

Chapter 1

General Introduction

1. Introduction

1.1. Bio-molecules and their sensing

Bio-molecules are the molecules that are present in all living organisms and are involved in the maintenance and metabolic processes of life. Carbohydrates, proteins, lipids and nucleic acids are the main bio-molecules in living systems. These macro bio-molecules are made up of several building blocks or subunits. For example, D-glucose is the building block of the polysaccharides starch and cellulose, amino acids are the building units of proteins, fatty acids are the monomeric units of membrane lipids, and pentose sugars, phosphate and nucleobases- adenine, guanine, thymine, cytosine, and uracil are the building blocks of the nucleic acids.¹ [Fig.1.1].

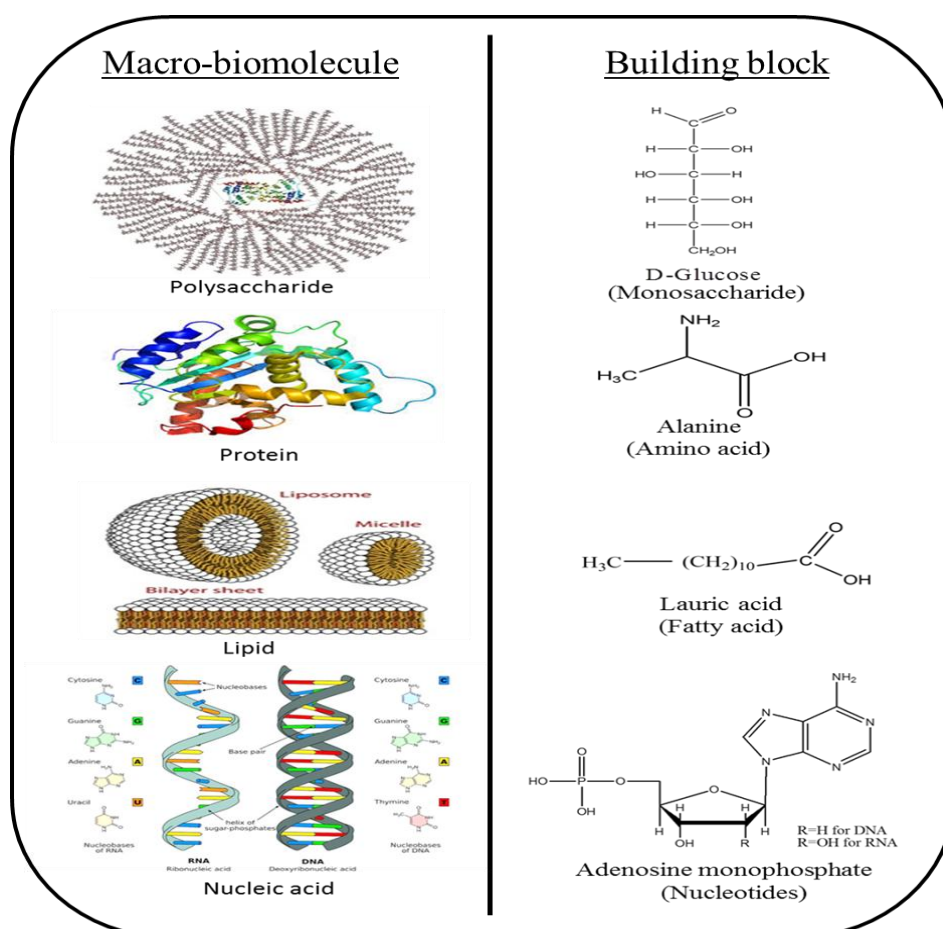


Fig. 1.1. Macro-biomolecules and their corresponding building block.

Along with these there are several other bio-molecules such as vitamins, hormones, neurotransmitters, caffeine, uric acid, hydrogen peroxide, adenosine triphosphate, nicotinamide adenine dinucleotide and many more in the living system. These bio-molecules are critically important for human health, a little abnormality causing several diseases. Some common examples like abnormal blood sugar levels cause diabetes which represents a leading cause of several problems for human health like complications of the retina, circulatory system, kidneys etc.²; high levels of uric acid in the blood are indications of some diseases like gout, kidney stone, hyperuricemia and Lesch-Nyhan syndrome;³ and the abnormal concentrations of different catecholamine neurotransmitters like dopamine, epinephrine etc. in the body fluids result in several neurological- diseases like Parkinson's disease, Schizophrenia, Huntington's disease, etc.⁴ Therefore, it is very important to detect and determine the amount of these important bio-molecules in a selective and accurate manner.

Various methods have been employed for the detection and quantitative determination of different biologically important molecules. Titrimetry,⁵ chromatography,⁶ spectrophotometry,⁷ fluorometry,⁸ chemiluminescence,⁹ flow injection method,¹⁰ and electrochemical techniques¹¹ are mostly employed to detect and measure different bio-molecules. Among them electrochemical techniques have received much attention due to their low cost, rapid response time, simplicity of construction, ease of miniaturization, need of small testing sample volume and most importantly, it's high selectivity and sensitivity.

Many biologically important molecules do not show any response when using solid electrodes within a potential window. Usually high overpotential (a potential exceeding the analytes' formal potential) is required for the electrochemical detection of such compounds.¹² At high potential, a large background current can be generated causing an inferior detection limit.¹³ The stability of the electrode response is also greatly affected due to oxide formation or adsorption of analyte or reaction products over the electrode surface. Determination of trace analyte in presence of large excess of coexisting component may severely interfere. To eliminate interfering components, complicated sample pre-treatments are often required.¹⁴

To overcome these problems and improve quantitative measurement, the chemical nature of the electrode surface can be controlled and manipulated by chemical modification.

1.2. Chemically modified electrodes

Murray and co-workers first introduced the term “chemically modified electrode” in the year 1975,¹⁵ since then, modification of electrode surface and electrochemical reactions at the interface have received considerable attention. When a metal (Pt, Au, etc.) or carbon electrode is dipped into an aqueous solution, a layer of water molecules covers its surface and sometimes, the added species or impurity present in the solution gets attached to the electrode surface. The adsorbed species often changes the electrochemical behavior of the electrode. For example, for a given electrochemical process, the adsorbed species may block the access of electroactive species to the electrode surface and decrease the observed oxidation and/or reduction current. Electrode surface modification with specific molecules in a controlled manner is of current attention as it can produce electrodes with new and interesting properties. These chemically modified electrodes may form the basis of different applications of electrochemistry such as in electro-synthesis, corrosion protection, electrochromic display, molecular electronics and sensing. Along with these, the fundamental studies of such modified electrodes give a better insight of the nature of charge transfer and charge transport process in thin film.¹⁶

1.3. Techniques of electrode modification

Different techniques have been used to modify electrodes surfaces. The techniques fall into four major categories and these are (a) incorporation of modifying materials into the electrode body, (b) direct attachment of modifying materials, (c) forming film from modifying materials and (d) incorporation of modifying materials into the supporting matrix.¹⁷ Mostly the layer on the electrode surface is electroactive and therefore it can exchange electrons with underlying substrate material (metal or carbon) and be oxidized or reduced.

1.3.1. Incorporation of modifying materials into the electrode body

In this technique the modifying species is directly incorporated into a carbon paste matrix. This technique is extremely attractive for repetitive or routine electrochemical measurements. The fabrication process is quite simple and can be controlled conveniently with renewable surface coverage.¹⁸ The main advantage is their wide flexibility for the attachment of different modifiers including organic and inorganic polymers, ion exchange resins and insoluble materials.¹⁹

1.3.2. Direct attachment of modifying materials

1.3.2.1. Chemisorption

Some molecular species are favored to stay at the surface of the electrode than in the bulk solution and attach spontaneously to the surface. Organic species, such as thiols (R-SH), sulphides (R-S-R) or disulfides (R-S-S-R) have strong tendency to chemisorb onto a soft metal surface (Au, Ag, Pt or Cu)²⁰ via the formation of metal-sulphur bond and spontaneously organize to form a dense monolayer called self-assembled monolayer (SAM) (Fig. 1.2).

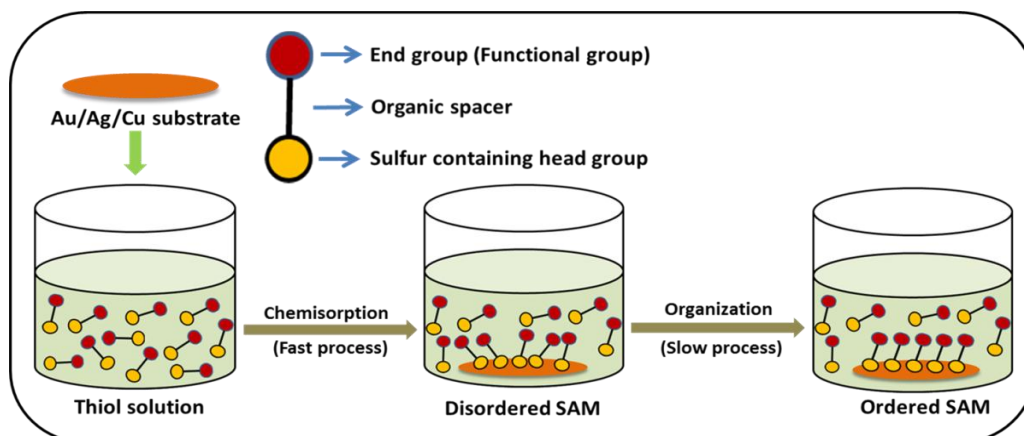


Fig. 1.2. Schematic representation of SAM formation on soft metal substrate.

Long chain alkane thiol forms densely packed, highly oriented and ordered monolayer on gold surface from its dilute solution whereas short chain alkane thiol forms thin monolayer.²¹ Functionalized SAM is formed when the layered molecules bear a chemically active group like NH_2 , COOH in addition to anchoring group thiol. The orientation of the monolayer is not influenced as long as the functional group is relatively

small such as NH_2 , OH etc. but the packing density as well as ordering is decreased when bulky groups like COOH , ferrocene are present.²² Functionalized SAMs can bind with metal ions, complexes or nanoparticles through electrostatic interactions. The immobilized redox centers act as electron transfer mediator which facilitates the charge transfer between the electrode and the analyte.²³

1.3.2.2. Covalent attachment

To form chemical bonds between a molecule and the metal or carbon electrode surface, chemical reactions are generally carried out. An electrode surface can be oxidized so that the surface can be considered as consisting of hydroxyl groups. This hydroxylated surface can react with organosilane, which can again react with another molecule of interest.²⁴ Here, the silane acts as a kind of adhesive for attaching the molecule to the electrode surface. This technique is expected to form a monolayer of molecules on the electrode (Fig. 1.3).

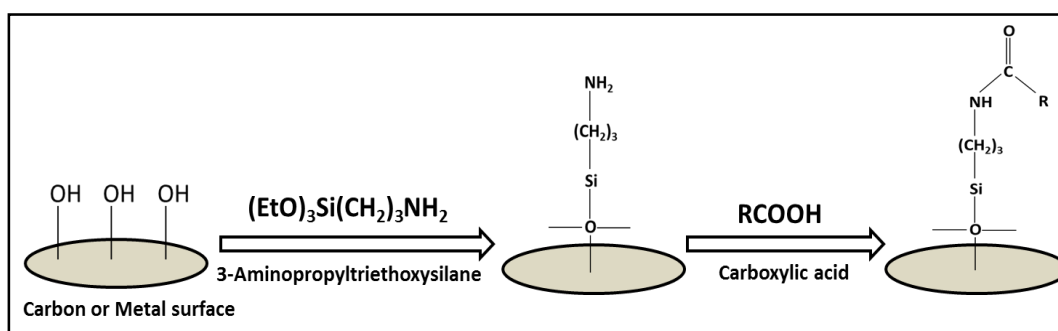


Fig. 1.3. Carbon or metal surface modification through covalent attachment of silane.

Polymerization is also observed during silanization reaction and a thicker layer which is equivalent to several monolayers is formed.

The electrochemical reduction of aryl diazonium salt is another technique for the covalent attachment of molecular species to the electrode surfaces. This process is usually termed electrografting.²⁵ The reduction of diazonium salt ArN_2^+ produces aryl radical. Such aryl radicals are unstable and immediately react with the metal or carbon or semiconductor electrode surface²⁶ to form covalent bonds. The grafting process is described in Fig. 1.4.

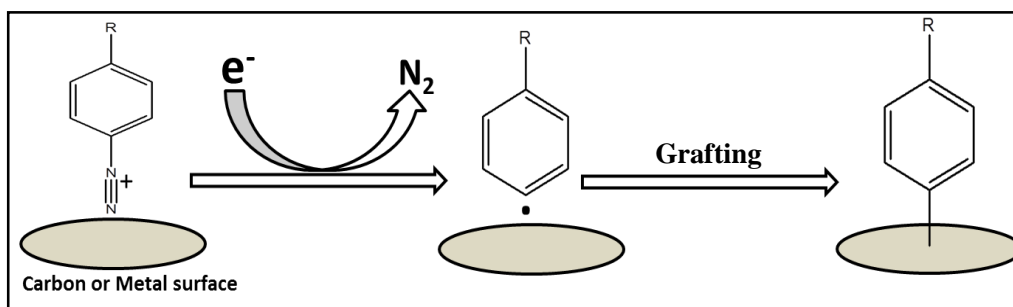


Fig. 1.4. Grafting process of aryl diazonium salt on carbon or metal surface.

1.3.2.3. Electrodeposition

Electrodeposition is referred to as either electroplating or electrophoretic deposition (EPD) on the electrode surface.²⁷ Electroplating process is based on ionic species in aqueous medium and there is a charge transfer during the deposition to produce the metal or oxide layer on the electrode whereas EPD occurs in a suspension of particles in organic solvent where the deposition occurs without any reaction. The main driving force for electrophoretic deposition is the charge and the electrophoretic mobility of the particles under the influence of the applied electric field.²⁸ The electroplating of metal oxides involves two consecutive steps. In the first step, metal hydroxide gets precipitate on the electrode surface due to the reaction of metal ions in alkaline solution and in the next step the metal oxide is formed through a condensation or dehydration process. The last step can occur during electrodeposition or by a subsequent annealing procedure.²⁹ Anodic oxidation is another alternative for metal oxide layer formation. Here metallic anode is the source of metal ions and the metal oxide layer is deposited on top of the metal electrode.²⁹

In electrophoretic deposition technique generally before deposition, the metal oxide nanoparticles are synthesized by sol-gel, precipitation or hydrothermal methods.³⁰ The main challenge of this process is the synthesis of a stable dispersion that forms a layer with uniformity, appropriate thickness and good properties. Presence of dispersants, binders or other additives influences the agglomeration and charge of the particles and can contribute to the tuning of the layer properties.²⁷ Electroless deposition (autocatalytic) is another deposition technique where a reducing agent dissolved in the

electrolyte is the electron source for the redox reaction and no external power supply is required.³¹

1.3.3. Film formation from modifying materials

1.3.3.1. Electropolymerization

A polymer film can be grafted on to an electrode surface using electrochemical techniques including galvanostatic (constant current), potentiostatic (constant potential) or potentiodynamic (such as CV) methods. Electrochemical polymerization of monomer is achieved when an electrode is immersed in a suitable electrolyte containing the monomers and the desired doping salt and an anodic potential or current is applied.³² The properties of polymers can be changed by attaching different chemical groups to the monomer before polymerization. The film thickness can be tuned by controlling the charge passed during deposition. The polymer film contains very low impurity as strong oxidizing agents are not used. Polymer films are usually porous and uneven when constant current or potential mode is applied for polymerization whereas smooth and compact films are formed using CV.³³ Several parameters such as nature of electrode, applied potential, solvent, nature and concentration of electrolyte, temperature, pH and mode of polymerization highly affect the electrochemical polymerization process.

1.3.4. Incorporation of modifying materials into the supporting matrix

1.3.4.1. Sol-gel techniques

In addition to the several electrode modification techniques, the sol-gel method is very promising for the fabrication of an integrated three dimensional network of materials like metal oxide from a colloidal solution at the electrode surface.³⁴ Various Inorganic and inorganic-organic composite films have been prepared through hydrolysis and condensation of metal alkoxides, most notably the silicon alkoxides that exhibit excellent thermal stability, chemical inertness and tunable porosity.³⁵ Nanomaterials have often been used in the fabrication of complicated sol-gel three dimensional networks. This technique provides a simple, renewable and convenient route to incorporate temperature sensitive materials including organic molecules and several bio-molecules like proteins,

enzymes onto such porous ceramic materials.³⁶ The resulting inert and stable matrices are very much useful for electrochemical sensing applications.

1.4. Characterization techniques of chemically modified electrode

After the modification of the electrode surface it is necessary to prove that the surface has been modified and also find out the nature of layer, surface morphology and its physical and chemical properties. As very little amount of material is attached onto the surface, therefore, very sensitive analytical techniques are needed for proper characterization.

1.4.1. Contact angle measurements

A contact angle measurement between the surface monolayer and a liquid gives the surface structure information. A contact angle is the angle that the liquid/vapor interface makes with the solid surface. Hydrophilic surfaces have small contact angles whereas hydrophobic surfaces have large ones.³⁷ A large number of contact angle studies is available in the literature. For example, electrode modification with 4-aminothiophenol (4-ATP) and gold nanoparticles can be monitored by contact angle measurement (Fig. 1.5).

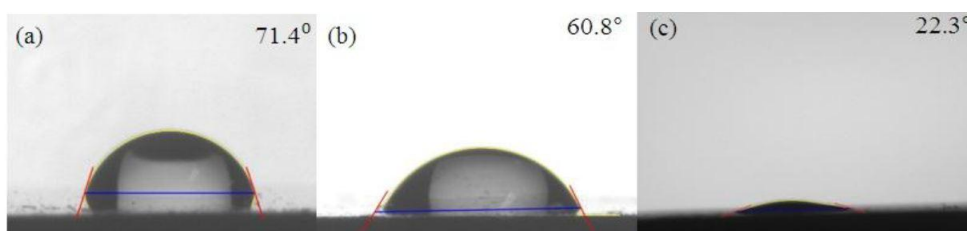


Fig. 1.5. Contact angles of the carbon plate (a), 4-ATP-GC (b) and AuNPs- 4-ATP-GC electrode. Reproduced from Ref. 38, Copyright (2014) ESG.

The water contact angle of the carbon plate electrode is 71.4° and after 4-aminothiophenol grafting on the electrode surface the contact angle decreased to 60.8° and when gold nanoparticles was immobilized on the 4-aminothiophenol monolayer modified electrode surface it further decreased to 22.3° .³⁸ From this observation it can be inferred that the stepwise modification was successfully done and hydrophilicity was introduced to the surface.

1.4.2. Electrochemical methods

Cyclic voltammetry is a useful technique which allows probing into the features of a modified electrode. The amount of material present on the electrode surface can be analyzed readily by this technique. Monolayer of material shows a peak with an integrated area in cyclic voltammetry which is equivalent to the material present on the electrode surface (Fig. 1.6).

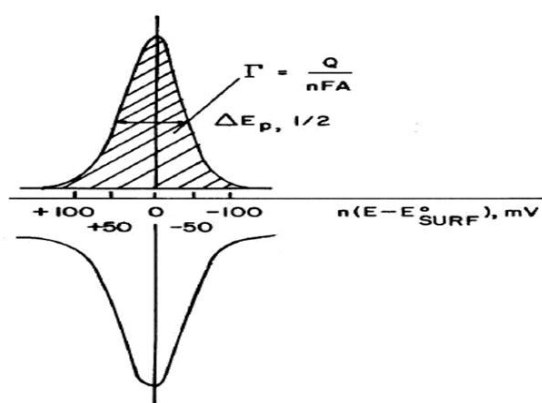


Fig. 1.6. Ideal cyclic voltammetric behavior for a surface layer on an electrode. Reproduced from Ref. 39, Copyright (1980) Elsevier.

When the thickness of the layer increases the integrated area becomes larger which represents the greater amount of material attached on the electrode surface than before. Surface concentration of the modifier can be calculated using the following equation $\Gamma = Q/nFA$, where Γ is the surface coverage, Q is the charge, n is the number of electrons involved, F is the Faraday constant and A is the electrode area.³⁹ Detailed study of the shape of the CV curve and nature of the response can provide a wealth of information about chemical interactions in the layer and the rate of charge transfer through them. To study electrode surface blocking behavior in different steps of sensor preparation, generally $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple is used.

Electrochemical impedance spectroscopy (EIS) is another effective technique for electrode surface characterization. For a given system the EIS data presentation is commonly known as Nyquist plot (imaginary component of impedance, $-Z''$ versus real component of impedance, Z') and every point on the Nyquist plot is characteristic of the complex impedance at the given frequency. There are two frequency regions in the

Nyquist plot, the high frequency region (kinetically controlled) where the semicircle is observed and the low frequency region (diffusion limited process) where Warburg line is observed. (Fig. 1.7a). The diameter of the semicircle correspond to the charge transfer resistance (R_{ct}) where smaller the semicircle, faster is the electron transfer.⁴⁰ A wide range of frequencies is used in this technique and therefore kinetic as well as mass transport parameters in electrode process can be conveniently determined. A typical example is shown in Figure 1.7a which exhibits that the 2-mercaptoethanol modified electrode has the lowest charge transfer resistance compared to those of the bare and complex immobilized gold electrodes.

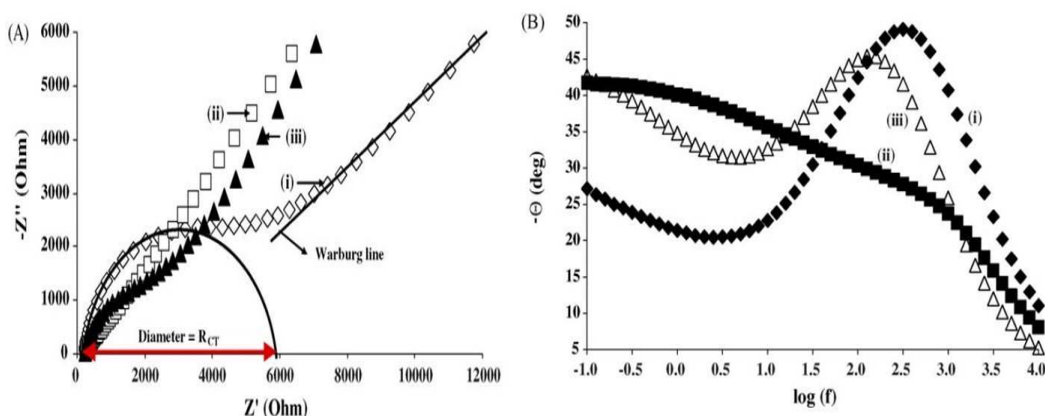


Fig. 1.7. Nyquist plot ($-Z''$ versus Z') (a) and Bode plot (b) obtained from impedance measurements in the presence of $1\text{mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$ (1:1) mixture in 0.1 M KCl solution for (i) Au (ii) 2-mercaptoethanol-Au (iii) iron tetra-carboxylic acid chloride phthalocyanine complex-2-mercaptoethanol-Au. Reproduced from Ref. 40, Copyright (2007) Elsevier.

From Nyquist plot, frequency information is not obtained which the Bode plot (phase shift; $-\theta$ versus $\log(\text{frequency})$) (Fig. 1.7b) can give. In Bode plot, the phase angle at low-to-medium frequency region gives the important results, for example the phase angle greater or equal to 90° means that the modified layer behaves like an ideal capacitor.⁴¹ This means that no current leakage occurs at the defect sites and that the film forms an insulating layer. If the phase angle is less than 90° ,⁴¹ then the layer is viewed as a

contaminated capacitor meaning the modified layer is permeable to solution ions. Fig. 1.7b shows that SAMs are either permeable to solution ions or are catalytic.⁴⁰

1.4.3. Spectral and Microscopic techniques

X-ray photoelectron spectroscopy is a powerful method for characterizing the modified electrode surface. For example, XPS of self-assembled 3-mercaptopropionic acid ($\text{SH}-(\text{CH}_2)_2-\text{COOH}$) on gold electrode surface shows the presence of sulphur - 2p (S_{2p}) at 162 eV (Fig. 1.8a).⁴² In this spectrum, the first binding energy of 162.1 eV is attributed to Au-S bond and the second higher binding energy of 163.5 eV is considered as adsorbed free thiol groups that are non-covalently bonded with gold electrode.

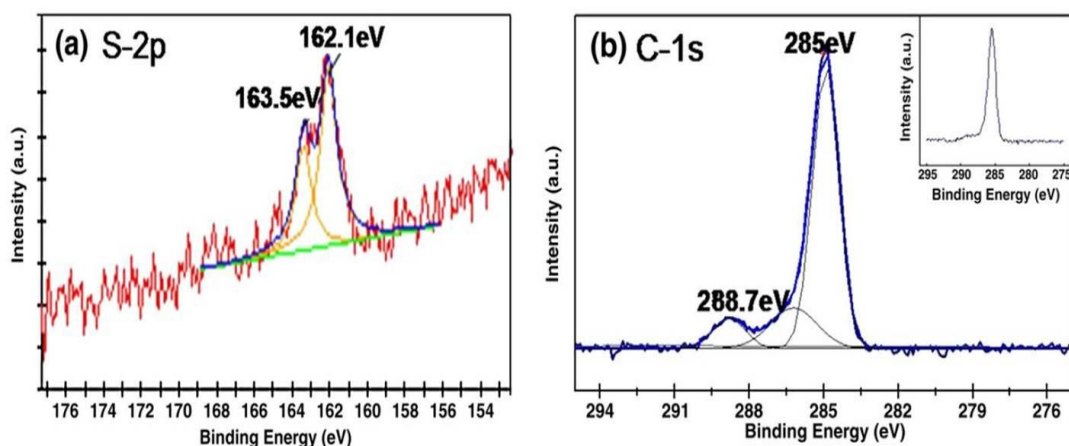


Fig. 1.8. HR-XPS spectra for 3-mercaptopropionic acid modified gold electrode. Reproduced from Ref. 42, Copyright (2010) Elsevier.

Fig. 1.8b shows the high energy carbon 1S (C_{1s}) spectrum of 285 eV attributed to the aliphatic carbon (CH_2 group) chain in 3-mercaptopropionic acid. The other peak observed at 288.7 eV (Fig inset) was due to the carboxyl group of the monolayer which is not observed at the bare gold electrode and probed the binding of 3-mercaptopropionic acid on gold electrode.

ATR-FTIR spectroscopy is used to characterize different functional groups present in the monolayer on the electrode surface and in turn is useful to monitor the stepwise surface modification process. For example, a comparison of ATR-FTIR spectra of 2-

mercaptoethanol film modified gold electrode and the only compound, 2-mercaptoethanol, can confirm that the electrode was modified or not. Fig. 1.9b shows a characteristic band 2555 cm^{-1} for $-\text{SH}$ group which is absent in Fig. 1.9a. This observation attributed that the S-H bond was cleaved and formed a new S-Au bond. This experiment proves that 2-mercaptoethanol has been assembled on the gold electrode.⁴³

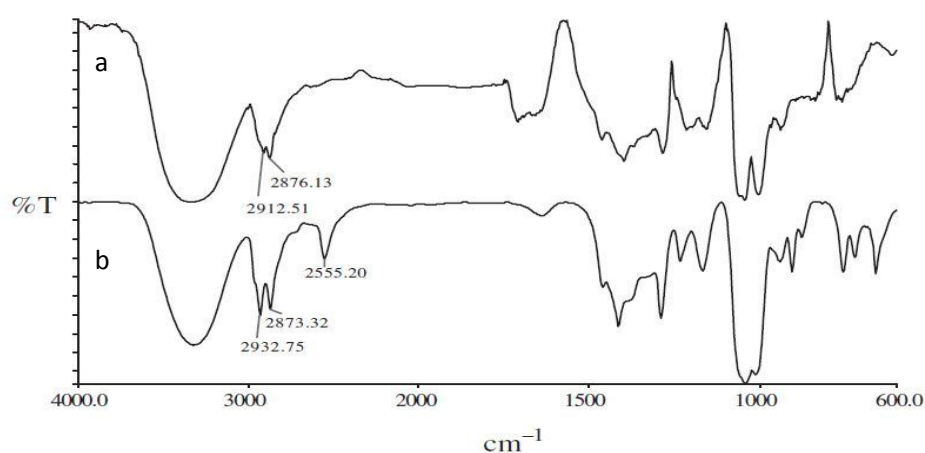


Fig. 1.9. ATR-FTIR spectra of 2-mercaptoethanol-Au electrode (a) and 2-mercaptoethanol (b) Reproduced from Ref. 43, Copyright (2002) Taylor & Francis.

Scanning electron microscopy (SEM) is often useful for providing information about the morphology and porosity of the layers on the surface. A rough estimate of the thickness of the layer over electrode surface can be possible using SEM.⁴⁴ The SEM instrument is usually equipped with an Energy dispersive X-ray analysis system to carry out the chemical compositional analysis of the surface layer.⁴⁵ Fig. 1.10 shows the characteristic SEM images and corresponding EDS analysis of ZnO nanotube array on ITO electrode and after modification with glucose oxidase and Nafion. SEM images revealed the tube like structure while the EDS analysis indicated that ZnO nanotubes consist of Zn and O whereas modified ZnO nanotubes consist of Zn, O, C and F. SEM images and EDS analysis confirmed the proper modification of ZnO nanotube array-ITO electrode surface with nafion and glucose oxidase.⁴⁶

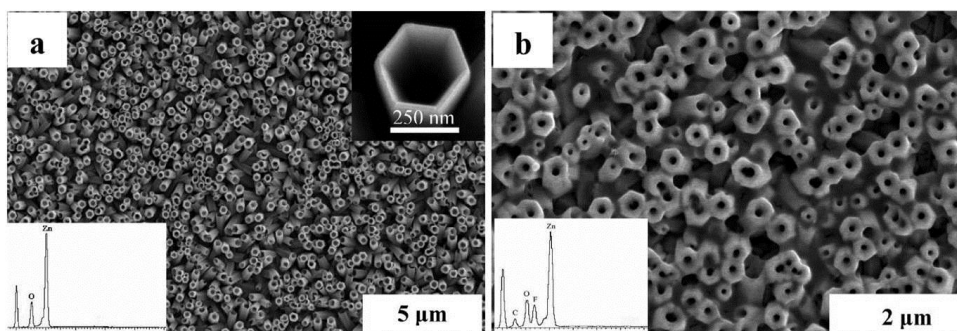


Fig. 1.10. SEM image and EDS analysis (inset) of ZnO nanotube array (a) and Nafion-GOx-modified ZnO nanotube array (b) on ITO coated glass electrode. Reproduced from Ref. 46, Copyright (2009) The American Chemical Society.

Atomic force microscopy (AFM) is a local probe technique used to investigate the surface structure of chemically modified electrode at molecular level. AFM can provide images in three-dimensional topography with resolution at the atomic scale. Figure 1.11 shows the AFM images of (3-mercaptopropyl)trimethoxysilane (MPS) modified gold electrode and it depicts a complete coverage of the self-assembled monolayer of MPS over the grain boundaries of the gold (111) surface and the formation of porous structure. The line structure of the MPS-SAM is observed with line spacing of 2-3 nm. The results confirm that a highly ordered MPS monolayer forms on gold surfaces.⁴⁷

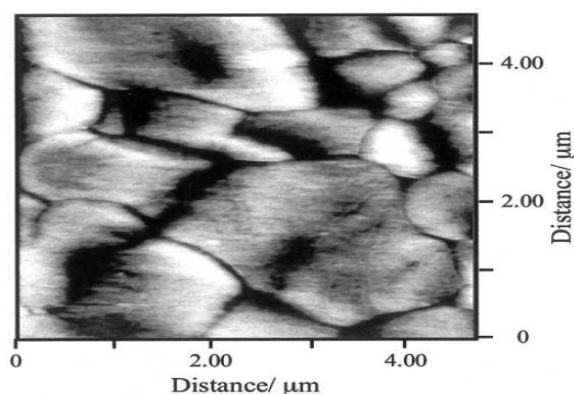


Fig. 1.11. AFM image of the (3-mercaptopropyl)trimethoxysilane modified Au (111) at $(5 \times 5) \mu\text{m}$ magnification. Reproduced from Ref. 47, Copyright (2003) Elsevier.

1.5. Electrochemical sensing of some selected bio-molecules using thiol SAM modified gold electrode - A short review

Self-assembled monolayer modified gold electrodes have widely been used in electrochemical sensing technology as they provide a convenient way of attaching modifying material at electrode surface and can tune the selectivity and/or sensitivity of the analysis. Among different chemically modified electrodes, self-assembled thiol monolayer modified gold electrodes are quite promising as they are easy to prepare, stable, selective and sensitive for such bio-molecule detection. Many review articles on electrochemical sensing of bio-molecules using chemically modified electrodes are available.⁴⁸ The following paragraphs describes the electrochemical sensing of some biologically important molecules such as ascorbic acid, uric acid, dopamine, epinephrine, glucose, hydrogen peroxide and nitrogenous bases using different thiol monolayer modified gold electrode.

Ascorbic acid (AA), a water-soluble vitamin, is present naturally in fruits and vegetables. It is an important preservative and antioxidant agent used in food industry, pharmaceutical formulations and cosmetic applications. Thus, the development of a simple and rapid method for its detection has attracted great attention. Ascorbic acid has been detected electrochemically using different thiol modified Au electrodes. R. S. Freire *et al.* reported a bis(2,2'-bipyridyl)copper(II) complex immobilized on 3-mercaptopropionic acid SAM modified gold electrode for the detection of AA. The sensor allowed a convenient quantification at the levels down to 0.08 μM .⁴⁹ Later, X. Lu *et al.* developed a thiol derivatized cobalt(II)porphyrine monomolecular film on gold electrode for the electrochemical sensing of AA. The catalytic current increased linearly with AA concentration in the range of 0.08 - 390.00 μM and the detection limit was 4.6 nM.⁵⁰ Recently, R. K. Shervedani and co-workers used a cytochrome C oxidase immobilized on gold-mercaptopropionic acid *via* Zr(IV) as a metal ion glue for the detection of AA. The substrate showed a linear response from 10.0 μM to 1.30 mM with a detection limit of 5.0 μM .⁵¹

The development of voltammetric sensors for the determination of uric acid (UA) in human fluid such as urine and serum has received considerable interest in recent years. The electrochemical response and the electrochemical reaction of UA on a penicillamine

(PCA) self-assembled monolayer modified gold electrode were studied by W. J. Kang *et al.* In phosphate buffer solution (pH 5.0), the peak current was proportional to the concentration of UA in the range of 60-700 μM and 20 -700 μM for the cyclic voltammetry and differential pulse voltammetry methods with the detection limits of 5.0 μM and 3.0 μM , respectively.⁵² In biological fluids UA is present with AA and moreover, at the bare electrode the oxidation of AA occurs at a potential close to that of UA. L. Wang and co-workers demonstrated the electrochemical behaviors of UA at the PCA self-assembled monolayers modified gold electrode (PCA/Au) in presence of AA and the modified electrode shows excellent sensitivity, good selectivity and antifouling properties. In DPV measurements, the PCA/Au electrode can separate the UA and AA oxidation potentials by about 120 mV and can be used for the selective determination of UA in the presence of AA. The detection limit was 1.0 μM .⁵³ C. R. Raj and T. Oshaka described the fabrication of a voltammetric sensor based on a SAM of mercaptobenzimidazole (MBI) for the determination of UA in the presence of AA. The MBI monolayer facilitates the oxidation processes of AA and UA. The voltammetric signals of UA and AA are well separated with a potential difference of 270 mV and AA was not interfered with the measurement of UA at the monolayer-modified electrode. The practical utility of this monolayer-modified electrode was also demonstrated by measuring UA in human blood serum.⁵⁴ Later, C. R. Raj and co-workers modified a gold electrode with the SAM of heterocyclic thiol, mercaptotriazole and used for flow injection amperometric sensing of UA and AA. This electrode could sense as low as 1.0 μM in the presence of 10 fold excess of interfering AA. The sensitivity (0.012 $\mu\text{A}/\mu\text{M}$) of the electrode towards AA was not changed in the presence and absence of AA.⁵⁵ Zhao's group studied the electrochemical behavior of UA and AA at the L-cysteine (L-Cys) SAM modified gold electrode (L-Cys/Au electrode). The modified electrode showed an excellent electrocatalytical effect on the oxidation of UA and AA. In DPV measurements, the L-Cys/Au electrode can separated the oxidation peak potentials of UA and AA present in homogeneous solution by about 236 mV though the bare electrode gave a single broad response. A successful elimination of the fouling effect by the oxidized product of AA on the response of UA was achieved at the L-Cys/Au electrode. The detection limit of UA and AA was 2.0 μM and 11.0 μM , respectively.⁵⁶

Dopamine (DA) is an important catecholamine neurotransmitter in the mammalian central nervous system playing a crucial role in brain activities like movement, emotional response, behavior and so on. Accurate detection and determination of dopamine is critically important for the treatment of different neurological diseases. Since DA is highly electroactive, electrochemical sensing is the best way for the detection purpose. However, it's very low concentration (10.0 – 1000.0 μM), high over potential and the large excess of interfering species such as UA and AA in biological fluids creates serious problems in electrochemical measurement of DA. T. Liu *et al.* studied the voltammetric behavior of DA on a gold electrode modified with the SAM of N-acetylcysteine and obtained a pair of well-defined redox peaks of DA at the SAM modified gold electrode. The oxidation peak current increased linearly with the concentration of dopamine in the range of 1.0 – 200.0 μM . The detection limit was 0.8 μM . Furthermore, the modified gold electrode was applied for the simultaneous detection of DA and AA.⁵⁷ A. Mohadesi and co-workers investigated the voltammetric behaviour of DA and AA on a gold electrode modified with the self-assembled monolayer of 2-mercaptoethanesulfonate. This negatively charged layer formed a discriminating layer against AA and DA based on the electrostatic interactions. Thus modified electrode enabled selective determination of DA in an excess of AA. The detection limit was 1.1 μM .⁵⁸ F. Malem *et al.* presented the ω -Mercapto carboxylic acid, monolayer modified gold electrodes for the electrochemical detection of DA in presence of large excess ascorbic acid.⁵⁹ C. Y. Liu *et al.* synthesized a new thiol compound, 2-amino-5-mercapto-1, 3, 4-triazole (MATZ) and used it to modify gold electrode surface. The modified electrode was used to determinate DA and UA simultaneously with a detection limit of 0.8 μM for DA and 1.0 μM for UA, respectively. The modified gold electrode was also successfully applied for the determination of the contents of DA and UA in synthetic urine sample.⁶⁰ A gold electrode modified with SAM of cysteamine conjugated with functionalized multiwalled carbon nanotubes (MWCNTs) was investigated by R. Saberian *et al.* for the simultaneous detection of DA and UA. The detection limit was obtained as 20.0 μM and 100.0 μM for DA and UA, respectively.⁶¹ Recently, M. Behpour and co-workers reported a comparative electrochemical behavior of SAMs of Schiff's base 2-[[*Z*]-1-(3-furyl)methylidene]amino]-1-benzenethiol for determination of DA in the presence of high concentration of AA and UA.⁶² U. E.

Majewska *et al.* investigated the influence of the per(6-deoxy-6-thio)- α -cyclodextrin monolayer modified gold electrode for the detection of DA in presence of AA.⁶³ G. Diao *et al.* fabricated a self-assembled thiolated calix[n]arene (n = 4, 6, 8) films on gold electrode and used it for the electrochemical determination of dopamine. The thiolated calix[8]arene-Au electrode showed an excellent detection capability for DA with high current density of $1.5 \text{ mA mmol}^{-1} \text{ Lcm}^{-2}$, broad linear range (1.0 – 1000.0 μM) and low detection limit 0.5 μM .⁶⁴

A hormone secreted by the medulla of adrenal glands, epinephrine (EP), is another important catecholamine neurotransmitter in the mammalian endocrine and central nervous systems. Various modified electrodes have been constructed to be used as electrochemical sensors for EP such as penicillamine modified Au electrode.⁶⁵ In presence of UA, EP was detected using meso-2,3-dimercaptosuccinic acid SAM on Au electrode. The detection limit of EP was 0.054 μM by cyclic voltammetry technique and 0.053 μM by DPV and that of UA is 0.084 μM by cyclic voltammetry and 0.042 μM by DPV techniques.⁶⁶ Later, gold nanoparticles, meso-2,3-dimercaptosuccinic acid and cystamine were used successively to modify gold electrode surface and applied for the quantitative determination of EP in the presence and absence of AA and UA.⁶⁷ The sensor showed a wide linear range 0.1 to 800.0 μM with high detection sensitivity $\sim 7 \text{ Acm}^{-2} \text{ mol}^{-1} \text{ dm}^3$ and low detection limit of 28.0 μM . Very recently, N F Atta *et al.* detected EP in presence of sodium dodecyl sulfate (SDS) using cysteine over Au-nanoparticles. The results showed that Au/Au_{nano}-Cys---SDS electrode can selectively determine epinephrine in the coexistence of a large amount of uric acid and glucose. In addition, a highly selective and simultaneous determination of tertiary mixture of AA, EP, and acetaminophen were explored at this modified electrode. Excellent recovery results were obtained for determination of EP in spiked urine samples at the modified electrode. Au/Au_{nano}-Cys--SDS electrode shows excellent reproducibility, sensitivity, and long term stability.⁶⁸

Detection and measurement of glucose is very important in the clinical diagnosis. Various functionalized thiol SAM modified gold electrodes were used for the selective and sensitive detection of glucose. Y. Kajiya *et al.* prepared a self-assembled 4-aminothiophenol monolayer modified gold electrode containing chemically bound glucose oxidase (GOx) and 2-aminoethyl ferrocene to detect glucose.⁶⁹ Under optimized

condition the sensor showed sensitivities more than $30 \mu\text{A cm}^{-2}$ for 20 mM glucose. Latter S. J. Choi *et al.* reported an electrochemical sensor for β -D-glucose using a SAM of thiolated α -cyclodextrin on gold electrode surface.⁷⁰ When this SAM modified electrode containing ferrocene is exposed to a glucose solution, the captured electroactive ferrocene molecules are replaced by the electroinactive glucose molecule, thus lowering the current of ferrocene oxidation. The decrease of current is directly proportional to the amount of glucose present and by this protocol glucose can be analyzed up to 0.8 mM. S. Yabuk *et al.* used polyglutamate-glucose oxidase complex to immobilize on a cysteamine modified gold electrode and monitored the glucose sensing behaviour. They reported that the response time was quite good ~ 15 s and the linear response range was between 5-100 μM glucose. They also showed that the complex based electrode enhanced the glucose response than the glucose oxidase modified gold electrode.⁷¹ V. Annandan and co-workers used two different chain length alkane thiol SAMs to immobilized the functionalizing enzyme glucose oxidase onto gold nanopillar (gold electrode with standing gold nanopillars forming three dimensional electrode) modified electrode and utilized it for the detection of glucose. The results showed that long chain alkane thiol modified electrode make a high surface coverage with less defect and a higher electron transfer resistance on the other hand SAM of short chain alkanethiol give rise to a higher detection sensitivity.⁷² Glucose oxidase - 3-mercaptopropionic acid modified nanopillar gold electrode exhibited a higher sensitivity of $3.31 \mu\text{A.mA}^{-1}.\text{cm}^{-2}$ which is much higher than that for a gold nanotube modified electrode ($0.4 \mu\text{A.mA}^{-1}.\text{cm}^{-2}$).⁷³ Recently, M. Karadag *et al.* reported a AuNPs / 6-(ferrocenyl)hexanethiol and cysteamine / polyaminoamine dendrimers / glucose oxidase modified gold electrode for glucose sensing. In optimized condition the linear range for the glucose was 1.0-5.0 mM with a detection limit of 0.6 mM (S/N = 3).⁷⁴ Very recently C. Hu *et al.* reported a convenient and effective glucose sensing matrix based on Pt@ bovine serum albumin nanocomposite on gold electrode along with covalent adsorption of GOx. The modified electrode was able to detect glucose in the linear range of 0.5 – 12.05 mM and an optimal detection limit of 15.0 μM .⁷⁵

Hydrogen peroxide is another biologically important molecule and is extensively used as an oxidizing agent. H_2O_2 is a catalytic by-product of different oxidases such as glucose

oxidase, cholesterol oxidase etc. and also a substrate for the enzyme horseradish peroxidase.⁷⁶ Excess H₂O₂ could damage DNA or proteins by causing oxidative stress.⁷⁷ Therefore, precise monitoring of H₂O₂ is of practical significance. H.-Y.Chen *et.al.* reported a sensor for the sensitive detection of H₂O₂ and the sensor was fabricated by using horseradish peroxidase (HRP)-labelled gold colloids immobilized on gold electrode surface by cysteamine monolayer. The sensor responded in the concentration range of 0.39 μM – 0.33 nM with the detection limit of 0.15 μM. They also reported that with the aid of electron mediator- catechol, the smaller sized HRP-labelled gold colloids exhibited a higher amperometric response to the reduction of H₂O₂ than that observed on the larger sized HRP-labelled gold colloids.⁷⁸ Later, H. Baccar *et al.* made a comparative study of H₂O₂ sensing by HRP enzyme-glutaraldehyde on 16-mercaptohexadecanoic acid modified gold and cysteamine functionalized palladium nanoparticles on modified gold surface and obtained the limit of detection 75.0 μM and 7.5 μM, respectively.⁷⁹ J. Liu *et al.* used poly(vinylpyrrolidone) protected Prussian blue nanoparticles onto L-cysteine SAM modified gold electrode to detect H₂O₂.⁸⁰ Recently, T. H. Lu and co-workers developed a ferric ion immobilized on three dimensional nanoporous gold films modified with 4-mercapto-3-(phosphonomethylamino)butanoic acid on gold electrode for the selective determination of H₂O₂ in the linear range of 0.9 to 500.0 μM. The limit of detection was 1.0 nM.⁸¹

The nitrogenous bases purines (guanine and adenine) and pyrimidines (thymine and cytosine) are two of the essential building blocks of deoxyribonucleic acid (DNA), the basic elements of the cell programming machinery. For the detection of nucleobases modified glassy carbon electrodes were most frequently used⁸² but very limited numbers of report are available on thiol modified gold electrodes. The self-assembled alkane thiol monolayer on gold electrode is a very good covalent linker to immobilize ssDNA probe over the electrode surface.⁸³ For the detection of adenine and guanine, nucleic acid immobilized gold electrode was demonstrated to be a very useful sensor⁸⁴ and also a useful tool for the detection of DNA hybridization and damage.⁸⁵ As guanine is the most electrochemically active nitrogenous base, DNA oxidation results in DNA damage occurs as guanine oxidation peak.⁸⁶ Recently, R. K. Shervedani *et al.* developed a functionalized mercaptopropionic acid self-assembled monolayer on gold electrode with 5-amino-1,10-

phenanthroline–Fe(II) complex for the selective electrochemical detection of guanine. The modified gold electrode showed a wide dynamic range of response with a linear range of 1.0 to 100.0 μM guanine and a detection limit of 0.17 μM .⁸⁷

1.6. Thesis work

The thesis focuses on the gold electrode surface modification with thiol containing molecule like L-cysteine, penicillamine, thiophenyl-azo-imidazole, 4-(pyridine-4'-amido)thiophenol and immobilization of metal ions, complexes or nanoparticles on the functionalized thiol modified electrode surface. The thesis consists of electrode modification, characterization and studies of electrochemical sensing behavior towards the biologically important molecules such as glucose, hydrogen peroxide, dopamine, epinephrine, ascorbic acid, uric acid, adenine and guanine.

References in this thesis are presented in the form of author's names, *journal name*, year, **volume**, initial page number.

1.7. References

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