changing and adaptation is hard. The allergic reactions occurs when body comes across an allergenic material and this plays a crucial role that the detection of allergenic reaction. Huang et al. find out a solution for point of care allergenic detection, which is based on impedance spectroscopy to determine Der f2. Firstly a glassy carbon electrode was modified by gold electrodeposition, subsequently Der f2 solution dropped on electrode and waited for a night to ensure that the active sides of nanogold were all occupied by allergen molecules. Then it was exposed to different concentration of murine monoclonal antibody solution. Electrochemical impedance spectrums were employed for calibration curve from 2μg/ml to 300μg/ml [12].

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4.3. Impedimetric DNA-aptamer based biosensors

DNA is known as double-stranded (ds) source of genetic information. This genetically occurred biomolecule includes base pairs, which are Adenine-Thymine and Guanine-Cytosine, phosphate backbone, which provides negative charge properties. These pairs occur on the dsDNA strand is only if specific its complementary DNA strand. This specificity is very important for label free molecule detection or DNA sequence detection. This specificity leads to utilize DNAs for biosensor, which DNA is used as biorecognition receptor. By using DNAs specificity, a new method has become revealed that is Aptamer usage as a biorecognition receptor. Aptamers are artificial nucleic acids with specific binding affinity to molecules, proteins, amino acids, drugs, pesticides, etc. Their advantages proposed them as alternatives of antibodies [13-16]. On the other hand their specific affinity to wide range of molecules, an aptasensor is only specific for one target. Thus, this limitation must be improved for detection of other molecules. Detection of more than one molecule by using aptamer is a challenging technique, because of its limitations. To detect one molecule a strand is enough, but to detect more than one molecule, DNA strand must be prolonged to bind two or more molecules. This prolongation, which contains more than one aptamer units, on DNA increases the flexibility of DNA strand and affects formation of aptamer-analyte molecule complex [17], or hybridization on same strand occurs. Aptasensors are widely used, however their ultrasensitive detections are limited because of their low association constants, for the signal amplification many methods have been proposed for aptasensor usage, such a rolling circle amplification [18,19], strand displacement amplification [20,21], enzyme label [22-24]. These methods are very advantageous for signal amplification, besides they are complicated and expensive.

Deng et al. have proposed a bifunctional aptasensor to detect lysozyme and adenosine. In this method, two DNA strands, which were used adenosine contained DNA and lysozyme aptamer. $[Ru(NH_3)_6]^{3+}$ was used as signaling transducer, moreover gold nanoparticles (AuNPs) were used to increase signal. DNA strands, which were used as biorecognition receptors, were modified thiol-terminated. According to self-assembly method (SAM), DNA (SAM) layers formed on gold electrode layer. Main idea of this study was a DNA was used as capture; a DNA was used as linker, and a DNA-AuNPs as used increase the signal. Negatively charged DNA strands were treated with $[Ru(NH_3)_6]^{3+}$ complexes. In any consideration, which adenosine was added on electrode surface, aptamer lysozyme complex released from surface, lysozyme was added on electrode surface, aptamer-adenosine complex preleased from surface. DNA-AuNPs complexes increase the selectivity by increasing the lysozyme aptamer formation signal. Analytes, lysozyme and adenosine, bind to electrode surface $[Ru(NH_3)_6]^{3+}$ complexes were released and amplify the electrical signal. All in all this bifunctionalaptamer biosensor is based on structure-switching properties. This signal changes were monitored by impedance spectroscopy to measure layer changes. Switching on the layer for any molecule will change the capacitive and resistive properties of the electrode and impedance becomes a prominence measurement technique and it provides label free and enzymless detection. Lysozyme and adenosine concentrations were detected 0.01μg mL^{-1} and 0.02nM, respectively [25].

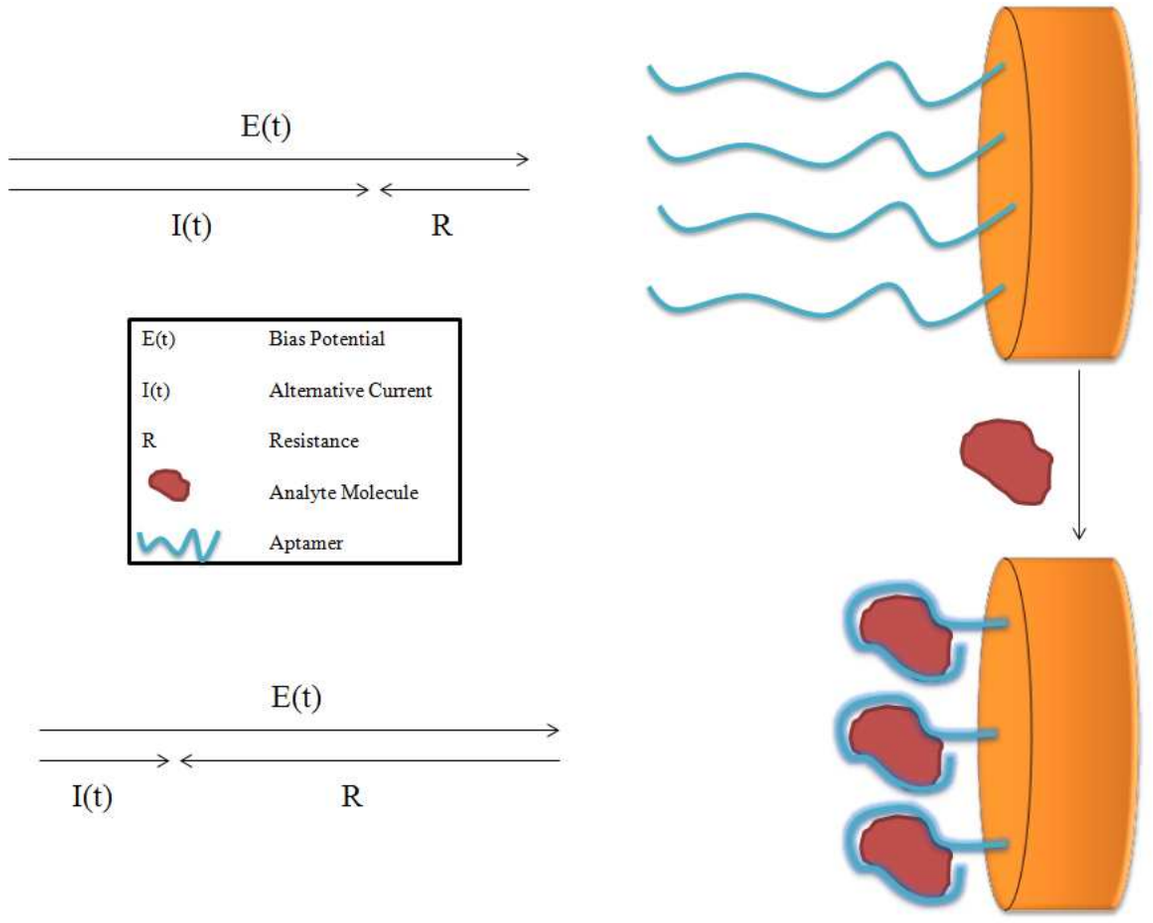


Figure 6. Schematic representation of impedimetricaptamer biosensor.

Ensafi et al. developed a DNA biosensor, which is based on DNA-DNA hybridization. This DNA hybridization was used for detection a cancer type that is chronic lymphocytic leukemia (CLL), which provokes the production of white blood cells called B lymphocytes in bone marrow. This biosensors system is based on porphobilinogendeaminase (PBGD) gene, which is associated with CLL cancer. A probe 25-mer DNA modified with thiol-terminated and binds to AuNPs as SAM. The target, complementary DNA was detected as through hybridization. The PBGD gene hybridizations were monitored impedance spectrums, by using redox probe, which is $[Fe(CN)_6]^{3-/4-}$. Deposited AuNPs on gold electrode, SAM of DNA layer formed and complementary DNA hybridized. This biosensors LOD result was calculated as 1 femtoM. By using impedance spectroscopy mismatched DNA strands was able to observed, even for one base mismatched. This showed that label-free detection is provided in higher sensitivity levels [26].

4.4. Other impedimetric biosensors

After most widely used biomolecules (DNA, Receptors and Antibodies) for biosensors, other biomolecules for impedimetric biosensor usage is presented in this part of the chapter. In this chapter two molecules, which show specific affinity themselves other than receptors,

DNAs and antibodies, will be revealed, such as specific protein-molecule, cell-molecule and protein-cell. When it is mentioned before that to develop an impedimetric biosensor, it is only necessary to find two molecules, which show affinity. Because of this affinity the binding will be come true, and impedimetric signal will be obtained. In order to illustrate some ides, this part will be written by giving examples about other impedimetric affinity biosensors.

Hu et al. has proposed a study, which is Multi-wall carbon nanotube-polaniline biosensor based on lectin-carbohydrate affinity for ultrasensitive detection of Con A. Concavalin A is a kind of allosteric protein, which has four affinity residues on it for binding specific atoms and molecules, which they are calcium and manganese cations to activate binding sides for carbohydrates, one is for hydrophobic recognition, one is for R-mannose or D-glucose [27]. Concavalin A is called in lectin family, which is specific protein family to carbohydrates, moreover it has an ability to activate and proliferate for mature T cells [28]. Lectin binding carbohydrate ligands, in other words carbohydrate specific proteins, show high affinity likewise antibody-antigen interactions. Lectins have been used for biosensor technology for detection of carbohydrate. In this study carbohydrate, D-glucose was used as recognition receptor for lectin detection. Using nonmaterials(PANI and CNT) increased the surface are of electrode. MWNT-PANI nanocomposite material was dropped on GCE and glucose added in 50 C for 6h through Schiff-base reaction. This modified electrode was exposed different concentration on Con A solutions and EIS spectrums were obtained. R.S.D was found as 2.1% and a calibration curve was constructed from 3.3pM to 9.3nM and LOD was 1.0pM [29]. MWNT-PANI nanocomposite layer was very promising modification step, in order to reach lowest limits.

Oliveira et al. published an article, which is an impedimetric biosensor based on self-assembled hybrid cystein-gold nanoparticles and CramoLLlectin for bacterial lipopolysaccharide recognition. As it is known lipopolysaccharides are endotoxines, structural components of gram-negative bacteria, which is common for humans, animals and plants [30]. In this study electrode surface was modified poly(vinyl chloride-co-vinyl acetate-co-maleic acid)(PVM), gold nanoparticles-cystein composite (AuNpCys), CramoLL, respectively. This electrode was specific for lipopolysaccharides. PVM layer was charged positively, this provides that attract the CramoLLlectin electrostatically. LPS detection depended on carbohydrate composition of LPS, and this limited the selectivity and binding abilities of biosensor. In this study, different cells, which had constructed LPS layer, were used to detection of any difference when LPS layer differs. Because of linear LPS layer composition of *S. marcescens* highest impedance value was obtained. The CramoLL-LPS binding process can be significantly inhibited by saccharide, glucose, providing further proof of the sensing mechanism on lectin interface. AuNpCys-PVM complex positively charged surfaces were suitable for CramoLLlectin immobilization. As it is known that cell surfaces are negatively charged [31].

Tlili et al. developed an impedimetric biosensor system, based on cell, which was Fibroblast Cells: a sensing bioelement for glucose detection by impedance spectroscopy. Fibroblast cells were used as biorecognition receptor for glucose detection. Diabetes is one of the most widely studied disaster in the world, in order to find diabetes, its markers must be deter-

mined, which is most important glucose. Several studies were carried out glucose biosensing. Using living cells as biorecognition receptors provides an opportunity for high sensitivity in a broad range of biologically active substances that affect the response of the cells. In this study, 3T3-L1 fibroblast cells are able to metabolise glucose through the activation of specific glucose transporters (Glut 1 and Glut 4). Biological cells are very poor conductors at low frequencies, for this reason, force electrical currents to bypass them. Impedance spectrums were proportional to fibroblast cell growth on electrode; especially gaps between cells will affect impedance spectrums. An ITO (Indium Tin Oxide) electrode was used for this biosensor, fibroblast cells were attached on electrode, after 2-4 days a thin layer was formed by fibroblast cells. The effect of additional glucose concentrations, Nyquist diagram was chosen, which allows more sensitive measurement. Glucose and cell interaction depended on the glucose intake ability of cells through Glut 1 and Glut 4. To examine the specificity of the biosensor, D-mannitol was chosen instead of glucose and no variation was observed. No signal variation was obtained by inhibition of glucose transporters, therefore glucose intake provides metabolic process in order to detect glucose molecules, not incorporated by increased osmotic pressure. Addition of carbohydrate not metabolized by cells did not give any signal variation in impedance curves. The calibration curve was constructed from 0 to 14mM glucose concentration [32]. On the other hand for this study, as a biorecognition receptor, cell surface transporters were used for biosensor indirectly. This provided selective measurement.

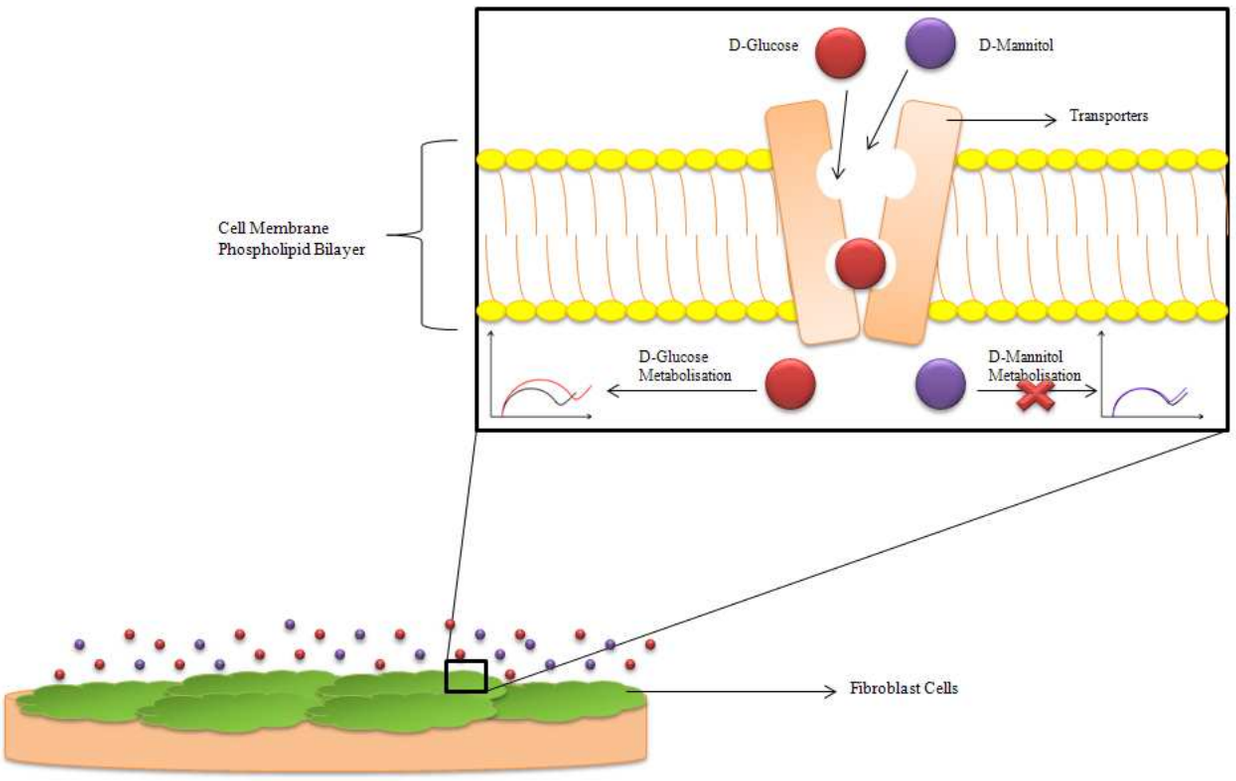


Figure 7. Schematic representation of fibroblast cell based impedimetric biosensor.

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Tong et al. have declared an annexin V-based biosensor for quantitatively detecting early apoptotic cells. Apoptosis is programmed cell death in organisms and it is necessary for organism homeostasis. Measurement of apoptosis can give clues about any metabolic process, for example anti-tumor therapies, drugs. Annexin V is a calcium dependent phospholipid-binding protein with high affinity for phosphatidylserine, which is altered in translocation in plasma membrane for early apoptotic cells [33]. Therefore this protein can be used as PS exposure marker upon cell membranes. In this study, the authors described first time annexin V-modified biosensor was developed for quantitatively detecting early apoptotic cells. Gold electrode was modified; 1,6-hexanedithiol, gold nanoparticles, annexin-V, respectively. Then PS exerted cells were detected by using this modified electrode. As you can a slight difference on electrode can be detected by using impedance spectroscopy and this detection provides apoptotic cell existence. This biosensor's signal was dependent upon calcium ion concentrations. Therefore, before PS detection, the modified electrode treated with 5μM Ca^{2+} for 1 h [34].

5. Some footnotes about impedimetric biosensors

One of the most important points of impedimetric biosensors is to find proper molecule that shows affinity to your analyte. Secondly your measurement process will be considered; especially modification steps and oxidation/reduction ability of redox probe will define experimental parameters such as frequency, bias potential, electrical circuit model. In order to obtain sensitive detection, faradaic impedance is proposed to reach lower frequencies, because of reduction/oxidation properties of redox probe, the electrons, which produced by this oxidation/reduction, can move easier. This transportation can be measured as electron transfer resistance. On the other hand non-faradaic impedance is only measure resistance and surface capacitance, that technique is for whole surface by electrical circuit model.

Electrical circuit model is constructed by characteristic of impedance curve, which depends on the electrical conductibility of electrochemical measurement solution, electrode surface and interaction between electrode-electrolyte. Impedance spectroscopy provides sensitive and label free detection by its sensitive electrode surface characterization ability. Especially for sensitive measurements faradaic experiments are considered that is better, by using redox probes, it is possible to reach lower frequencies, thus lower detection limits can be reached by examination of the modified electrode surface in lower frequencies. Electrode surface modification plays a crucial role for impedimetric measurements that inhomogenicity on electrode surface, pin holes or a direct interaction with bare electrode surface after a failed modification and electrolyte, the electrons, which through electrode surface without confront any electrical resistance, will give a false impedance spectrum. In this case of inhomogenity, capacitance element of circuit model is redefined as constant phase element to solve inhomogenity problem. In case of lower or less altered signals, impedance spectroscopy provides signal amplification by modifying electrode surface with a molecule or electrode surface can be charged same as redox probe's charge. Another challenging factor for impedimetric biosensor is the affinity, low affinity or affinity for wide range will reduce the importance or usefulness of the impedimetric biosensor.

Analyte	Bioreceptors	LOD	Linear Range	Reference
17β-estradiol	Estrogen Receptor-α	$1.0x10^{-13}$M	$1.0x10\text{-}1.0x10^{-11}$M	[8]
AIV H5N2	Monoclonal Anti-H5 Antibody	$1x10^{1.2}ELD_{50}$/ml	$1x10^{1.2}ELD_{50}$/ml-$1x10^{5.2}ELD_{50}$/ml	[36]
Atrazine	Anti-atrazine Antibody	10 fg/ml	10 fg/ml-1ng/ml	[11]
Chronic Lymphocytic Leukemia Gene Sequence Mutation	25-Mer PorphobilinogenDeaminase (PBGD) Gene	1.0×10^{-12}M	7.0×10^{-12}–2.0×10^{-7}M	[26]
Concavalin A	Glucose	1.0 pM	3.3pM-9.3nM	[29]
Der f2	Murine Monoclonal Antibody	2.0 µg/ml	2-300 µg/ml	[12]
Glucose	3T3-L1 Fibroblast Cells		0-14 mM	[32]
Lipopolysaccaride Layer of *S. marcescens*	CramoLLLectin	25 µg/ml	25-200 µg/ml	[31]
Lysozyme and Adenosine	Lysozyme and Adenosine Specific Two Aptamers	0.01µg mL^{-1} and 0.02nM, respectively	0.2-40 nM Adenosine	[25]
PS exerted cells	Annexin V	5 Fu		[34]
Thrombin	Thrombin Aptamer	0.013 nM	0.1-30 nM	[35]
VEGF	VEGFR-1	100 fg/ml	100-600 fg/ml	[4]

Table 1. Comparison of Some Impedimetric Biosensor Systems

6. Conclusion

Consequently, biosensor systems have been developing, the most important factors are selectivity and low cost. These specialties are very important of Point-Of-Care usage. Electrochemical impedance spectroscopy is very effective technique for label-free molecule detection. It can provide sensitivity, low cost and selective biosensor systems. As you can read from above, by using specific molecules such as DNA, Aptamer, Receptor, Antibody, Specific Proteins etc. it is possible to construct selective and sensitive impedimetric biosensor systems.

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