

CHAPTER 7

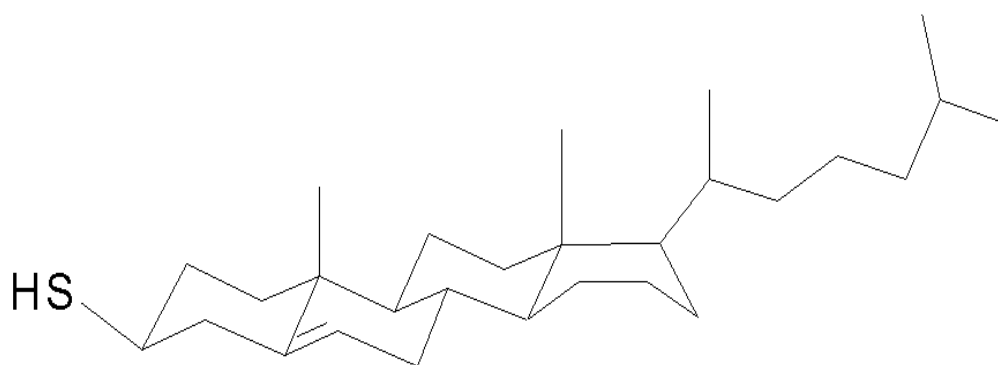
Self-Assembled Monolayers of Thiocholesterol on Gold: A potential application for electroanalytical sensors

7.0 Introduction :

Self-assembled monolayers of alkanethiols form a ($\sqrt{3} \times \sqrt{3}$) R 30° overlayer structure on Au (111) surfaces. The monolayers provide an ideal model systems for studying the role of different molecules in biological membranes and have contributed to a large extent in the field of long range electron transfer [1,2], wetting [3,4], protein adsorption [5], and molecular recognition [6]. However, SAMs are seldom defect free. Structural defects in most SAMs are due to surface roughness of gold substrate [7], mismatching in chain orientation of adjacent molecular domains [8], and etching process during the self-assembly of thiol molecules on gold [9].

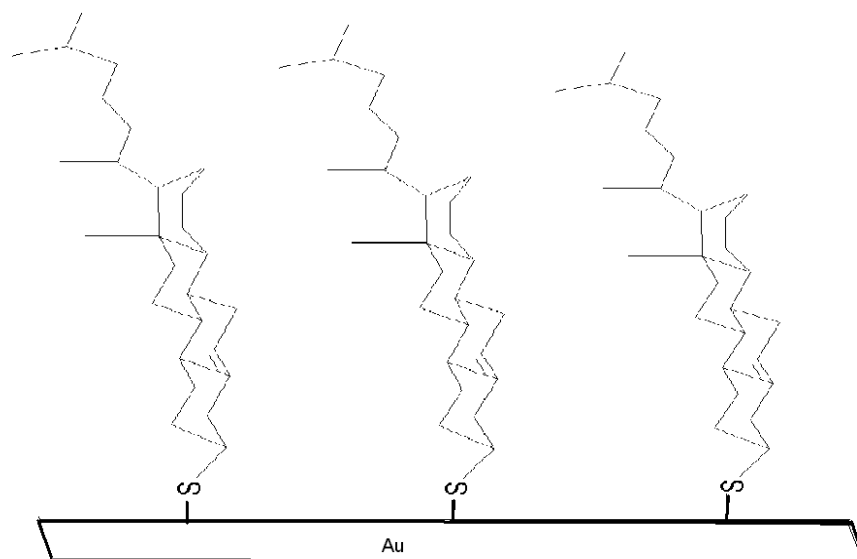
Cholesterol plays very important role in biological membranes. A large number of model systems of biological membranes have been examined to understand the effect of cholesterol. The effect of cholesterol on the properties of membranes depends on the concentration and orientation of cholesterol in the membranes [10,11]. Recently, Yang et al.[12,13] have studied the SAMs of Thiocholesterol (TC) molecules on gold. TC is a cholesterol molecule containing a thiol moiety in place of a hydroxyl group at the 3- β position. The molecular structure is a nearly polycyclic steroid

ring with branched aliphatic chain. Hence, TC SAM on gold provides an ideal model system to investigate biomembranes and to study a variety of molecular recognition processes at surfaces. Due to the size and irregular shape of the TC molecule, a large number of defects are created on the surface of gold, which is very much unlike the well ordered densely packed monolayer of alkanethiol molecules [12].



Molecular structure of thiocholesterol

The defects in TC SAM on gold are periodic in nature, whereas the defects in alkanethiol SAM are more random.



Schematic diagram of thiocholesterol SAM on gold showing periodic defects

Yang et al. [12] have studied the formation of thiocholesterol SAM on gold by ellipsometry, contact angle measurements, infrared spectroscopy

and cyclic voltammetry. The TC monolayer exhibits only 65% of average surface coverage than that of alkanethiol SAM with large number of molecular defects. These defects exist because of a mismatch between the size and shape of TC molecule and the pinning distance at the Au (111) crystal lattice. The size of these defects are 5-8 Å. The cyclic voltammetric study of TC coated Au electrode shows microelectrode array behaviour [12]. Yang et al. [12,13] have formed mixed monolayer of thiocholesterol (TC) and 11-mercapto undecanoic acid (MUA) on gold and studied the influence of TC on the molecular composition and orientation in the mixed monolayer by using infrared reflection-absorption spectroscopy (IRAS), X-ray photoelectron spectroscopy (XPS), ellipsometry, contact angle measurements and cyclic voltammetry. Their results indicate that the TC molecules maintain their orientation in the mixed monolayer. This is in contrast to MUA molecules in the mixed monolayer, which exhibit more disordered orientation [13]. Their cyclic voltammetric results [13] show that the mixed monolayers are more densely packed and impermeable than the pure TC and MUA monolayers. The mixed monolayer of TC+MUA provides ideal model systems of biological membranes as it is just the thiol analogues of cholesterol and fatty acid. Yang et al. [14] have also studied the influence of mixed monolayers on the heterogeneous electron transfer process of $[\text{Fe}(\text{CN})_6]^{3-}$. The effect of TC on the electron transfer kinetics of ferricyanide redox reaction was studied by Tafel plots. In a pure MUA SAM, the electron transfer is governed by penetration through the monolayer. However, introduction of TC into the SAMs give rise to diffusion controlled electron transfer in addition to penetration. By increasing the TC content, the rate of electron transfer process is enhanced due to diffusion. This behaviour was explained by a model, where there is a

transition from defect free monolayer (pure MUA SAM) to a defect rich structure containing an array of ultramicroelectrodes (Mixed MUA+TC SAM) which is reflected in the electron transfer kinetics of ferricyanide redox reaction. The defect rich structure of TC SAM can be potentially used for electroanalytical and biosensors applications. Yang et al. [15] have utilized the defect rich hydrophobic surface of thiocholesterol SAM in batch and Flow Injection Analysis (FIA) of hydrophobic analytes. Yang et al. [15] have quantified several phenothiazines in FIA in a broad linear concentration range of 0.5-100 μM .

In our study, we have formed mixed hydrophobic SAM of TC +HDT (hexadecanethiol) and study its effect on the electron transfer kinetics of ferrocyanide redox reaction. We have determined the surface coverage of TC coated gold surface from the measurement of true surface area by potential cycling in 0.1 M perchloric acid. We have formed TC SAM on 40 μm diameter Au microdisk electrode and studied the electron transfer properties of ferrocyanide redox reaction. We find from literature that the TC SAM has not been used for metal ions detection so far. In this context, we have used the TC SAM as sensors for detection of Cu^{+2} ions. We have also studied the ethanol oxidation reaction on TC SAM coated gold as this will help in understanding the electron transfer processes of small organic molecules in defect sites.

7.1 Experimental :

7.1.1 Electrodes and cells

Gold working electrode was constructed by proper sealing of 99.99% pure gold wire (Arora Mathey) of 0.5 mm diameter with soda lime glass having

thermal expansion coefficient close to that of gold. The electrode has a geometric area of 0.002 cm^2 . This small area working electrode has highly reproducible true surface area (as measured by potential cycling in 0.1 M perchloric acid) even after repeated usage. A conventional three electrode electrochemical cell was used in this study. A platinum foil of large surface area was used as counter electrode. A saturated calomel electrode (SCE) was used as reference electrode. Evaporated gold substrates on glass were used as working electrode in some of our studies. Evaporation of gold ($\sim 100 \text{ nm}$ thickness) on glass was carried out using vacuum evaporation unit (Hindhivac) at a pressure of $2 \times 10^{-5} \text{ mbar}$. Chromium underlayers ($2\text{-}5 \text{ nm}$ thickness) were deposited on glass before gold evaporation to improve the adhesion of gold on glass. The gold samples were used as strips for electrochemical studies with a well defined area exposed to the electrolyte solution, rest of the portion being insulated with parafilm and teflon. These gold samples were cleaned with Piranha solution (mixture of concentrated sulphuric acid and 30% hydrogen peroxide in $3:1$ ratio) for 30 s before each experiment and finally rinsed in millipore water. During the experiment, a blanket of nitrogen gas was maintained on top of the solution. The cell was cleaned thoroughly before each experiment and kept in a hot air oven at 100°C for at least one hour before the start of the experiment.

7.1.2 Electrode pretreatment and thiol adsorption

Immediately before use, the gold electrode was polished using aqueous slurries of progressively finer alumina ($1.0, 0.3, \text{ and } 0.05 \text{ }\mu\text{m}$ sizes), sonicated to remove alumina particle and finally etched using dilute aqua regia ($3:1:4$ ratio of concentrated HCl , concentrated HNO_3 and water) for one minute. TC SAM was formed by dipping the gold electrodes in to 1 mM

TC solution in ethanol for 1 hour and then rinsed in ethanol and millipore water.

7.1.3 Instrumentation

Cyclic voltammetry was carried out using an EG&G potentiostat (Model 263A) interfaced to a PC through a GPIB card (National Instruments). The potential ranges and scan rates used are shown in the respective figures. Electrochemical impedance studies were performed using an EG&G potentiostat (Model 263A) along with a two phase lock-in amplifier (Stanford Research Systems Model SR830). A pure sine wave of 10 mV amplitude was derived from the lock- in amplifier for application to the cell. Impedance studies were carried out at formal potential of the respective systems as measured from the cyclic voltammograms.

7.1.4 Chemicals

Ethanol (Emerck), sodium hydroxide (BDH), mercaptobenzothiazole (BDH), sodium fluoride (Qualigens), potassium ferrocyanide (Loba), potassium ferricyanide (Qualigens), copper sulphate (Merck), sodium acetate (Qualigens), sulphuric acid (Merck), perchloric acid (Ranbaxy), thiocholesterol (TC), hexadecanethiol (Aldrich) were used in our studies.

7.2 Results and discussion :

7.2.1 Determination of surface coverage of TC SAM :

The surface coverage of TC SAM on gold was determined by measuring the charge of gold oxide stripping peak by potential cycling in 0.1 M perchloric acid. Figure 1 shows the cyclic voltammogram of 1 mM TC (thiocholesterol coated Au electrode) in 0.1 M perchloric acid. Figure 1 (Inset) shows the voltammogram for bare gold electrode. We have determined the fractional

surface coverage of the adsorption of thiocholesterol onto gold surface using the integrated charge values from gold oxide stripping peak. Fractional surface coverage θ , is given by

$$\theta = \frac{Q_B - Q_M}{Q_B} \times 100$$

Where, Q_B = charge of oxide stripping peak for bare Au electrode (uncoated) $1285 \mu\text{C cm}^{-2}$. Q_M = charge of oxide stripping peak for TC coated Au electrode $370.5 \mu\text{C cm}^{-2}$. We have obtained a fractional surface coverage of 71 % of

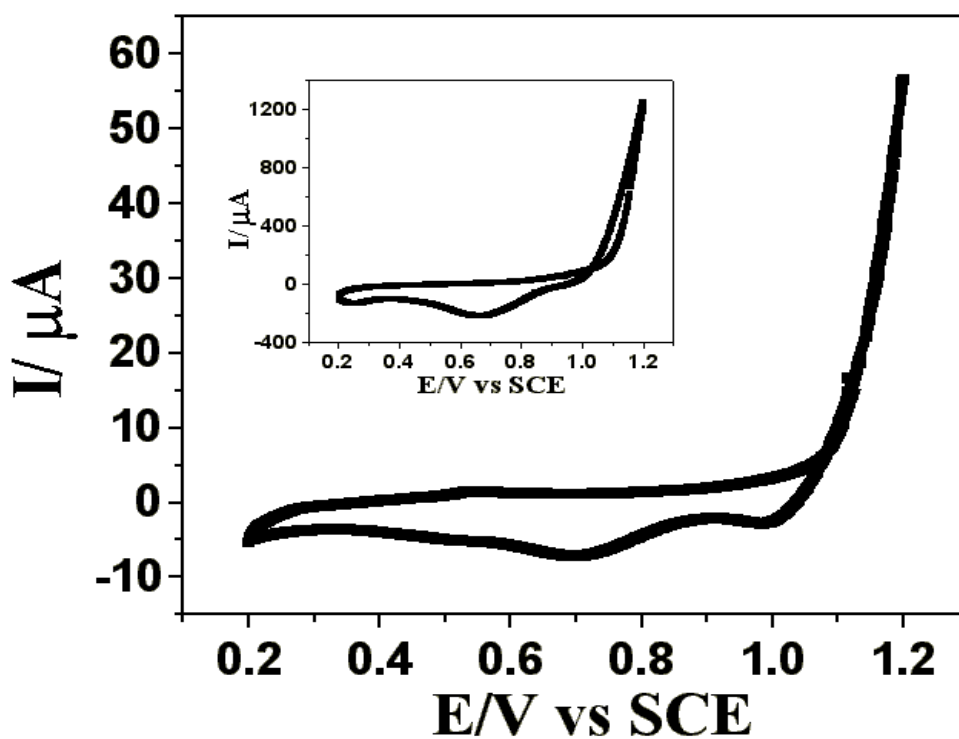


Figure 1

Cyclic voltammogram of 1 mM TC (thiocholesterol) coated Au electrode in 0.1 M perchloric acid. Scan rate = 50 mV s^{-1} . Inset shows the voltammogram for bare gold electrode.

TC molecules on gold surface which suggests the presence of a large number of molecular defects. This result is in good agreement with the value

obtained by Yang [12] et al. Thus, TC coated Au surface behaves as a nanoporous material.

7.2.2 Study on the barrier property of TC SAM :

7.2.2.1 Ferrocyanide redox reaction

We have studied the ferrocyanide redox reaction with TC coated Au electrode. Figure 2 shows the cyclic voltammograms of bare (scan rate = 50 mV s^{-1}) and 1 mM TC coated Au disk electrode (geometric area 0.002 cm^2)

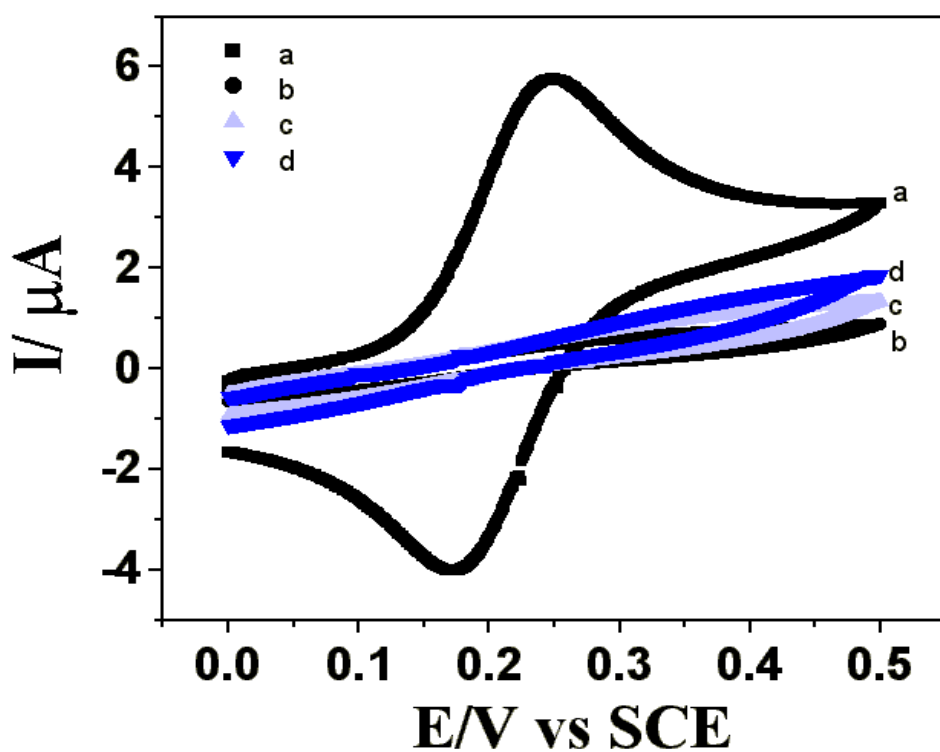


Figure 2

Cyclic voltammograms of a) bare (scan rate = 50 mV s^{-1}) and 1 mM TC coated Au disk electrode (geometric area 0.002 cm^2) for 10 mM ferrocyanide in 1 M NaF in water. b) scan rate = 50 mV s^{-1} , c) scan rate = 200 mV s^{-1} , d) scan rate = 500 mV s^{-1}

for 10 mM ferrocyanide in 1 M NaF in water. The voltammograms of TC coated gold electrode at different scan rates (from 50 to 500 mV s^{-1}) are almost independent of scan rate. This kind of electrochemical behaviour

resembles that of an array of microelectrode [16] which indicates the presence of structurally induced defects present in TC SAMs.

We have also formed hydrophobic mixed SAM by immersing the TC coated Au electrode in neat and 1 mM (ethanolic) HDT solution. We have studied the barrier property of the mixed TC+HDT SAM using cyclic voltammetry and electrochemical impedance spectroscopy. Figure 3 shows the cyclic voltammograms of pure TC and mixed TC + HDT SAM for ferrocyanide redox reaction.

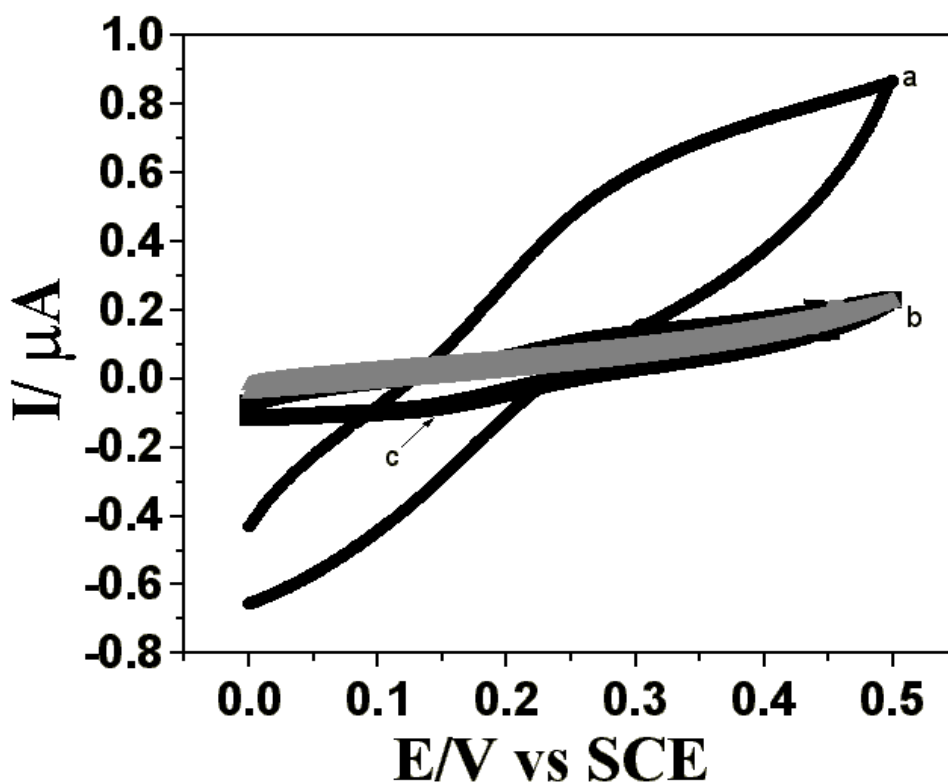


Figure 3

Cyclic voltammograms of gold disk electrode for 10 mM ferrocyanide in 1 M NaF in water. Scan rate = 50 mV s^{-1} , a) 1 mM TC coated. b) TC+ neat HDT coated. c) TC + 1 mM HDT (ethanolic) coated gold electrode.

It can be seen that mixed SAM of TC + neat HDT (Figure 3b) shows better barrier properties towards ferrocyanide redox reaction compared to either

pure TC SAM (Figure 3a) or mixed TC + 1 mM HDT SAM (Figure 3c). These results confirm that the defects in TC SAMs can be filled by HDT, which is known to form ordered densely packed SAM.

Our cyclic voltammetric results were supported by the impedance results. Figure 4 shows the Nyquist plots of pure TC and mixed TC+HDT SAM for 10 mM ferrocyanide/ferricyanide in 1 M NaF measured at 0.240 V vs SCE. From the Nyquist plot, (Figure 4) it can be seen that a larger semicircle (with larger value of R_{ct}) is exhibited by the mixed SAM of TC+HDT, which indicates better blocking of ferrocyanide redox reaction compared to pure TC SAM (smaller semicircle with lower value of R_{ct}).

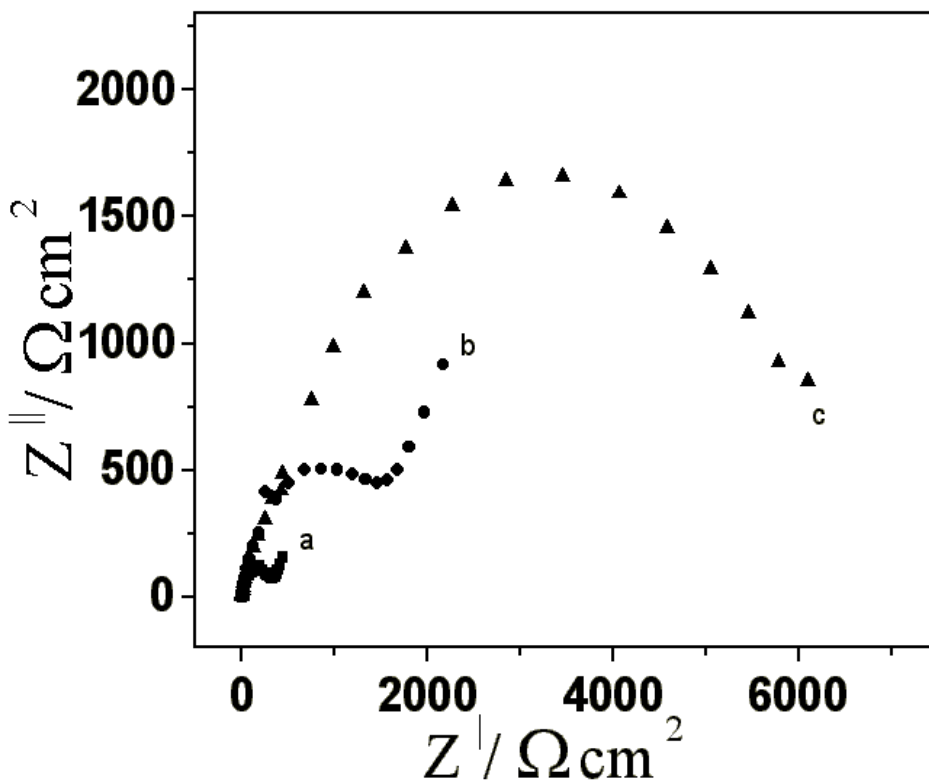


Figure 4

Nyquist plots of pure TC and mixed TC+HDT SAM on gold electrode for 10 mM ferrocyanide +10 mM ferricyanide in 1 M NaF measured at 0.240 V vs SCE. a) TC SAM. b) TC + 1 mM HDT (ethanolic) SAM. c) TC + neat HDT SAM.

The impedance results are in good agreement with our previous cyclic voltammetric results.

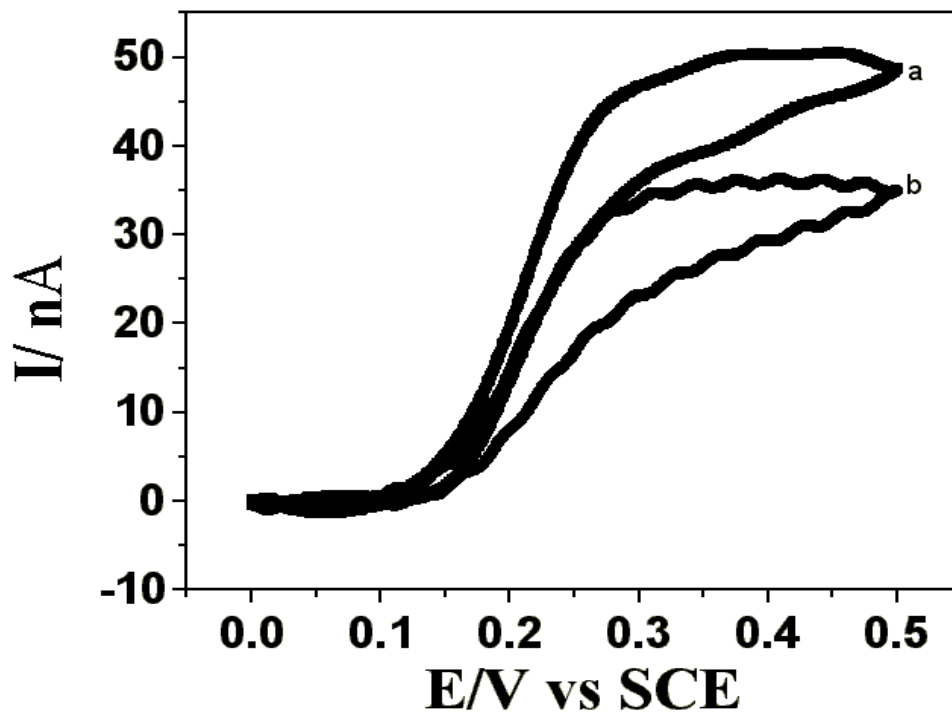


Figure 5

Cyclic voltammograms of a) bare and b) TC coated Au micro disk electrode (40 μm diameter) for 10 mM ferrocyanide redox reaction in 1 M NaF. Scan rate = 5 mV s^{-1}

Figure 5 shows the cyclic voltammograms of bare and TC coated Au micro disk electrode (40 μm diameter) for the ferrocyanide redox reaction. We have observed s-shaped voltammograms with TC coated Au microdisk electrode (Figure 5 b), in which the ferrocyanide redox reaction is almost unimpeded (as the current is almost same as bare gold electrode). This suggests that the TC SAM formed on microelectrode is highly disordered with more number of molecular defects. The voltammograms are independent of the scan rate in the range from 5 to 100 mVs^{-1} . This behaviour indicates that TC coated Au microdisk electrode exhibits nanoelectrode array characteristics.

7.2.2.2 Ethanol oxidation reaction

Various studies deal with the electro-oxidation of ethanol [17-20], with the purpose of using them in a direct alcohol fuel cell using platinum and platinum based alloys as electrocatalyst. On the other hand, gold is generally considered as a poor electro-catalyst in acidic medium, but its reactivity in alkaline medium is much greater, due to the absence of formation and consequent adsorption of poisoning species like CO on gold surfaces [21]. We have performed ethanol oxidation on bare and TC coated Au surface in

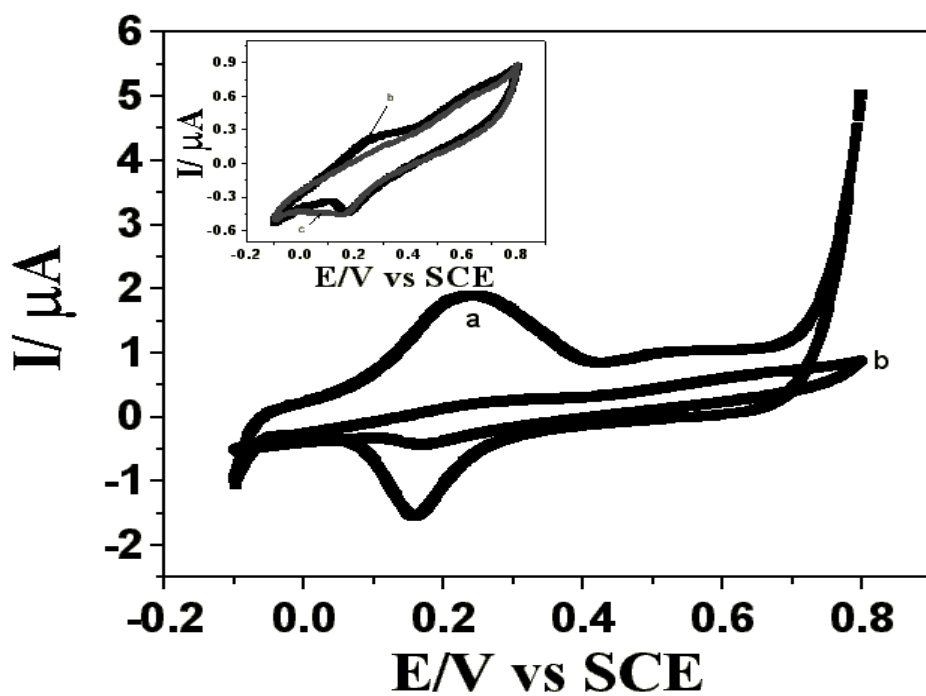


Figure 6

Cyclic voltammogram of ethanol oxidation (0.1 M ethanol) reaction in 0.1 M NaOH. a) bare gold electrode. b) TC coated gold electrode. Scan rate = 50 mV s⁻¹. Inset : b) voltammogram of TC coated gold electrode in 0.1 M NaOH (with 0.1 M ethanol) and c) without ethanol.

alkaline medium. Figure 6a shows the ethanol oxidation peak at 0.240 V vs SCE for bare gold electrode in 0.1 M ethanol in 0.1 M NaOH. The cathodic peak corresponds to gold oxide stripping peak. Figure 6b shows the voltammogram for TC coated gold electrode in 0.1M ethanol in 0.1M

NaOH. It can be seen from voltammogram that there is a marked decrease in the ethanol oxidation and oxide stripping current. Although, only 65 % of Au surface is covered by TC molecules, the size limitation of the defects (size of these defects are 5-8 Å) present in TC SAM allows the partial access of electroactive ethanol molecules or hydroxyl ions. However, these defects will not allow the access of larger electroactive species like ferrocene or benzoquinone molecules. Hence, TC SAM exhibits excellent barrier properties towards ferrocene and benzoquinone redox reaction as we found from our experiments. This experiment actually demonstrates the effect of size of electroactive species on the barrier properties of TC SAM on gold. (Inset illustrates the voltammogram of TC SAM in 0.1 M NaOH without ethanol (Figure 6c),)

7.2.2.3 Application of TC SAM for Cu⁺² ion detection

Figure 7A shows the voltammogram of 1 mM TC coated evaporated gold electrode in 0.5 M NaOAc solution. The TC coated gold electrode was previously dipped in 10 mM CuSO₄ +0.5 M H₂SO₄ solution for 30 minutes and then it was transferred into the sodium acetate solution (without any copper ions). The electrode was kept at negative potential (-0.3 V vs SCE) for five minutes. The voltammogram in Figure 7A clearly shows a broad double peak in the anodic region. The broad double peak corresponds to two step oxidation of Cu to Cu⁺² (Cu → Cu⁺¹ & Cu⁺¹ → Cu⁺²).

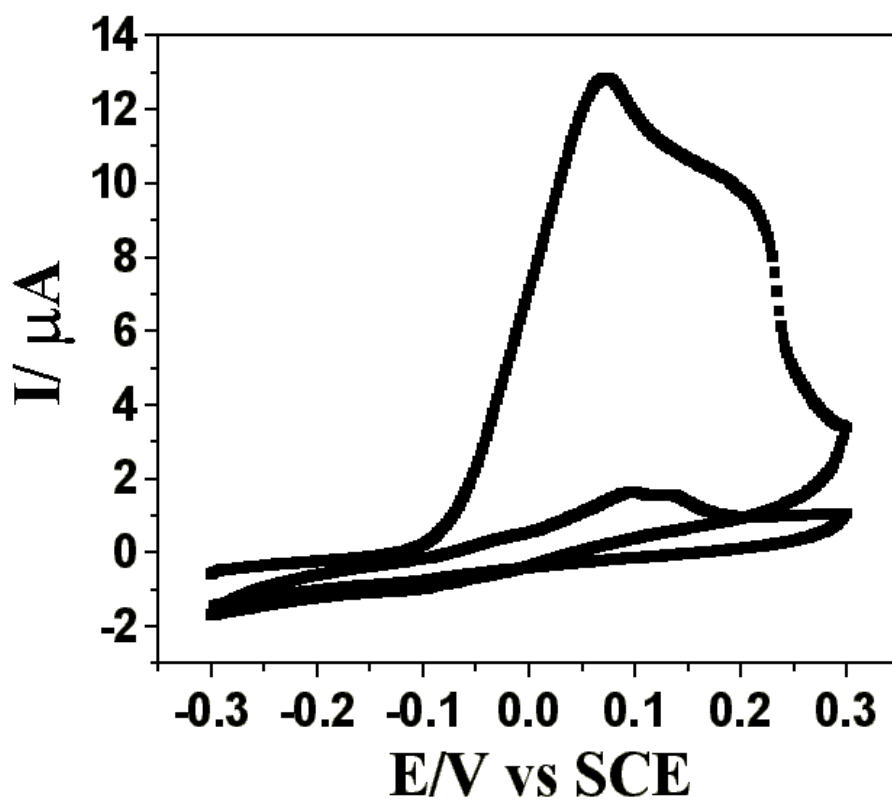
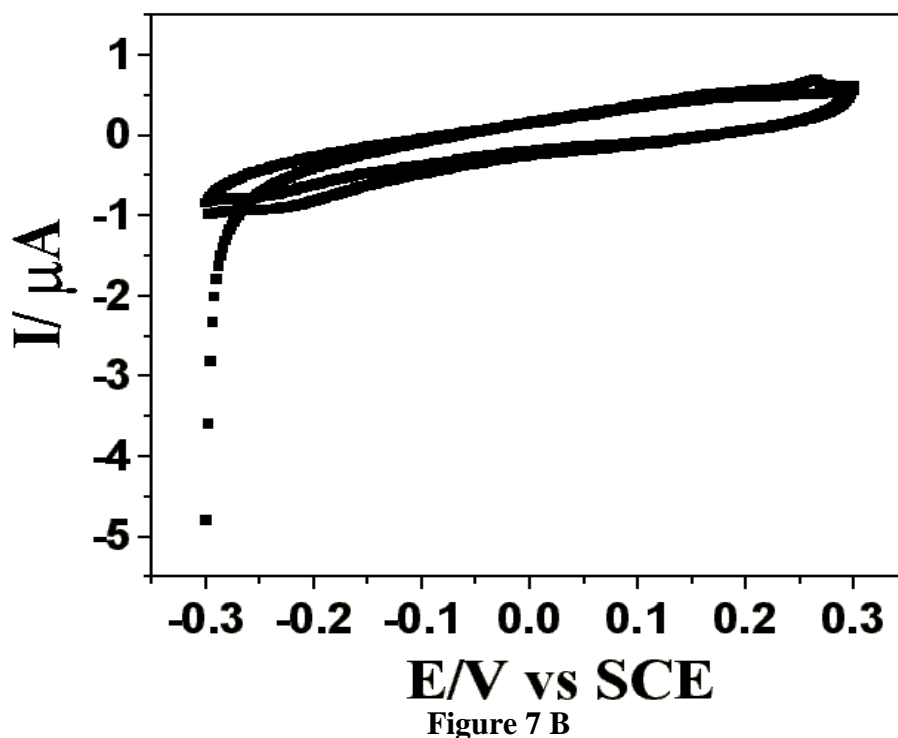


Figure 7 A

Cyclic voltammogram of TC coated evaporated gold electrode in 0.5 M NaOAc solution. Scan rate = 50 mV s⁻¹. The voltammogram shows copper oxidation peak in the 1st scan.

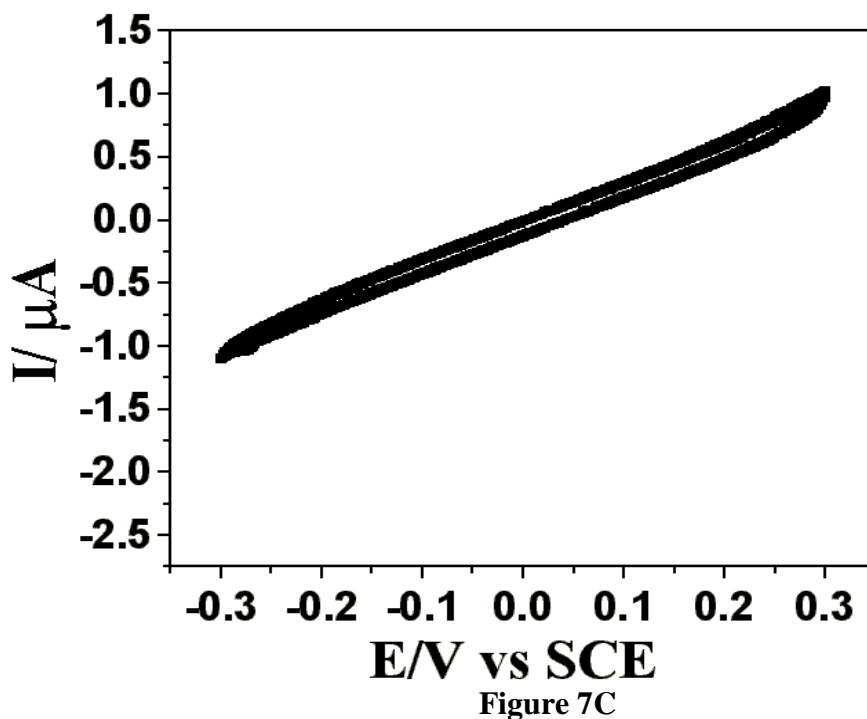
After a few scans, (around 10 scans) there is a large decrease in the oxidation current compared to 1st scan. This can be attributed to the loss of Cu⁺² ions due to gradual oxidation of deposited copper metal. Once it gets oxidized, it is released from the TC SAM and goes into the solvent medium. After 30 scans no copper oxidation peak was observed, indicating complete removal of Cu⁺² ions from the nanoporous assembly of TC SAM on gold (Figure 7 B). The above set of experiments prove the presence of Cu⁺² ions in the large defect sites of nanoporous assembly of TC SAM.



Cyclic voltammogram of TC coated evaporated gold electrode in 0.5 M NaOAc solution. Scan rate = 50 mV s⁻¹. The voltammogram shows no copper oxidation peak in the 30 th scan.

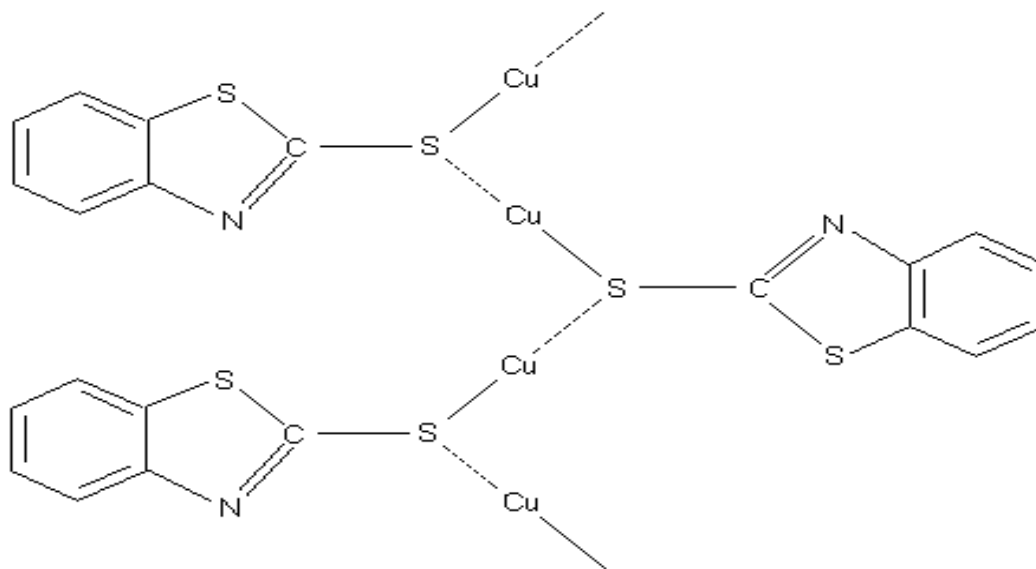
To further verify the presence of Cu⁺² ions inside the nanoporous assembly of TC SAM, we have dipped the TC SAM coated gold electrode into a solution of complexing agent. The complexing agent can form strong coordination complexes with copper ion and release the metal ions from the nanoporous assembly into the solution. It is well known that mercaptobenzothiazole (MBT) form strong coordination complexes with copper (Cu⁺¹) ions. We have taken the TC SAM coated Au electrode for further experiment which was previously dipped in 10 mM CuSO₄ + 0.5 M H₂SO₄ solution for 30 minutes and was kept in 0.5 M NaOAc solution at a negative potential for 5 minutes to reduce the copper ions into metallic copper. This electrode was now dipped into a solution of 1 mM MBT (mercaptobenzothiazole) in 0.1 M NaOH for 15 minutes and then it was scanned in sodium acetate solution. Figure 7C shows the corresponding

voltammogram, which does not exhibit any copper oxidation peak. This suggests the absence of copper inside the nanoporous assembly of TC SAM on gold.



Cyclic voltammogram of TC coated evaporated gold electrode in 0.5 M NaOAc solution. Scan rate = 50 mV s⁻¹. The voltammogram shows no copper oxidation peak.

It is well known that MBT form strong coordination complexes with Cu⁺¹ through the S atom of the thiocarbonyl group (C=S group). Ohsawa et al. [22] proved this by the infrared and XPS studies on Cu-MBT system. They found that a water insoluble polymeric film of mercaptobenzothiazolato-Cu(I) is formed by the surface reaction of cuprous ion with adsorbed MBT. The structure of the polymeric film proposed by them is shown in the figure below. The deposited copper on the defect sites of nanoporous assembly of TC SAM is always covered with a layer of Cu₂O under ambient conditions, which provides a source of Cu⁺¹ ions for MBT to react. Our results suggest that the Cu⁺² ions which are reduced and deposited



Cu (I)- MBT complex

as copper metal in the defect sites of TC SAM are released from the TC SAM due to the strong affinity of copper towards MBT molecules in alkaline medium. This shows that a strong ligand like MBT can easily extract copper from the TC SAM through strong coordination complex formation. Thus, TC SAM coated gold can be used as a potential sensor for the detection of Cu^{+2} ions. Further experiment needs to be carried out for other metal ions to demonstrate that the TC SAM has potential application as a sensor for metal ion detection.

7.3 Conclusion :

The following conclusions can be inferred from our studies :

1. TC SAM on gold acts as a nanoporous molecular assembly with large number of molecular defects with a size of about 5-8 Å and periodic in nature. These defects arise due to the size and irregular shape of the molecule. We find that the TC SAM has a surface coverage of 65-70 %, which is very low compared to ordinary alkanethiol SAM on gold.

2. The TC SAM shows array of microelectrode behaviour. We have utilized this property of TC SAM to form mixed monolayer of HDT+TC. Our cyclic voltammetric and impedance results show that the mixed SAM exhibits better barrier property towards ferrocyanide redox reaction compared to pure TC SAM. This is due to the filling up of the defects presence in TC SAM by HDT molecules.
3. The TC SAM can be used a potential sensor for the detection of Cu^{+2} ions and possibly other electroactive metal ions within the potential range of gold electrode. This can be achieved by just dipping the nanoporous assembly of TC in a solution containing Cu^{+2} ions and the captured ions can be deposited by application of proper potential in a suitable medium in the absence of those metal ions. This was confirmed by our cyclic voltammetric results, which exhibit copper oxidation peak.

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