Chapter 12
Self-Assembled Monolayers, Biological Membranes, and Biosensors

Adsorbed organic layers on surfaces are models for biological bilayers, lipid membranes, and biosensors. EIS is an important tool in the study of these materials. In this chapter, the study of self-assembled monolayers, bilayers, and biosensors will be presented.

12.1 Self-Assembled Monolayers

Self-assembled monolayers (SAMs) [482–484] are usually formed by a spontaneous reaction of alkanethiols with solid metal surfaces (e.g., Au, Ag, Cu). They can also be prepared using the Langmuir-Blodgett method. They form well-ordered, close-packed monolayers and may be applied in the control of wetting and adhesion, chemical resistance, photosensitization, molecular recognition for sensor applications, in fundamental studies of electron transfer, and other applications. The chains might be easily functionalized with various groups, e.g., hydrophilic or redox groups, or become biocompatible. SAMs form a single hydrophobic layer of hydrocarbon chains, usually strongly linked to the metallic support; however, multiple layers might also be deposited. Thiols form chemically bonded monolayers Me–S–R (Fig. 12.1) where metal, Me, is gold.

Usually, quite compact layers are obtained. The simplest electrical equivalent model represents the solution resistance in series with the capacitance of a SAM, R_s C_{SAM} (Fig. 12.2a). More detailed analysis reveals that the layers are rarely purely capacitive and their capacitance is in parallel with their resistance, R_{SAM}, leading to a circuit: $R_s(C_{SAM}\,R_{SAM})$. Moreover, a diffuse double layer exists at the SAM/solution interface [485, 486]. In such a case, the electrical equivalent circuit contains a diffuse-layer capacitance, C_{dl}, in parallel with the resistance, R_{dl} (Fig. 12.2b).

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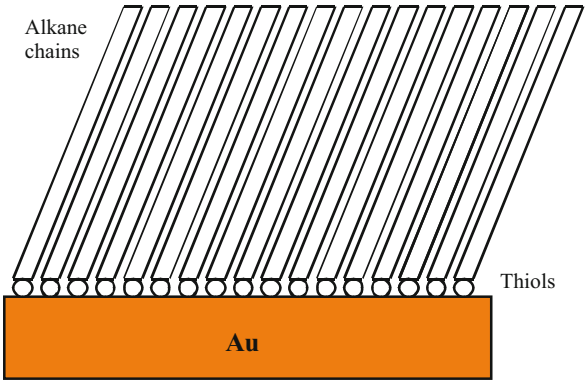

Fig. 12.1 Schematic representation of SAM at gold substrate

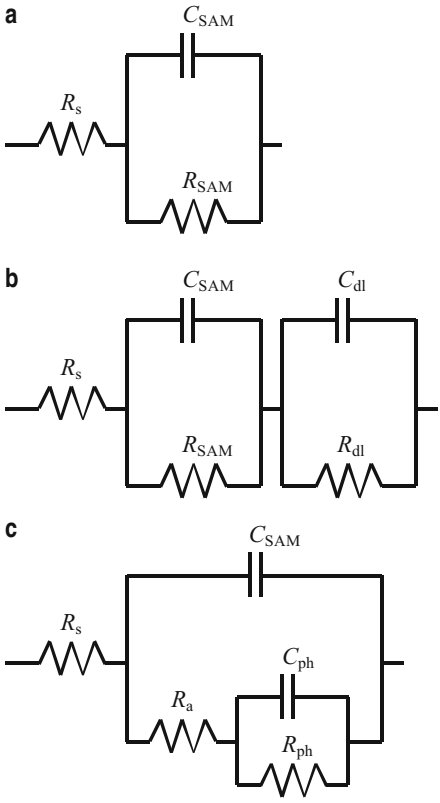

Fig. 12.2 Electrical equivalent circuits of SAMs: (**a**) simplified model, (**b**) model including diffuse double layer at SAM/solution interface, (**c**) SAM in presence of pinholes

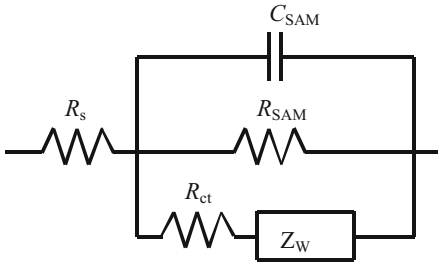

Fig. 12.3 Electrical equivalent system for SAMs with pinholes in presence of redox process

However, double-layer capacitance is usually neglected because it is much larger than that of the SAM (the dielectric constant of the organic layer is much lower than that of water), $C_{dl} \gg C_{SAM}$. In a series connection of capacitances, the resulting capacitance, $1/C_{eq} = 1/C_{dl} + 1/C_{SAM}$, and only a small capacitance, becomes important. In this case, the model reduces to that in Fig. 12.2a. This effect could be more important when ionized groups are attached to alkyl chains [485]. It is interesting to add that the reciprocal of the interfacial capacitance is linear with the number carbon atoms for longer chains, confirming that the capacitance is inversely proportional to the layer thickness [483, 487].

However, the SAMs formed are rarely ideal and they contain small defects called pinholes, e.g., bare metal sites or other defects. In such cases, an additional branch must be added in parallel (Fig. 12.2c) consisting of the resistance, R_a, in series with the parallel connection of the pinhole resistance, R_{ph}, and its capacitance, C_{ph} [488, 489]. The surface coverage of the pinholes is usually very small and does not influence very much the electrode capacitance. The presence of such pinholes can be easily detected using cyclic voltammetry or EIS [483, 490, 491]. The simplified electrical equivalent model of the redox reaction in the presence of pinholes is displayed in Fig. 12.3 [490, 492].

The resistance of SAMs is rarely observed [490]. The faradaic impedance of pinholes represented as charge transfer resistance, R_{ct}, in series with the Warburg mass transfer impedance, Z_W, should be modified to account for the diffusion layer overlap. In fact, an electrode covered with pinholes resembles a microarray electrode [493], and the corresponding impedance developed for microarray electrodes should be used [490, 494, 495]. On well-prepared SAMs a linear relationship between the logarithm of the heterogeneous rate constant, ln k_0 and the number of CH_2 groups exists for thicker layers. Protsailo and Fawcett [492] found such a relationship for SAMs consisting of thiols C_9–C_{18} at monocrystalline gold electrodes using $[Ru(NH_3)_6]^{3+/2+}$ as a redox species. However, a better model to study electron tunneling through SAMs are SAMs with attached redox centers [483, 496, 497]. Redox processes at SAMs were recently reviewed [498].

EIS allows for a good characterization of SAMs in the absence and presence of redox species in solution and attached to alkyl chains. These studies allowed for testing the theory of electron tunneling. SAMs are also used in biosensors (see below).

12.2 Lipid Bilayers

A lipid bilayer is a thin, polar membrane made of two layers of lipid molecules. These molecules contain one polar group attached to a nonpolar hydrophobic chain and typically contain phospholipids. A schematic diagram of such a bilayer is displayed in Fig. 12.4. In polar solvents, the polar groups are directed toward the solution and hydrophobic tails toward the core of the bilayer. The cell membranes of almost all living organisms and many viruses, as well as the membranes surrounding cell nuclei and other subcellular structures, are made of lipid bilayers [499]. However, most biological membranes are extremely complex structures consisting of hundreds or thousands of different molecules. Bilayer lipid membranes (BLMs) prepared in vitro resemble biomembranes and are used as models to understand their functioning. Studies of a planar BLM model system led to their applications in the fields of, for example, specific electrodes, biosensors, biomolecular electronic devices, and solar energy transductors [500, 501].

BLMs may be prepared as supported or freestanding membranes. Membranes deposited on solid supports are easier to prepare and more stable and have been studied extensively [502–508]; however, the use of an unsupported bilayer membrane separating two solutions is more important [509–517]. The resistances of the membranes are large and the capacitances are on the order of approximately $1\ \mu\text{F cm}^{-2}$.

The impedance of freestanding membranes may be studied using the system displayed in Fig. 12.5. It uses a homemade differential amplifier. Similar possibilities occur with the use of two potentiostats, allowing for the grounding of the working electrodes (e.g., from Bank Elektronik [518]) or using a Solartron dielectric interface [519, 520].

For a simple planar bilayer membrane the impedance studies produce one semicircle on the complex plane plots according to the model $R_s(R_mC_m)$, from which the resistance, R_m, and capacitance, C_m, of the membrane can be simply determined.

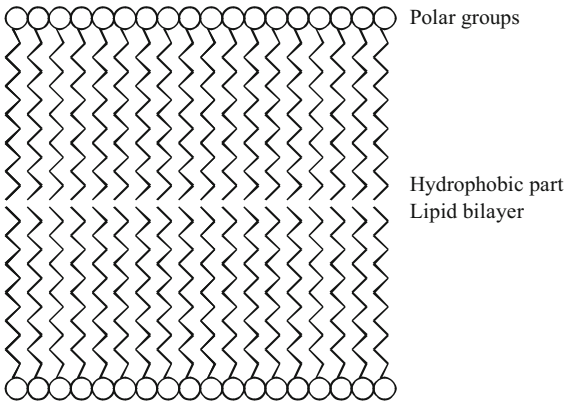

Fig. 12.4 Schematic representation of bilayer lipid membrane

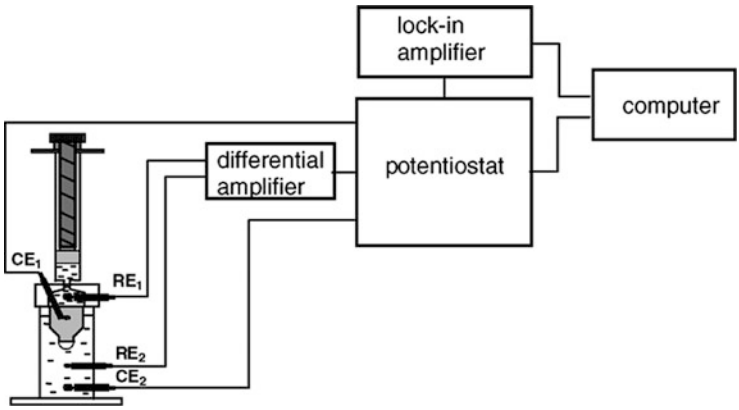

Fig. 12.5 System allowing measurements of membrane impedance containing differential amplifiers (From Ref. [512], copyright (2005), with permission from Elsevier)

Interesting studies were performed on membranes composed of two components, where the formation of complexes or domains was possible [510, 511, 514–516, 521–525].

Let us look at the example of interactions between phosphatidylethanolamine and α-tocopherol in bilayer membranes [515]. In this case, the formation of lipid domains consisting of phosphatidylethanolamine, 1, and phosphatidylethanolamine-α-tocopherol, 3, of a certain composition is observed. Assuming that only these two domains exist in the membrane and that the electrical parameters are additive, one can write an equation for the capacitance and resistance of the membrane:

$$C_{\mathrm{m}} = C_1 c_1^{\mathrm{S}} S_1 + C_3 c_3^{\mathrm{S}} S_3; \quad \frac{1}{R_{\mathrm{m}}} = \frac{1}{R_1} c_1^{\mathrm{S}} S_1 + \frac{1}{R_3} c_3^{\mathrm{S}} S_3 , \tag{12.1}$$

where C_1 and C_3 are the capacitance of the membrane composed of pure components 1 and 3 (in $\mathrm{F\,cm^{-2}}$), R_1 and R_3 are the specific resistances of these components ($\Omega\,\mathrm{cm^2}$), $c_{\mathrm{i}}^{\mathrm{S}}$ are the surface concentrations of these components (mol $\mathrm{cm^{-2}}$), and S_{i} are the surface areas occupied by 1 mol of pure components ($\mathrm{cm^2\ mol^{-1}}$). Introducing the molar fraction of the components and the relation between surface concentrations,

$$x_1 = \frac{c_1^{\mathrm{S}}}{c_1^{\mathrm{S}} + c_3^{\mathrm{S}}}; \quad x_3 = \frac{c_3^{\mathrm{S}}}{c_1^{\mathrm{S}} + c_3^{\mathrm{S}}}; \quad c_1^{\mathrm{S}} S_1 + c_3^{\mathrm{S}} S_3 = 1, \tag{12.2}$$

and eliminating $c_{\mathrm{i}}^{\mathrm{S}}$ it is possible to obtain relations for the membrane capacitance and conductance:

$$C_{\mathrm{m}} = \frac{C_1 S_1 + (C_3 S_3 - C_1 S_1) x_3}{S_1 + (S_3 - S_1) x_3}; \quad \frac{1}{R_{\mathrm{m}}} = \frac{R_1^{-1} S_1 + \left(R_3^{-1} S_3 - R_1^{-1} S_1\right)}{S_1 + (S_3 - S_1) x_3}. \tag{12.3}$$

These equations can be simplified for small values of x_3 to

$$\frac{C_{\mathrm{m}}}{x_3} = \frac{C_1}{x_3} + \frac{(C_3 - C_1) S_3}{S_1}; \quad \frac{1}{R_{\mathrm{m}} x_3} = \frac{1}{R_1 x_3} + \frac{\left(\frac{1}{R_3} - \frac{1}{R_1}\right) S_3}{S_1}. \tag{12.4}$$

These equations represent linear plots versus ${x_3}^{-1}$. It was found that these plots were linear, from which the surface area of the domain $S_3 = 320$ Å^2 was determined indicating that a stoichiometry of the phosphatidylethanolamine-α-tocopherol domain of 4:1 was obtained [515]. A similar formalism was used for systems containing three components when 1:1 complexes were formed in the membrane.

Ionic transport across membranes is of fundamental importance in physiology. To model such processes, lipid bilayers without [526] and with the addition of complexing compounds, such as crown ethers [527, 528], valinomycin [508, 513], or gramicidin [502, 504, 529–531], were also studied.

12.3 Biosensors

Biosensors are analytical devices that are able to detect biological components. SAMs and lipid bilayers are often used as platforms for the immobilization of biosensors [532–534], but other supports, such as $Si-SiO_2$ [535–537], TiO_2 [538], and polymers [539, 540], can also be used. Electrochemical biosensors [541, 542] might use potentiometric, field-effect transistor, amperometric, or impedimetric transducers.

Impedimetric [535, 543–547] biosensors monitor biointeractions using impedance. Biomaterials that can interact with electronic transducers include proteins such as, for example, enzymes, antibodies or antigens, and oligonucleotides or DNA fragments. This is a relatively new domain, and a Scopus search for *impedimetric biosensor* shows less than 200 citations.

Although they have been successfully applied at the academic level, commercial devices are being developed [548, 549]. Impedimetric immunosensors could potentially be used for qualitative purposes, such as, for example, in the detection of bacteria, pregnancy tests, allergy screening tests, etc [546].

Impidimetric biosensors can be:

a. Capacitive (nonfaradaic), measuring changes in the electrode capacitance in the absence of redox reactions; for example, antibody-antigen interactions cause adsorption and a decrease in the electrode capacitance.

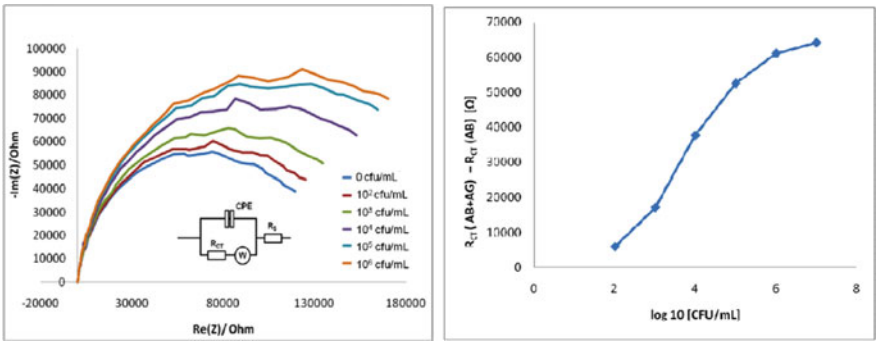

Fig. 12.6 *Left*: Complex plane plots obtained for a sensor with antibodies immobilized by covalent bonding as function of *E. coli* DNA. *Right*: Increase in R_{ct} versus logarithm of concentration. Concentrations are in colony-forming units per milliliter (From Ref. [552], copyright (2009), with permission from Elsevier)

b. Faradaic, in the presence of redox reactions measuring changes in the charge transfer resistance. These changes occur during adsorption/desorption of biomaterials.

In what follows, examples of the application of these methods will be presented. It should be added that these methods might be used in solutions or in microfluidic conditions [547].

An example of a capacitive biosensor is detection nucleic acids. SHARP Laboratories [548, 549] developed low-cost, selective, and multiuse sensors based on gold interdigitated microelectrodes with a microfluidic chamber. Gold electrodes were functionalized with DNA oligonucleotide (complementary to the targets) bound to the surface with thiol groups. They were applied to detect genes from *Escherichia coli* bacteria. An increase in impedance (measured at one frequency) with time was observed as the selective adsorption proceeded. After measurements the chambers were washed and regenerated. The response was nonlinear, and the slopes $\Delta Z/\Delta t$ versus log concentration were analyzed. The detection limit was 5–10 nM for DNA targets.

An inverse process of enzyme-catalyzed dissolution of a polymer coating [550, 551] leads to an easily detected increase in electrode capacitance. Such a process was studied for urea and immunoglobulin G (IgG) [550] and urea and creatinine in serum [551].

An example of redox sensors is a bacteria biosensor [552]. Gold working electrodes were covered with SAMs of mercaptohexadecanoic acid, and antibodies were attached covalently to the surface. $K_3[Fe(CN)_6]$ was used as the redox probe. Bacteria attached to the surface receptor, causing an increase in the charge transfer resistance. The increase in R_{ct} was plotted versus the logarithm of bacteria concentration (Fig. 12.6), and it presented a nonlinear calibration curve with a detection limit of 10–100 colony-forming units/mL.

The use of nanomaterials [545] such as gold nanoparticles and carbon nanotubes increases the sensor surface and enhances analytical detection. The use of PEGylated arginine functionalized magnetic nanoparticles for early detection of cervical cancer has been reported [553]. This sensor displayed good selectivity and sensitively down to 10 cells mL^{-1}.

Impedimetric biosensors represent a new and rapidly developing research area that permit the fast detection of biomaterials. Commercialization of such sensors is only beginning. More applications may be found in recent reviews [535, 543, 544, 546].

12.4 Conclusions

The area of impedance studies of self-assembled monolayers, bilayers, and biosensors is among the recent applications of EIS. They represent an area under development that has interesting fundamental and possibly practical applications. Research in this area falls somewhere between electrochemistry and biology and requires a sound knowledge of both domains. Apparently, knowledge of EIS is often insufficient.