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A Comparative Study of Thiols Self-Assembled Monolayers on Gold Electrode

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Abstract: Self-assembled monolayers (SAMs) were performed on the surface of screen-printed gold electrode (AuE). Three different thiols were compared by cyclic voltammetry (CV) and scanning electron microscopy (SEM). AuE were modified with aromatic thiol (6-mercaptonicotinic acid, 6-MNA), aminethiol (thiourea, TU) and aliphatic thiol (11-mercaptoundecanoic acid, 11-MUA). The surface morphology and electrochemical characteristic of SAM-modified AuE such as electron transfer blocking properties, fractional surface coverage, stability and reproducibility were investigated. Based on the results, the electron transfer properties and fractional surface coverage of 11-MUA was the highest, followed by TU and 6-MNA. The stability and reproducibility of 11-MUA and TU were almost the same. 11-MUA which forms well-ordered and highly compact monolayer among three SAMs was selected for measurement of naphthalene.

Key words: Electrode Modification · Alkanethiols · Screen-Printed Electrode · Naphthalene

INTRODUCTION

A dynamic area of research in electrochemistry is the development of electrodes produced by chemical modification of different conductive substrates [1]. A mode of attachment for electrode modification is by self-assembled monolayer (SAM). The main function of SAM is to form a surface coating for binding of bio-recognition elements such as protein and antibodies onto metal substrates [2]. This modification of SAMs of sulfur-based molecules on gold substrates has generated great attention in this recent decade [3]. One of the most commonly SAMs used in gold electrode (AuE) modification is alkanethiol. The high affinity between sulfur atom and gold atom with the addition of intermolecular interactions between the alkyl chains, form a very stable SAM [4].

Many researchers had employed an alkanethiol, 11-mercaptoundecanoic acid (11-MUA) as the coating on the electrode surface for adsorption of bio-molecules due to its stability and this type of thiol had been investigated extensively [5]. However, there are also researchers that suggested other type of thiol reagent such as thiourea (TU) (amine-thiol) as alternative thiol for surface modification of gold electrode due to its cheaper price [6]. Besides, it is of interest to investigate the electrochemical behaviour of aromatic thiols like 6-mercaptonicotinic acid (6-MNA) due to its intermolecular interactions are expected to be stronger than those between the alkanethiols. The electrochemical characteristic of 6-MNA was only first been studied in year 2005 [7]. If the TU-modified electrode has the electrochemical properties and other analytical parameters similar with other thiol reagents, TU which is cheaper can be chosen as an alternative thiol. This can reduce cost in the production of electrochemical sensors or biosensors. However, the SAM modified electrode with better performance should be chosen in order to improve the performance of the sensors.

In this study, gold electrodes were modified with different type of thiols, which were 11-MUA, TU and 6-MNA. The surface morphology of modified gold electrodes were studied with scanning electron microscopy (SEM), whereas the electrochemical characteristics of these modified gold electrodes were investigated by cyclic voltammetry (CV). CV technique was chosen as it has rapid and simple implement. Besides, CV is the most well-known electrochemical technique that can be used to study the redox process at SAM modified electrodes [8]. It measures the electron transfers between a compound in solution and the gold electrode provides versatile information on the surface coverage or the

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compactness of SAM [9]. Several analytical parameters including surface coverage, repeatability, stability and reproducibility based on voltammogram obtained were also be compared in this study.

The SEM modified electrode with the best performance based on the characterization using CV was selected to be tested on the entrapment of naphthalene. This is to develop a baseline study in order to investigate the research opportunities of the best SAM. Naphthalene was selected as analyte because it is the most vital compound of polycyclic aromatic hydrocarbons in industry. It is largely used in the manufacture area such as in plastics and dyes. Red blood cell will be destructed if one is exposed to high concentration of naphthalene. Kidney and liver might be damaged if naphthalene is inhaled for a long period of time [10].

MATERIALS AND METHODS

Materials: Screen-printed gold electrodes (AuE) model C220AT were purchased from DropSens, Spain. 11-MUA, TU, 6-MNA, absolute ethanol, methanol, isopropanol, acetone, deionized water, potassium ferricyanide ($Fe(CN)_6^{3/4-}$) and potassium chloride (KCl) were purchased from Sigma-Aldrich, Switzerland, while naphthalene was purchased from Merck (KGaA, Belgium).

Instrumentations: All experiments involving cyclic voltammetry were conducted at room temperature using AutoLab potentiostat PGSTAT30 from Methrohm (Switzerland). AuE was connected to the potentiostat by DRP- CAC connector which was purchased from DropSens, Spain. Surface morphologies of the electrodes were studied by JEOL scanning electron microscopy (SEM) JSM-6360LA. AuE was cleaned with JAC Ultrasonic Cleaner 2010P, Jinwoo Engineering Co. (Korea).

Preparation of Gold Electrodes: C220AT AuE was ultrasonic cleaned in acetone for 5 minutes, followed by isopropanol 5 minutes and lastly in DI water for 5 minutes.

SAMs Formation of Gold Electrode: Clean AuE was immersed in 11-MUA solution for 20 hours at room temperature. This MUA solution was prepared by dissolving 11-MUA in absolute ethanol to reach the concentration of 5 mM. The residual MUA molecules were rinsed off with absolute ethanol. For surface modification with TU, clean AuE was soaked in 250 mM thiourea solution at room temperature for 24 hours. Then, it was washed thoroughly with distilled water and dried. The surface of AuE was modified with 6-MNA by immersing clean AuE in methanol solution of 5 mM MNA for 20 hours. The schematic of surface modification with different types of thiol is illustrated in Figure 1.

Immobilization of Naphthalene: MUA-modified electrode was immersed in freshly prepared 1 mM naphthalene for 3 hours. Then, the electrodes were rinsed with distilled water and dried at room temperature.

Voltammetric Monitoring of the AuE: The potential was scanned at the potential ranging from -0.2 to +0.6 V, with the scan rate of 100 mVs^{-1} in 5 mM $Fe(CN)_6^{3/4-}$. 0.1 M KCl redox couple was used as supporting electrolyte. The voltammogram of unclean and clean bare AuE had been investigated. Then, the voltammetric responses of clean

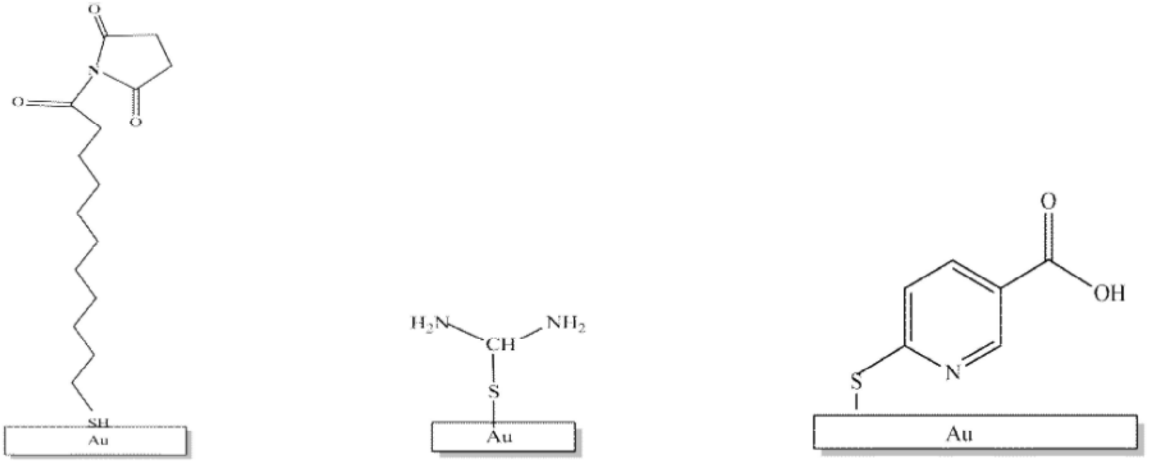

Fig. 1: Schematics represent the surface modification of AuE by SAM (Left to right: 11-MUA, TU and 6-MNA).

bare AuE and three types of modified-AuE (11-MUA, TU and 6-MNA) have been investigated and compared. The stability of modified electrodes were studied by comparing the ten cycles of CV. Reproducibility of three different modified electrodes were also tested by repeating the experiments for three times (n=3). Measurement of modified electrode immobilized with naphthalene was carried out using the CV procedure for bare electrode.

Surface Characterization of Modified AuE using Scanning Electron Microscopy: The surface of AuE was observed using scanning electron microscope (SEM). The surface morphology of the unclean bare AuE and clean bare AuE have been observed and compared. Then, SEM image of SAMs-modified AuE also have been investigated and compared before and after the modification.

RESULTS AND DISCUSSION

Electrochemical Characterization of Modified Electrode: The voltammetric responses of three types of thiol-SAMs (11-MUA, TU and 6-MNA) modified AuE were compared with the voltammetric response of bare AuE, is shown in Figure 2.

For electrochemical characterization of 11-MUA, the bare AuE demonstrated a 'quasi-reversible' electrochemical characteristic with a ?E_p value is approximately 120 mV. The peak morphology was absence after the modification of AuE with 11-MUA SAM. This CV result reveals that voltammetric process was clearly irreversible as there was no redox reaction noticed. The electron-transfer kinetic of $Fe(CN)_6^{3-/4-}$ redox couple was greatly decrease. These conditions are due to the blocking of access of $Fe(CN)_6^{3-/4-}$ redox couple to the gold electrode by 11-MUA SAM [11]. In addition, the disappearance of peak-shaped morphology of 11-MUA modified AuE shows that the SAM was well-packed on the AuE. The pinholes sites were believed to cover very less on the electrode surface by comparing the response of the bare gold electrode. The result obtained was in good agreement with the ones reported by Ahmad & Moore, 2012 [5].

The electrochemical properties of TU SAM were also investigated using CV with $Fe(CN)_6^{3/4}$ as redox couple. The peak-to-peak separation of TU is bigger compared to bare gold electrode. The current response of redox peaks TU also decreased after TU was self-assembled on the surface of AuE. The peak current reduced accompanied with a significant increase in ΔE_p (approximately 350 mV) indicated the electron transfer kinetic is comparative slow. The reason is TU SAM hinders the electron transfer $Fe(CN)_6^{3-/4-}$ redox couple toward the electrode surface [12]. The insulating effect was increased after the modification of AuE with TU. However, the oxidation and reduction peak still can be observed in the voltammogram of TU. This shows that some of the electron transfer might occur through bare spot on the electrode or by tunnelling across the SAM [13]. The result support with the observation reported by Zhang &Zhuang, 2010 [10].

Similar to 11-MUA and TU, 6-MNA was studied using CV with $Fe(CN)_6^{3-/4}$ as redox couple. The voltammogram of 6-MNA modified electrode also demonstrated lower peak current and bigger peak-to-peak separation when the voltammetric response compared to bare AuE. This suggested that electron transfer kinetic of $Fe(CN)_6^{3-/4-}$ at 6-MNA electrode was relatively slow. Slower electron transfer kinetic can be related to electrostatic repulsion. Carboxyl (-COOH) groups of the SAM on the electrode surface can undergo ionisation and become negative charged. This made negatively charge redox couple difficult to approach the surface of electrode due to electrostatic repulsion. Similar to TU, the redox peaks still can be seen from the voltammogram of 6-MNA.

All of the three types of SAM modified electrode demonstrated certain degree of insulating properties on the electrode as the peak currents were reduced.

If the peak shape morphology was fully suppressed, this shows that the SAM was closed-pack, has less defect sites, high insulating effect and thus inhibited electron transfer process significantly. This is because the presence defect sites or permeation was normally contributed to the peak-shape morphology. On the other hand, the tendency of voltammogram to form sigmodial curve similar to the standard potential of the $Fe(CN)_6^{3-/4-}$ redox couple indicates the presence of pinholes and the electrons transfer occurring at these pinholes sites.

Therefore, the CV result reveals that the insulating and electron transfer blocking properties of 11-MUA was the highest, followed by TU and 6-MNA, based on the peak current and peak-shaped morphology of respective SAMs according to Figure 2. This is due to 11-MUA is a type of long-chain alkanethiols. Long-chain (n= 10) thiols will form a very stable and well order monolayer on the electrode surface. It is a relatively compact monolayer which will act as ionic insulator on gold electrode. It will has less defect and high fractional coverage (vide infra). Thus, more 11-MUA molecules can hinder the route for electron transfer and suppressed the current response most significantly [13].

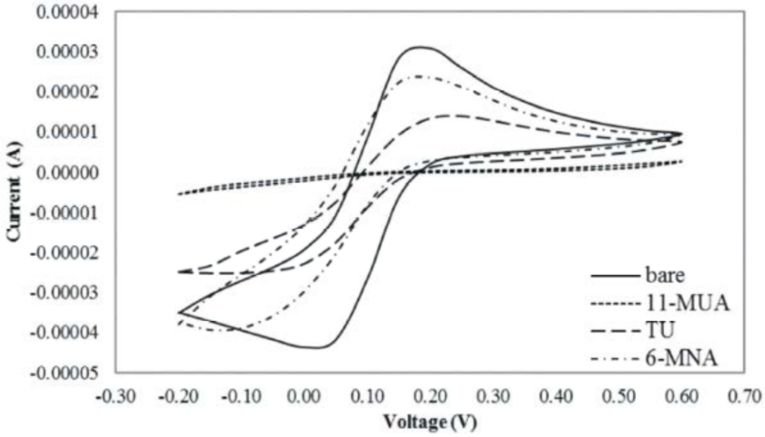

Fig. 2: Cyclic voltammogram of 5mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl solution at a scan rate of 0.05 Vs^{-1} on a clean bare gold electrode, modified with 11-MUA, TU and 6-MNA.

This less blocking effect and faster electron transfer of TU might due to TU was a type of short chain thiol. This short chain length of thiol normally formed disorder and low degree of surface coverage of monolayer on electrode then long chain thiol [14]. Moreover, the result of TU is in good agreement with Limbut *et al*. (2006a) as 1-dodocanethiol was used in their research as final capping of the electron surface to increase the degree of insulation [6].

For 6-MNA, the molecule was usually form a less compact monolayer on gold electrode due to the presence of bulky carboxyl (–COOH) group. The orientation of –COOH groups is face away from each other which means the orientation of 6-MNA molecules were less compact on the surface of electrode.

In addition, the length of alkane chains is not the only reason that affected the ion permeability and electron transfer process. The terminal group of SAMs also has a great effect on the redox response and electron transfer kinetic due to the electrostatic force between the terminal groups of SAMs and ionic species of redox couple. For instance, the voltammetric response of $Fe(CN)_6^{3-/4-}$ redox couple is reduced in the order of NH_2 > OH > COOH [15]. It is also one of the factors that affect the voltammetric behaviour of thiols modified AuE.

Surface Morphologies of SAMs: Both the surface morphology of bare AuE and SAMs-modified AuE were studied using SEM. The attachment of SAM layer can be further confirmed by SEM images. The accelerating voltage used was 15kV.

The magnification level and resolution used were 1, 000 and 10 µm respectively for both bare AuE and 11-MUA modified AuE. Figure 3 shows the surface of AuE was difference before and after the modification with 11-MUA SAM. These scanning electron images shows 11-MUA molecules are attached on surface of AuE.

For TU, the magnification level and resolution used was 1, 500 and 10 µm respectively for both bare AuE and TU modified AuE. These scanning electron images presented in the Figure 4 further confirmed the attachment of TU molecules on the surface of electrode.

For 6-MNA, the magnification level and resolution used were 1, 500 and 10 µm respectively for both bare AuE and 6-MNA modified AuE. Attachment of 6-MNA was further confirmed by these SEM images based on Figure 5.

Fractional Surface Coverage: The fractional surface coverage of the monolayer, θ^{i}_{CV} was related to the reduced observed in the peak current of the oxidation/reduction of a redox probe in the solution. θ^{i}_{CV} can be calculated using the following equation and assuming the diffusion to uncovered parts of AuE is linear:

θ^{i}_{CV} = 1- (Error! Reference source not found.)

where ip^{AuE} is the peak current obtained at the bare AuE and ip^{SAM} is the peak current obtained in the same conditions at the SAM-modified AuE. The percentage surface coverage can be calculated by comparing the percentage of ratio of peak current of bare AuE with peak current of SAM-modified AuE under the same conditions.

From Table 1, the fractional coverage of 11-MUA monolayer was the highest, followed by TU and 6-MNA was the lowest. This result supports CV result observed, in which the greater the peak shape morphology in voltammogram suppressed by SAM, the more compact

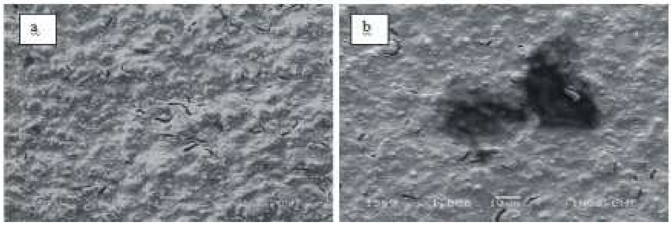

Fig. 3: SEM images of a) clean bare AuE and b) 11-MUA modified AuE.

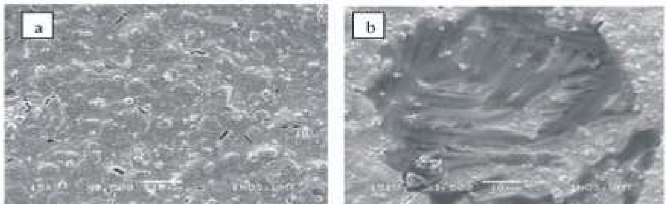

Fig. 4: SEM images of a) clean bare AuE and b) TU modified AuE.

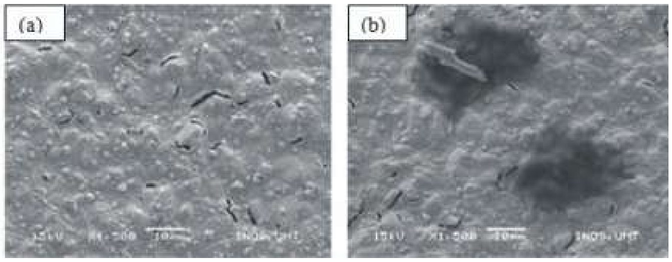

Fig. 5: SEM images of the a) clean bare AuE and the b) 6-MNA modified AuE.

and well-packed the SAM on the surface of AuE. However, the θ^{i}_{CV} of stable monolayer, which is 11-MUA, is theoretically more reliable than the short chain SAM (TU and 6-MNA). This is due to the electron transfer on SAM-modified AuE can arise by tunnelling through the SAM molecule [16]. The limitations of CV such as nonlinear diffusion to pinhole and overpotential also affect the accuracy of θ^{i}_{CV} of the short chain SAM. Thus, CV should be complementing with various type of spectroscopy such as electrochemical impedance spectroscopy EIS to obtain result which is more precise [17].

Reproducibility: The reproducibility of SAMs modified AuE was studied using distinct working electrodes which was modified with the same procedure. The percentage of current change of bare AuE and SAMs modified AuE was compared and the relative standard deviation (RSD) was calculated. The RSD for 11-MUA, TU and 6-MNA modified AuE were 0.23%, 2.53% 12.67%, respectively.

Reproducibility was greatly associated with the precision of the results obtained. The reproducibility of 11-MUA and TU modified AuE were quite high and the RSD can be ignored. However, the reproducibility of 6-MNA modified AuE was relatively high. The reproducibility varied due to the presence of contaminants, human error and condition of the lab place. All those physical factors will definitely affect the thickness of coating of SAMs on the electrodes.

Stability: The repetitive measurements were conducted through CV for ten successive scans on AuE modified with SAMs. The RSD of ten successive scans of 11-MUA, TU and 6-MNA was 5.16%, 3.13% and 9.50%, respectively.

Electrochemical Evaluation of Naphthalene Immobilisation on 11-MUA: 11-MUA, which was the best performance among the three thiols measured was selected to be tested on the possibility for the immobilization of naphthalene. This can act as the baseline testing for the further application such as in forming molecular-imprinting SAM or other types of sensor.

Capability of MUA to trap naphthalene in water was investigated and recorded in Table 2. Two comparisons were made; AuE without SAMs and AuE with SAM. Both AuEs, after being immersed in naphthalene in water, displayed lower current value compared to current value before immersion. This implies that naphthalene was

Table 1: Fractional Coverage, θ^{i}_{CV} of 11-MUA, TU and 6-MNA

SAM	Fractional Coverage of Monolayer, θ^{i}_{CV}	% Surface Coverage of Monolayer
11-MUA	0.9903 ± 0.0023	99.03
TU	0.6148 ±0.0155	61.48
6-MNA	0.2539±0.3288	25.39

Table 2: Fractional coverage of naphthalene on AuE without SAMs and AuE with SAMs

Types of SAM	I_i (A)	I_f (A)	Fractional of coverage
Bare (without SAM)	4.79E-05	4.34E-05	9.39
MUA	3.36E-06	2.09E-06	37.80

successfully entrapped on the electrode surface. However, the shape of bare electrode does not change despite the slight reduction in current values, which means that no disturbance in electron transfer. This is might due to the temporary attachment of naphthalene since it is quite impossible for naphthalene to bond itself to the gold surface. For MUA-modified electrode, there is considerable reduction of current and the shape of voltammogram is vaguely shifted into more flat shape along the horizontal axis compared to before immersion in naphthalene solution.

However, voltammograms only prove the entrapment of naphthalene without showing the percentage of naphthalene captured on the electrode. In order to calculate the percentage of naphthalene coverage, equation 1 was applied, using the data collected from cyclic voltammetry measurements. Unfortunately, this equation cannot predict the exact amount of naphthalene molecules, but it can be used to calculate the approximate coverage of naphthalene on the electrode surface.

From Table 2, AuE with MUA-SAMs displays higher percentage compared to AuE after immersed in water containing naphthalene. It is understandable that bare electrode has low percentage value due to no SAMs to capture naphthalene. For MUA-SAMs, the carboxyl functional group, which exposed to naphthalene environment, is free to bond with naphthalene molecules. However, due to the large size of the molecules, only a few molecules can bond with the carboxyl group, thus explains the voltammograms and percentage of coverage.

CONCLUSION

Long-chain alkanethiol, 11-MUA is the best among the three types of thiols been investigated due to highest blocking effect for electron transfer process, highest surface coverage, high reproducibility and high stability based on electrochemical characterization. Good blocking characteristic for the electron transfer process indicates the monolayer formed is highly ordered, well-packed and compact on the AuE surface. Moreover, naphthalene was successfully entrapped on the surface of 11-MUA modified. This indicates a lot of research opportunities to be explored by using 11-MNA to functionalize the surface of electrode.

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