CHAPTER 111

Self-Assembly of Mercaptothiadiazole Compounds on Au Electrode and Their Electrocatalytic Applications
3.1. Introduction

The heteroaromatic dithiol, 2,5-dimercapto-1,3,4-thiadiazole (DMT) and its derivatives (Chart 3.1) have been extensively studied as chelating agents and cathode-active material for lithium secondary batteries [1-5]. In comparison with many other thiol compounds, a distinguishing feature of DMT and its derivatives is the formation of tautomeric structures having either the thioamide [-NHC(=S)-] or the thiol [-NC(SH)-] form (Scheme 3.1) [6,7]. The tautomerization influences the reactivity of the thiadiazoles, which has been demonstrated for polymerization

Scheme 3.1. Proton dissociation equilibria of DMT
process [8] and substitution reactions [9]. In the solid state, DMT exists as a thione-thiol tautomer [6], while in solution a solvent-dependent equilibrium exists between all forms (Scheme 3.1). DMT has acid dissociation constants of $pK_a^f = -1.36$ and $pK_{a2} = 7.5$, making its neutral form a very strong acid and its anionic form a weak acid [5,10].

Oyama and Buttry have extensively studied how the acid-base chemistry of DMT affects the spectral and redox properties of DMT in different solvents containing weak bases and acids [8,11-15]. On the basis of the spectral and electrochemical studies, they concluded that DMT exists as both undissociated (dithiol) and dissociated (thiolate) forms in ethanol and DMSO, since these solvents are capable of dissociating DMT into thiolate species while it exists exclusively in dithiol form in acetonitrile (AN), which is not a sufficiently strong proton acceptor for the deprotonation of DMT [8,11-15]. Although the spectral and electrochemical properties of DMT were not studied in an aqueous medium previously, it is expected that water would act as a slightly better proton acceptor than AN and be capable of deprotonating DMT.

Recently, the adsorption behavior of $\omega$-alkanedithiols and aromatic dithiols on Au, Ag and Pt surfaces has been reported [16-42]. One report has shown that 1,6-hexanedithiol adsorbs flat on Au surface via both the sulfurs [26]. However, other reports suggest that it adsorbs through one of the two SH groups and its molecular axis is oriented perpendicular to the metal surface [27-31]. Several studies have shown that various structures and coverages can be formed by
dithiols, and their adsorption geometry depends on the conditions of deposition [22,26,32-35]. The adsorption behavior of DMT and its derivative, 5-methyl-2-mercapto-1,3,4-thiadiazole, on Au and Cu surfaces has been studied recently [37-40]. The objective of the present chapter is to examine the adsorption behavior of DMT on Au surface from different solvents. Since DMT mainly exists in dithiol form (Scheme 3.1) in AN, it is interesting to know whether it chemisorbs on Au electrode through both the S-H groups or one of the two S-H groups. If it adsorbs on Au surface via one of the two S-H groups, can the other S-H group engage in the formation of a multilayer through S-S linkage like «-hexanedithiol [30] or aromatic dithiols? [23,39]. To keep this objective in mind, the adsorption of behavior of DMT on Au surface from different solvents was studied.

### 3.2. UV-Visible Spectra of DMT in Different Solvents

The existence of DMT in dithiol or thiolate form can easily be identified from its characteristic absorption peak in the UV-visible absorption spectrum. Figure 3.1 shows the absorption spectra for DMT in ethanol, AN and water. It shows a predominant peak at 318 nm in AN, characteristic of the dithiol form (curve a) [14,43]. In ethanol, DMT shows a predominant peak at 335 nm due to the dithiol form and a shoulder wave at 261 nm due to thiolate form, indicating that DMT exists as both dithiol and thiolate forms (curve b) [14,43]. In water, a predominant peak at 329 nm with a shoulder band at 257 nm was observed (curve
c), and these bands were assigned to the dithiol and thiolate forms of DMT, respectively. To confirm that the two bands observed are due to dithiol and thiolate, we have added different concentrations of HCl to an aqueous solution of DMT. While increasing the concentration of HCl, the intensity of the band due to thiolate decreased, whereas the intensity of the band due to dithiol increased (Figure 3.2). After the addition of 6 N HCl, the band due to thiolate vanished. These results clearly confirmed that DMT predominantly exists as dithiol form in water. The observed differences in the position of absorption peaks of DMT in three different solvents can be easily explained by the proton acceptor properties of the solvents. Since ethanol is a sufficiently strong base to serve as a proton acceptor, deprotonation of DMT occurs and it exists in a dissociated state (CH$_3$CH$_2$OH$_2^+$ and DMT$^-$) [14,43]. Thus, DMT exists as both dithiol and thiolate
forms in ethanol (curve b). AN is not a strong base like ethanol for the deprotonation of DMT, and thus the spectrum corresponds to the dithiol form of DMT (curve a) [14]. As water is a slightly better proton acceptor than AN, DMT exists in both thiolate and dithiol forms, as expected.

3.3. Electrochemical Oxidation and Reductive Desorption of DMT Adsorbed on Au Electrodes from Acetonitrile

Figure 3.3A shows the first run of linear sweep voltammograms (LSVs) obtained for DMT adsorbed for 30 min and 40 h in a completely deaerated 1 mM DMT in acetonitrile (AN) on Au electrodes in 0.20 M phosphate buffer (PB) solution at a scan rate of 50 mV s$^{-1}$. It is evident from Figure 3.3A that bare Au electrode does not show any electrochemical response in 0.20 M PB solution.
However, DMT adsorbed from AN for 30 min shows two oxidation waves at 0.15 and 0.38 V (curve b). This is due to the fact that, as evidenced from absorption spectrum (Figure 3.1a) in AN, DMT exists in dithiol form and it gets adsorbed on Au electrode via one of its -SH groups, leaving the other -SH group free. This free -SH group is oxidized to disulfide at two different potentials in 0.20 M PB solution (Figure 3.3A). Further, the observed oxidation waves in Figure 3.3A clearly suggest that the adsorbed DMT does not undergo tautomerism as it does in solution [6,7]. The cause for the oxidation of -SH at two different potentials may be due to the adsorption of DMT at different crystallographic planes of the Au surface [44-46]. When DMT was adsorbed from AN for 40 h, it is observed that, except a small increase in the oxidation peak currents, no significant change in the oxidation behavior occurred (curve c). To confirm the aforesaid oxidation behavior of DMT adsorbed on Au surface, we have studied the electrochemical behavior of two thiadiazole derivatives, viz., 5-methyl-2-mercapto-1,3,4-thiadiazole (MMT) and 5-amino-2-mercapto-1,3,4-thiadiazole (AMT), adsorbed on Au electrodes from AN solution. No oxidation wave was observed for either MMT or AMT within the potential region of -0.10 to +0.70 V, indicating that the observed oxidation waves for DMT (Figure 3.3A, curves b and c) correspond to the -SH oxidation. The LSVs recorded for the reductive desorption of DMT adsorbed from AN for 40 h in 0.10 M KOH “before” and “after” electrochemical oxidation in 0.20 M PB solution in the potential window of -0.10 V to +0.70 V are shown in Figure 3.3B. DMT adsorbed from AN shows a
reductive desorption wave at -0.35 V along with a shoulder wave around -0.90 V (curve a). The reductive desorption of DMT, after electrochemical oxidation in 0.20 M PB solution, shows an identical desorption wave (curve b) as observed for

**Figure 3.3** (A). LSVs obtained for (a) bare Au and DMT adsorbed on Au electrodes from 1 mM of DMT in AN for (b) 30 min and (c) 40 h in 0.20 M PB solution at a scan rate of 0.05 V s\(^{-1}\). (B). LSVs obtained for the reductive desorption of DMT adsorbed on Au electrodes for 40 h from AN before (a) and after electrochemical oxidation (b) in PB solution at a scan rate of 0.10 V s\(^{-1}\) in 0.1 M KOH.

directly desorbed DMT, except for a slight increase in the desorption current at -0.35 V. That is, the reductive desorption behavior was the same for both direct desorption and desorption after electrochemical oxidation. This indicates that the adsorbed DMT layer was stable and not desorbed from the electrode surface after electrochemical oxidation. The appearance of two waves in Figure 3.3B was attributed to the desorption of DMT adsorbed on different crystallographic
domains of the polycrystalline Au electrode [44-46]. It is well-known that polycrystalline Au surface is composed of three low-index crystallographic faces [47]. The charge under the reductive desorption waves was used to calculate the surface coverage. Integration of reductive desorption waves in Figure 3.3B (curve b) based on one-electron reduction gives a charge density of 36 μC cm\(^{-2}\) without subtracting the roughness factor. This yields the surface coverage of \(4.70 \times 10^{10}\) mol cm\(^{-2}\) for DMT, which is in the sub-monolayer range.

3.4. Electrochemical Oxidation and Reductive Desorption of DMT Adsorbed on Au Electrodes from Aqueous Solution

Figure 3.4A shows the first run of LSVs obtained for DMT adsorbed on Au electrodes from a \textit{completely deaerated} aqueous solution at various time intervals. DMT adsorbed on Au electrode for 15 min shows an oxidation wave at 0.47 V (curve a). In addition, one more oxidation wave also appeared at 0.58 V for DMT adsorbed for 3 h on Au electrode (curve b) and which was shifted to 0.62 V when adsorption of DMT was increased on Au electrodes (curves c-f). Further, the oxidation peak current increases when the adsorption of DMT on Au electrode increases. However, it started to decrease after 40 h adsorption. The observed decrease in the oxidation peak current is likely that the conductivity of the DMT multilayer may decrease due to the formation of a thick film after 40 h of adsorption. Unlike DMT adsorbed from ethanol, DMSO, AN, and chloroform, the

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observed increase in the oxidation peak currents in aqueous solution (Figure 3.4A) indicates that the adsorption of DMT increases on Au electrodes with an increase in the soaking time. The charges calculated for DMT adsorbed on Au electrodes at different time intervals due to the oxidation of -SH are given in Table 3.1. The increase in the charges suggests the formation of DMT multilayer on Au electrode. Although DMT adsorbed from ethanol, AN, DMSO and chloroform solutions exhibit oxidation waves for -SH, we have not observed any significant increase in the peak current when Au electrode was soaked in these solvents, even for a
longer time. This indicates that medium plays an important role in the formation of DMT multilayer on Au electrode. As spelled out earlier, since water is a relatively 

**Table 3.1. Electrochemical oxidation and reductive desorption charges of DMT adsorbed on Au electrodes from an aqueous solution at various time intervals**

<table>
<thead>
<tr>
<th>Soaking time (hours)</th>
<th>Charges for electrochemical oxidation(^a) (\times 10^6) C/cm(^2)</th>
<th>Charges for reductive desorption(^b) (\times 10^6) C/cm(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>34</td>
<td>47</td>
</tr>
<tr>
<td>3.0</td>
<td>68</td>
<td>84</td>
</tr>
<tr>
<td>6.0</td>
<td>278</td>
<td>284</td>
</tr>
<tr>
<td>6.0(^c)</td>
<td>---</td>
<td>48(^c)</td>
</tr>
<tr>
<td>14.0</td>
<td>1201</td>
<td>1218</td>
</tr>
<tr>
<td>24.0</td>
<td>1907</td>
<td>1921</td>
</tr>
<tr>
<td>40.0</td>
<td>2104</td>
<td>2119</td>
</tr>
</tbody>
</table>

\(^a\)Based on two-electron oxidation  
\(^b\)Based on two-electron reduction  
\(^c\)Charge obtained from direct reductive desorption

weak proton acceptor, the dithiol form of DMT may present predominantly and thus DMT gets adsorbed on Au electrode via one of the two -SH groups, leaving the other -SH group free from binding. Thus, the surface pK\(_a\) of the adsorbed DMT was different from that in solution. It is most likely that the hydrogen bonding between water molecules and the free -SH groups of the adsorbed DMT are responsible for the formation of a multilayer. The involvement of hydrogen bonding between water molecules and the -SH groups was confirmed by ATR FT-
IR and XPS studies (*vide infra*). Further, the formation of multilayer via -SH group was also confirmed from the decreased oxidation peak current due to -SH oxidation when the multilayer electrode was immersed in 0.1 M NaOH (Figure 3.5). When DMT multilayer was immersed in NaOH solution, the hydrogen bonding is lost due to the dissociation of the -SH group. This leads to the removal of DMT from the multilayer, and thus, the oxidation current due to S-H oxidation was observed to decrease. Since our main intention is to show the involvement of S-H groups in the formation of multilayer, we have restricted the potential window between -0.10 and +0.80 V (Figure 3.4A). Actually, we observed a reduction wave at -0.40 V due to the reduction of S-S when we extend the potential to -0.60 V (Figure 3.6). Further, the electrochemical oxidation of S-H to S-S and the absence
of S-S linkage in the multilayer formation were confirmed from the observed huge differences in the reductive desorption charges of DMT multilayer in 0.10 M KOH ‘before’ and ‘after’ electrochemical oxidation. After electrochemical oxidation in the potential region of -0.10 to +0.80 V in 0.20 M PB solution, the multilayer electrodes were immediately transferred to 0.1 M KOH for reductive desorption measurements, and the corresponding LSVs are shown in Figure 3.4B. As mentioned earlier, immersion of multilayer electrode in an alkali solution leads to the removal of DMT molecules from the multilayer. Therefore, we have carried out the electrochemical reductive desorption measurements immediately in 0.10 M KOH. A reductive desorption wave around -0.40 V along with a shoulder wave at -0.84 V were observed for DMT adsorbed for 15 min on Au electrode (curve a),

![Graph](image)

**Figure 3.6.** CV obtained for 3 h adsorbed DMT on Au electrodes in 0.20 M PB solution at a scan rate of 50 mV s⁻¹.
which is similar to the reductive desorption of DMT adsorbed from AN (Figure 3.3B). Unlike AN, the potential of the reductive desorption waves was shifted to more negative potentials, and their currents were increased as the adsorption of DMT from aqueous solution increases (curves b-f). For DMT adsorbed for 40 h, the reductive desorption waves were observed at -0.47 and -1.10 V (curve f). The reductive desorption charges calculated at different time intervals after electrochemical oxidation are given in Table 3.1. The identical charges for the reductive desorption waves corresponding to monolayer and multilayer of directly desorbed DMT in 0.10 M KOH indicates that the amount of thiolate chemisorbed on Au electrode is the same for both of them. For example, direct desorption of DMT adsorbed for 6 h on Au electrode shows the same reductive desorption waves (curve g) as observed for DMT adsorbed for 15 min (curve a). This indicates that in both the cases the consumed charge corresponds to the reduction of Au-S. However, the reductive desorption of multilayer in 0.10 M KOH after electrochemical oxidation in 0.20 M PB solution shows higher charges than the directly desorbed DMT multilayer. For example, the reductive desorption of DMT adsorbed for 6 h on Au electrode after electrochemical oxidation shows much enhanced reductive desorption waves (curve c) than the directly desorbed DMT prepared under identical conditions (curve g). The charge estimated from the reductive desorption waves after electrochemical oxidation was nearly $\epsilon$-fold higher than that obtained for the directly desorbed DMT on Au electrode (Table 3.1). After electrochemical oxidation, the -SH groups present in the multilayer
were oxidized to S-S in 0.20 M PB solution and reductively desorbed in 0.1 M KOH at the same potential where Au-S reduction was observed. These results once again confirmed that the DMT multilayer was formed via S-H groups. Parallel observation was made recently in the study of monolayer and multilayer formation of mercaptoferrocene derivatives on Au(111) electrode. It is reported that identical reductive desorption charges are obtained for the monolayer and multilayer of mercaptoferrocene, suggesting that the amount of thiolate chemically bonded to Au electrode for a multilayer and monolayer is the same [48].

![Graph A](image1.png)  
![Graph B](image2.png)

**Figure 3.7** (A). LSVs obtained for DMT adsorbed on Au electrodes from an aqueous solution of 1 mM DMT for 6 h (a) before and after exposed to air for (b) 10 and (c) 20 min in 0.2 M PB solution at a scan rate of 50 mV s$^{-1}$. (B) LSVs obtained for the reductive desorption of DMT adsorbed on Au electrodes from an aqueous solution of 1 mM DMT for 6 h (a) before and (b) after electrochemical oxidation in PB solution and 6 h adsorbed electrodes after exposed to air for (c) 10 and (d) 20 min in 0.1 M KOH at a scan rate of 100 mV s$^{-1}$.

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reductive desorption of electrochemically generated bilayers of 1,4-benzenedimethanethiol (BDMT) on Au electrode has been reported [49]. It is shown that Au-S bond is reduced before the S-S bond, leading to direct desorption of the bilayer, and this is supported by the identical reductive desorption charges obtained for the monolayer and bilayer of BDMT [49]. For DMT multilayer, both Au-S and S-S reductions occur at the same potential in the present study, and thus we observed relatively higher reductive desorption charges. Very recently, the reductive desorption behavior of 1-octanethiol and 1,8-octanedithiol on Au(111) electrode has been reported [50]. It was found that the reductive desorption charge for 1,8-octanedithiol is greater by 32 \% than that for 1-octanethiol, implying that for a fraction of dithiol molecules both sulfur atoms are involved in the reduction process [50]. In the present study, the contribution of S-S charge was confirmed from the observed higher reductive desorption charge for DMT multilayer in 0.1 M KOH after electrochemical oxidation, in contrast to the directly desorbed multilayer. The role of -SH in the formation of DMT multilayer was also verified from the differences in the electrochemical oxidation behavior of multilayer ‘before’ and ‘after’ exposure to air. The DMT adsorbed for 6 h shows two oxidation waves at 0.50 and 0.59 V before exposure to air (curve a, Figure 3.7A). However, for the electrodes prepared under identical conditions, when exposed to air for 10 and 20 min, the oxidation peak currents were decreased (curves b and c). It is likely that the aerial oxidation of S-H leads to the formation of S-S, and thus a decrease in the oxidation peak current was observed. The formation of S-S after
aerial oxidation was confirmed from the observed stretching frequency at 537 cm⁻¹ in Raman spectrum (data shown below). The reductive desorption of DMT multilayer in 0.1 M KOH ‘before’ and ‘after’ exposure to air for 10 and 20 min is shown in Figure 3.7B. The direct desorption of DMT shows a reductive desorption peak at -0.42 V along with a shoulder wave at -0.84 V (curve a). However, when the electrodes were exposed to air, the reductive desorption currents were significantly increased (curves c and d), similar to desorption of electrochemically oxidized DMT multilayer (curve b). The observed identical reductive desorption characteristics for both aerial and electrochemically oxidized DMT multilayer suggest that both aerial and electrochemical oxidation of DMT multilayer leads to the formation of S-S.

3.5. SEM Images of DMT Multilayer Assemblies on Au Surface

The morphology of the DMT multilayer formed on Au surface was examined by SEM. The SEM images of DMT adsorbed on Au surface from aqueous solution for 6 and 40 h are shown in parts A and B of Figure 3.8, respectively. It is interesting to note that DMT adsorbed for 6 h shows a leaflike structure with island growth (Figure 3.8A). The thickness of each leaf was found to be ~0.8-1.0 μm. As the adsorption time increases from 6 to 40 h, densely packed leaves were formed with a thickness of 2-2.5 μm (Figure 3.8B). The SEM images support that adsorption of DMT on Au surface from an aqueous solution leads to the formation of multilayer assembly with a leaflike structure.
Figure 3.8. SEM images obtained for DMT adsorbed on Au surface for (A) 6 h and (B) 40 h

3.6. ATR-FT-IR and Raman Spectral Studies of DMT Adsorbed on Au Surface

Figure 3.9A shows the ATR-FT-IR spectra for DMT adsorbed on Au surface from 1 mM aqueous solution for 3, 12 and 40 h. For comparison, the FT-IR spectrum for solid DMT is shown in Figure 3.9B. The various vibrational frequencies observed for DMT adsorbed on Au surface and solid DMT and their assignments are given in Table 3.2. It can be seen from Figure 3.9A that the signals of all the stretching bands were increased when the adsorption of DMT on Au surface increases, indicating that adsorption of DMT increases when the soaking time increases. It is well-known that O-H stretching frequency is very sensitive to hydrogen bonding [51,52]. When O-H was not involved in hydrogen bonding, a sharp band will be observed around 3600 cm\(^{-1}\) [51-53]. The involvement of water molecules in the formation of multilayer assembly through intermolecular hydrogen bonding with free SH groups of DMT can be easily
identified from the appearance of a broad band in the region from 3400 to 3100 cm\(^{-1}\), corresponding to O-H stretching. A significant increase in the intensity of the broad band corresponding to hydrogen bonded O-H with respect to increased

**Table 3.2. Vibrational data and their assignments for DMT/KBr and DMT adsorbed for 40 h on Au surface.**

<table>
<thead>
<tr>
<th>DMT/KBr</th>
<th>ATR DMT/Au</th>
<th>Assignment</th>
<th>Marked in the spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>----</td>
<td>3300</td>
<td>O-H</td>
<td>1</td>
</tr>
<tr>
<td>3058</td>
<td>2921</td>
<td>(\nu(N-H))</td>
<td>2</td>
</tr>
<tr>
<td>2480</td>
<td>2478</td>
<td>(\nu(S-H))</td>
<td>3</td>
</tr>
<tr>
<td>1498</td>
<td>1538</td>
<td>(\nu(C=\text{N}))</td>
<td>4</td>
</tr>
<tr>
<td>1444</td>
<td>1460</td>
<td>(\delta(N-H))</td>
<td>5</td>
</tr>
<tr>
<td>1256</td>
<td>1225</td>
<td>(\nu(C-N))</td>
<td>6</td>
</tr>
<tr>
<td>1112</td>
<td>1160</td>
<td>(\nu(N-N))</td>
<td>7</td>
</tr>
<tr>
<td>1052</td>
<td>1053</td>
<td>(\nu(C-S))</td>
<td>8</td>
</tr>
</tbody>
</table>

**Figure 3.9 (A)** ATR spectra obtained for DMT adsorbed on Au surface from 1 mM aqueous solution for (a) 3 h (b) 12 h and (c) 40 h. Inset: Magnified spectra in the region of 2550 to 2400 cm\(^{-1}\) and 1100 to 980 cm\(^{-1}\). (B) FT-IR spectrum recorded for solid DMT in KBr pellet.
adsorption time clearly shows that water molecules are responsible for the formation of DMT multilayer. The increased intensity of the O-H band (Figure 3.9A) does not result from the adsorbed water molecules, as the adsorption of DMT on Au surface makes the film become more and more hydrophobic. As can be seen from the inset of Figure 3.9A (2550-2400 cm\(^{-1}\)), the intensity of the stretching band due to SH at 2478 cm\(^{-1}\) was also increased as the adsorption time increases from 3 to 40 h. Further, the stretching bands observed for solid DMT are retained for DMT adsorbed on Au surface with small shifts in their frequencies. On the basis of the earlier reports, we assigned the strong signals observed between 2950 and 2800 cm\(^{-1}\) to overtones and Fermi resonances [54-56]. A band observed at 1538 cm\(^{-1}\) corresponds to C=N stretching and the band at 1225 cm\(^{-1}\) to C-N stretching frequency. The bands at 1160 and 1053 cm\(^{-1}\) are assigned to N-N and C-S stretching frequencies, respectively [13,39,51,55,56].

Raman spectroscopy is one of the sensitive techniques to identify disulfide species, because it shows a strong signal around 540 cm\(^{-1}\) for disulfide [38]. The Raman spectra obtained for DMT multilayer prepared by 6 h adsorption on Au surface before exposure to air are shown in Figure 3.10a. The stretching frequencies at 1418, 1043, 737, and 675 cm\(^{-1}\) correspond to \(\nu(C=N)\), \(\nu(N-N)\), \(\nu_{as}(C-S-C\ endocyclic)\) and \(\nu_{s}(C-S-C\ endocyclic)\), respectively [13]. The absence of a band around 540 cm\(^{-1}\), characteristic of the S-S stretching mode, confirms that the DMT multilayer assembly is not formed by S-S linkage. However, the appearance of a band at 537 cm\(^{-1}\) for the same multilayer after exposure to air
suggests that only aerial oxidation of DMT multilayer leads to the formation of S-S (Figure 3.10b).

![Raman spectra](image)

**Figure 3.10.** Raman spectra obtained for DMT adsorbed on Au surface from 1 mM aqueous solution for 6 h (a) before and (b) after exposed to air

### 3.7. XPS of DMT Multilayer on Au Surface

XPS has been extensively used to characterize SAMs formed from organosulfur compounds terminated with different functional groups [57-59]. It is considered as one of the most sensitive methods to provide information about the oxidation states of adsorbed species [58]. Figure 3.11 depicts the deconvoluted high-resolution XPS obtained for DMT adsorbed for 3 and 6 h on Au surfaces in the S 2p, N 1s, and O 1s core level regions. The peak-fitting results and their assignments for DMT adsorbed for 3 h are given in Table 3.3. It was found that the intensities of S 2p, N 1s, and O 1s core level regions were increased from
500.1, 300.0, and 470.2 (a, c, and e) to 600.0, 490.1, and 1880 (b, d, and f), respectively, while increasing the adsorption time from 3 to 6 h. We have calculated the thickness \((d)\) of the DMT multilayer formed from 3 and 6 h adsorption using relative intensities \((I)\) of the Au (4f) and C (1s) peaks with the corresponding Scofield factors employing the following equation [60]. It was assumed the same photoelectron escape depths of gold and carbon for DMT multilayer film were \(\lambda_{\text{Au}} = 45 \, \text{Å} \) at 1400 eV and \(\lambda_{\text{C}} = 35 \, \text{Å} \) at 1202 eV. From the XPS spectrum, we obtained \(I_{\text{Au}}\) values of \(48 \times 10^3\) and \(50 \times 10^3\) and \(I_{\text{C}}\) values of \(120 \times 10^2\) and \(300 \times 10^2\) for the DMT multilayer adsorbed from 3 and 6 h soaking time. Thickness \((d)\) values of 7.63 and 12.44 \(\mu\text{m}\) were obtained for DMT adsorbed for 3 and 6 h, respectively. On the basis of XPS study, Woll and co-workers have

\[
\frac{I_{\text{C}}}{I_{\text{Au}}} = \frac{\sigma_{\text{C}}}{\sigma_{\text{Au}}} \frac{\lambda_{\text{C}}(E_{\text{C}})}{\lambda_{\text{Au}}(E_{\text{Au}})} \left[ 1 - \exp \left( -\frac{d}{\lambda_{\text{C}}(E_{\text{C}})} \right) \right] \exp \left( -\frac{d}{\lambda_{\text{Au}}(E_{\text{Au}})} \right)
\]

(3.1)

reported that DMT forms a very thin layer (20.9 Å) on Au substrate when it was adsorbed for 15 h in ethanol solution [39]. However, the observed higher thickness for DMT, in the present study, clearly suggests that DMT forms a multilayer assembly on the Au electrode when it was adsorbed from an aqueous solution. Inspection of the S 2p spectrum (Figure 3.11a) reveals four distinct sulfur species with different intensities located at 162, 162.9, 163.7, and 164.5 eV. It has been reported that the binding energies at 162.0 and 162.9 eV were attributed to the spin-orbit split doublet of S(2p3/2) and S(2p1/2) and were assigned to the gold-bound sulfur. The binding energies at 163.7 and 164.5 eV, corresponding to the
Figure 3.11. High resolution XPS obtained for DMT adsorbed on Au surface for 3 and 6 h in the S 2p (a and b), N 1s (c and d) and O 1s (e and f) regions.
Table 3.3. Peak fitting results for positions (eV) and their assignments of the XPS obtained for DMT adsorbed on Au substrate for 3 h.

<table>
<thead>
<tr>
<th>Components</th>
<th>Binding energy (eV)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S 2p</td>
<td>162.0, 162.9</td>
<td>gold bound sulfur</td>
</tr>
<tr>
<td></td>
<td>163.7, 164.5</td>
<td>unbound sulfur</td>
</tr>
<tr>
<td>N 1s</td>
<td>397.1</td>
<td>-N=</td>
</tr>
<tr>
<td></td>
<td>399.2</td>
<td>weakly hydrogen accepting nitrogen</td>
</tr>
<tr>
<td></td>
<td>400.6</td>
<td>strongly hydrogen accepting nitrogen</td>
</tr>
<tr>
<td>O 1s</td>
<td>532.1</td>
<td>-OH</td>
</tr>
<tr>
<td></td>
<td>533.4</td>
<td>H-donating H₂O</td>
</tr>
</tbody>
</table>

spin-orbit split doublet of S(2p3/2) and S(2p1/2), were assigned to the unbound sulfur [33,59,61]. The N 1s spectrum of DMT multilayer was deconvoluted into three component peaks at 397.1, 399.2 and 400.6 eV and were attributed to –N=, a weakly hydrogen accepting nitrogen, and a strongly hydrogen accepting nitrogen, respectively (Figure 3.11c) [62]. The observed binding energies at 399.2 and 400.6 eV suggest that nitrogen atoms may also be involved in the hydrogen bonding with water molecules for the formation of DMT multilayer. Further, the O 1s region was recorded to reveal the involvement of water molecules in the formation of DMT multilayer on Au surface. The O 1s spectrum exhibits two components at 532.1 and 533.4 eV (Figure 3.11e). Adsorption of water on various metal oxide surfaces and their characterization by XPS have been extensively investigated [62-65]. It has been reported that adsorbed water molecules in their native
environment are typically observed above 534 eV, while the dissociated products of hydroxyl and hydrogen-donating water molecules show peaks at lower binding region [62-64]. For example, in the case of TiO₂ anatase (101) surface, the O 1s peak due to adsorbed water is observed around 534.5 eV, and when the coverage of water on the surface increases, the peak was shifted to lower binding energies by ~1 eV [63,65]. Recently, the binding energy of 533-533.5 eV was reported for the hydrogen-donating water molecules to the nitrogen atom of pyridine [62]. Hence, we assigned the binding energy of 533.4 eV observed in the present work to the hydrogen-donating water molecules binding to the sulfur atom of DMT [62,65-69]. The -OH group of the water molecule shows a binding energy around 531.7-532.2 eV [62]. Thus, we assigned the binding energy at 532.1 eV in the

**Scheme 3.2. Structure for DMT multilayer assembly on Au surface**

![Scheme 3.2. Structure for DMT multilayer assembly on Au surface](image)
OIs region to the OH group of water molecules. XPS studies strongly support the important role played by the S-H groups and water molecules for the formation of the multilayer assembly of DMT on Au surface.

On the basis of CY, ATR-FT-IR, Raman spectroscopy and XPS results, we proposed the structure for DMT multilayer assembly on Au surface shown in Scheme 3.2. When Au electrode was immersed in an aqueous solution of DMT, one S-H group of DMT was chemisorbed on Au electrode while the other S-H is pointing away from the electrode surface. The presence of free S-H groups on the Au surface was confirmed from the CV, XPS and ATR-FT-IR measurements (data shown above). These free S-H groups on the Au surface form a hydrogen bond with the water molecules in solution. Subsequently, DMT molecules in solution form a hydrogen bond through one of its S-H groups with the water molecules already hydrogen bonded with DMT molecules, while the other S-H group of DMT forms a hydrogen bond with the water molecules in solution, and this type of intermolecular hydrogen bonding network goes on increasing when the adsorption time of Au electrode in an aqueous solution of DMT increases. The hydrogen bonding of thiol hydrogen with the oxygen atom of water molecule has been reported earlier [70]. As evidenced from the XPS results, in addition to thiol, the nitrogen atoms of DMT were also involved in hydrogen bonding with water molecules.
3.8. Simultaneous Determination of AA and UA

This section deals with the simultaneous determination of AA and UA using sub-monolayer of DMT and monolayers of AMT and MMT modified Au electrodes.

3.8.1. Electrochemical Oxidation of AA at Sub-Monolayer, Monolayer and Multilayer of DMT Modified Au Electrodes

Figure 3.12 shows the LSVs obtained for the oxidation of AA at bare, sub-monolayer, monolayer and multilayers of DMT on Au electrode in 0.20 M PB solution containing 0.5 mM AA. At sub-monolayer of DMT modified electrode, AA oxidation occurs at 0.24 V (Figure 3.12b) in contrast to bare Au electrode where oxidation of AA occurs at 0.48 V (Figure 3.12a). The reason for the 240 mV negative potential shift in the oxidation potential of AA is due to the possible

![Graph showing LSVs](image)

**Figure 3.12.** LSVs obtained for (a) bare Au, (b) sub-monolayer, (c) monolayer and (d) multilayers of DMT on Au electrode in 0.2 M PB solution containing 0.5 mM AA at a scan rate of 50 mV s\(^{-1}\).
hydrogen bonding between the carbonyl group at C1 in AA and DMT (Scheme 3.3). It has been already reported similar negative potential shift in the oxidation potential of AA at a poly(4-vinylpyridine)-coated carbon paste electrode [71]. It is suggested that the electron density of carbonyl group goes to DMT through the hydrogen bonding and increase the acidity of hydroxyl group at C3. As a result, the hydroxyl group becomes more acidic and eases in oxidation of AA. Thus, AA oxidized at less negative potential when compared to the bare electrode [71]. In the case of monolayer and multilayer, AA oxidation potential is shifted to more

**Scheme 3.3. Schematic representation for the hydrogen bonding between DMT and AA**

![Diagram showing hydrogen bonding between DMT and AA](image)

positive potential with decrease in the peak current when compared to sub-monolayer of DMT. It is obvious that the electron transfer will be slower when the layer thickness increases from sub-monolayer to multilayers. Thus, very good electrochemical response was observed for AA at sub-monolayer of DM in contrast to monolayer and multilayer of DMT modified Au electrodes.
3.8.2. Electrochemical Oxidation of UA at Sub-Monolayer, Monolayer and Multilayer of DMT Modified Au Electrodes

Figure 3.13 shows LSVs obtained for UA at bare, sub-monolayer, monolayer and multilayers of DMT modified Au electrodes in 0.20 M PB solution containing 0.5 mM UA. At bare Au electrode, UA oxidation occurs at 0.49 V (Figure 3.13a) whereas at sub-monolayer of DMT, UA oxidation occurs at 0.45 V with enhanced oxidation peak current (Figure 3.13b). The observed 40 mV negative shift in the UA oxidation potential and increase in the current response may be due to weak hydrogen bonding between the sub-monolayer of DMT with urate anion. Unlike AA oxidation potential (Figure 3.12b), only a small negative shift in the oxidation potential of UA was observed at sub-monolayer of DMT. Though hydrogen bond is formed between the carbonyl group of UA and DMT,

![Figure 3.13](image)

**Figure 3.13.** LSVs obtained for (a) bare Au, (b) sub-monolayer (c) monolayer and (d) multilayer of DMT on Au electrode in 0.2 M PB solution containing 0.5 mM UA at a scan rate of 50 mV s$^{-1}$
whose interaction is far from the reaction center [71] and thus only a marginal negative potential shift was observed for UA at sub-monolayer of DMT. Figures 3.13c and 3.13d show the oxidation of UA at monolayer and multilayer of DMT. The UA oxidation potential was shifted to more positive potential at monolayer and multilayer of DMT when compared to bare Au electrode and sub-monolayer of DMT modified Au electrodes. Since the electron transfer is expected to be slower when the thicknesses of the DMT layer increases, the oxidation potential of UA is shifted to more positive potential.

3.8.3. Simultaneous Determination of AA and UA at Sub-Monolayer, Monolayer and Multilayer of DMT Modified Au Electrodes

Figure 3.14 depicts the LSVs obtained for the mixture of 0.5 mM each AA and UA at bare Au, Au electrodes covered with a sub-monolayer, monolayer and multilayer of DMT in 0.20 M PB solution. As shown in Figure 3.14a only one oxidation peak was observed for both AA and UA at bare Au electrode. However, two oxidation peaks were observed for AA and UA at 0.26 and 0.47 V, respectively at a sub-monolayer of DMT on Au electrode (Figure 3.14b). The observed 210 mV potential difference is more than enough to determine the concentration of AA and UA simultaneously. When the thickness of DMT increases, the oxidation peaks of AA and UA were shifted to more positive potential and only one oxidation wave was observed. For example, at multilayer of DMT, only one oxidation wave was observed at 0.54 V (Figure 3.14d) whereas at monolayer, a small shoulder wave was observed around 0.30 V in addition
Figure 3.14. LSVs obtained for (a) bare Au, (b) sub-monolayer (c) monolayer and (d) multilayer of DMT on Au electrode in a mixture of 0.50 mM AA and UA in 0.20 M PB solution at a scan rate of 50 mV s\(^{-1}\).

to the oxidation wave at 0.52 V (Figure 3.14c). These results show that monolayer and multilayers of DMT cannot be used for the simultaneous determination of AA and UA. It is well established that the electrode passivation by thick monolayers slows down electron transfer, resulting in small currents and poor sensitivity [72]. On the other hand, these problems can be diminished on a thin monolayer, where the current is controlled by mass transport [72]. Very recently, the influence of orientation and monolayer thickness for the determination of AA and UA has been demonstrated using heteroaromatic thiol monolayers of mercaptobenzimidazole (MBI), mercaptobenzothiazole, mercaptomidazole and mercaptopyrimidine on Au electrode [73]. It is concluded that MBI forms a thin layer with flat orientation favors weak hydrogen bonding interactions with AA and UA and therefore it separates the voltammetric signals of AA and UA. On the other hand, since MBI
derivatives form thick layers with perpendicular orientation MBI, they fail to separate the voltammetric signals of AA and UA [73]. Recently, the electrochemical studies of electron transfer through SAMs with covalently bonded and hydrogen bonded electroactive groups were reported. It is found that the rate of electron transport through hydrogen bonded interface is only slightly lower than for covalently bonded system [74]. Likewise, fast electron transfer with favorable weak hydrogen bonding interactions with AA and UA would be expected at a sub-monolayer of DMT when compared to monolayer and multilayers of DMT. Further the stability of sub-monolayer DMT for the simultaneous determination of AA and UA was studied. The stability of a sub-monolayer of DMT for the simultaneous determination of AA and UA was tested by recording the LSV at different time intervals. Stable signals of AA and UA were observed without any significant shift in the oxidation potentials of AA and UA at a sub-monolayer of DMT for 17 repeated measurements. The observed stable signals of AA and UA indicate that sub-monolayer of DMT prevents the fouling effect caused by the oxidized products of AA, since fouling effect usually deteriorates the voltammetric signals of AA and UA [73].

Figure 3.15a shows differential pulse voltammogram (DPV) obtained for 10 fM of UA in the presence of 200 nM of AA in 0.20 M PB solution at sub-monolayer of DMT on Au electrode. It can be seen from Figure 3.15a that a very clear signal was observed for UA in the presence of 20-fold higher concentration of AA, which indicates that sub-monolayer of DMT can sense UA in the presence
of high concentration of AA. The observed sensitivity of sub-monolayer of DMT towards UA is more than enough to determine the concentration of UA in real samples because the normal UA levels in serum range from 240 µM and in urinary excretion is typically 1.4-4.4 mM. Further addition of 50 µM of UA into 200 µM

Figure 3.15. DPVs obtained for a sub-monolayer of DMT in a mixture of 200 µM of AA and 10 µM of UA (a) and 200 µM of AA and 50 µM of UA in 0.20 M PB solution.

of AA increases the voltammetric signal of UA without affecting the voltammetric signal of AA (Figure 3.15b). The differential pulse voltammetric measurements were also carried out by simultaneously changing the concentration of both AA and UA at sub-monolayer of DMT (Figure not shown). The peak currents of both AA and UA increase linearly with the concentration and the calibration plot is

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linear with correlation coefficients of 0.9952 and 0.9922 for UA and AA, respectively.

3.8.4. Determination of UA in Human Urine Samples Using the Sub-Monolayer of DMT Modified Au Electrode

The practical application of sub-monolayer of DMT modified Au electrode is tested by measuring the concentration of UA in human urine samples. The human urine samples were collected from the laboratory co-workers and were diluted to 100 times with PB solution without any treatment. DPV obtained for the urine sample in 0.20 M PB solution is shown in Figure 3.16a. It shows an oxidation peak at 0.44 V, which is assigned to the oxidation of UA. A small shoulder wave appears around 0.2 V in Figure 3.16a may be due to the oxidation of AA. To confirm the observed oxidation peak at 0.44 V is due to UA, the

![Graph showing DPV](image)

**Figure 3.16.** DPVs obtained for a sub-monolayer of DMT in 0.20 M PB solution for (a) 100 times diluted human urine sample and (b) after the addition of commercial UA to (a).
sample is spiked with certain amount of commercial UA and the resulting DP Vs is shown in Figure 3.16b. The increase in the peak current of UA after the addition of UA to the urine sample (Figure 3.16b) proved that the oxidation peak at 0.48 in Figure 3.16a for the human urine sample corresponds to the oxidation of UA. The same experiment was repeated for two more human urine samples collected from the laboratory co-workers. The recovery rates of the spiked samples were determined and ranged between 99% and 98% for urine samples. When compared to the recently reported MBI monolayer for the determination of UA [73], the sub-monolayer of DMT is stable for two days if it is kept in PB solution, whereas MBI monolayer is stable for only one day [73]. After 2 days, a 5% decrease in the peak

Table 3.4. Determination of UA in human urine samples using the sub-monolayer of DMT modified Au electrode.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Urine 1</th>
<th>Urine 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original value (µM)</td>
<td>5.24 ± 0.2</td>
<td>6.52 ± 0.2</td>
</tr>
<tr>
<td>Spike (µM)</td>
<td>0.72</td>
<td>1.20</td>
</tr>
<tr>
<td>After spike (µM)</td>
<td>5.92 ± 0.2</td>
<td>6.60 ± 0.2</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>98</td>
<td>99</td>
</tr>
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</table>

*Three replicate measurements were made on the sample

currents was observed. The possible oxidation of thiol into disulfide is the reason for the instability of sub-monolayer of DMT. Though we have not used blood
serum sample for the analysis of urine, our recovery rates is more than 98% when compared to MBI monolayer. The major advantage of the present electrode system is the less time consumption for the preparation of sub-monolayer of DMT. We found that 30 s to 300 s is enough for the formation of a sub-monolayer of DMT whereas 2 hours needed for the formation of MBI monolayer [73].

3.8.5. Electrochemical Oxidation of AA and UA in a Mixture at AMT and MMT Modified Au Electrodes

Figure 3.17A displays the LSVs obtained for the mixture of 0.50 mM each AA and UA at AMT monolayer modified Au electrode in 0.20 M PB solution. It resolved the voltammetric signals of AA and UA in a mixture with a potential difference of 270 mV in the first cycle (curve a in Figure 3.17A). The AA and UA oxidations occurred at 0.12 and 0.39 V, respectively. However, in the subsequent cycles, both AA and UA oxidation potentials were shifted to more positive potential and after 4 cycles AA oxidation was completely vanished (curve a in Figure 3.17A). However, UA oxidation was occurred around 0.50 V with decreased current response. Figure 3.17B shows the LSVs obtained for 0.50 mM each AA and UA in a mixture at MMT monolayer modified Au electrode in 0.20 M PB solution. The Au/MMT electrode could not separate the voltammetric signal of AA and UA in a mixture and it shows only one oxidation peak for both AA and UA at 0.48 V (curve a, Figure 3.17 B). Further, in the subsequent cycles, the oxidation peak was decreased. These results revealed that both AMT and MMT
monolayer modified Au electrodes are not suitable for the determination of AA and UA. The electrochemical behaviors of other biologically important molecules such as FA, DA, EP, NEP, XN, DOPAC, CY and HCY were also investigated at AMT and MMT monolayer modified Au electrodes. These electrodes could not show stable electrochemical response towards these analytes. The sub-monolayer DMT modified electrode did not show significant electrocatalytic activity towards aforesaid analytes.

Figure 3.17. (A) LSVs obtained for 0.5 mM each AA and UA at AMT monolayer modified Au electrode (a) 1\textsuperscript{st} (b) 2\textsuperscript{nd} (c) 3\textsuperscript{rd} and (d) 4\textsuperscript{th} cycles in 0.2 M PB solution (pH 7.2). (B) LSVs obtained for 0.5 mM each AA and UA at AMT monolayer modified Au electrode (a) 1\textsuperscript{st} (b) 2\textsuperscript{nd} and (c) 3\textsuperscript{rd} cycles in 0.20 M PB solution (pH 7.2) at a scan rate of 50 mV s\textsuperscript{-1}. 
3.9. Conclusions

Adsorption of DMT on Au surface from a completely deaerated aqueous solution led to the formation of a multilayer assembly via hydrogen bonding with water molecules, whereas adsorption from acetonitrile, ethanol, dimethyl sulfoxide, and chloroform solutions leads to the formation of a monolayer. CV, ATR-FT-IR, Raman spectroscopy, XPS and SEM were used to characterize the monolayer and multilayer assemblies of DMT on Au surface. Since DMT contains two S-H groups, it chemisorbs on Au surface through one of its two S-H groups, while the other S-H group is pointing away from the surface. The presence of free S-H groups on the Au surface was confirmed by CV, ATR-FT-IR and XPS. It is presumed that the free S-H groups of DMT on the Au surface form a hydrogen bond with the water molecules. Subsequently, DMT molecules in solution form a hydrogen bond with the water molecules attached with DMT on Au surface, and this type of hydrogen bonding network goes on increasing when the soaking time of the Au surface in an aqueous solution of DMT increases. The involvement of water molecules in the multilayer formation was confirmed from the appearance of a broad stretching band around 3300 cm\(^{-1}\) corresponding to the hydrogen bonded O-H in the ATR-FT-IR spectrum, in addition to a binding energy peak at 533.4 eV due to hydrogen donating water molecules in the O 1s region of XPS. The absence of a stretching band characteristic for S-S at 537 cm\(^{-1}\) in the Raman spectrum confirmed that the multilayer assembly was not formed via S-S linkage. However, exposure of DMT multilayer to air showed a stretching band
characteristic of S-S, indicating that aerial oxidation leads to the formation of S-S bond. SEM images showed that DMT forms a leaflike structured multilayer assembly on Au surface.

The sub-monolayer of DMT separated the voltammetric signal of UA from AA by 210 mV whereas monolayer and multilayers of DMT failed to separate them. The voltammetric signals of AA and UA were highly stable and reproducible at a sub-monolayer of DMT modified Au electrode. Fast electron transfer, weak hydrogen bonding interactions with AA and UA and prevention of fouling effect caused by the oxidized products of AA can be achieved at sub-monolayer of DMT and thus it successfully separated the voltammetric signals of AA and UA. On the other hand, AMT and MMT monolayer modified electrodes were not separated the voltammetric signals of AA and UA in a mixture. The practical application of the sub-monolayer of DMT modified Au electrode is demonstrated by measuring the concentration of UA in human urine samples without any pretreatment.
3.10. References


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