

The optimization of self-assembled monolayer of thiols on screen-printed gold electrode

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ABSTRACT

The activated gold-modified electrode surface for self-assembled monolayer (SAM) is suitable for N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), 16-mercaptohexadecanoic acid (16-MDA), and N-hydroxysuccinimide (NHS). The various substrates could potentially be used for surface functionalization that binds to one another. Using modified screen-printed gold electrodes, 16-MDA, EDC, and NHS, we developed a self-assembling monolayer. Different 16-MDA concentrations were applied to the surface of a screen-printed electrode surface to enhance the sensitivity of the working electrode. The impact of different EDC, NHS, and 16-MDA concentrations (0.4 M, 0.8 M, 1 M, 1.2 M, 1.4 M, and 1.8 M) and incubation times between 5 and 30 minutes were examined and compared. The binding surface of the screen-printed gold electrode was characterized using the differential pulse voltammetry (DPV) technique. It has been demonstrated that the substrate concentrations at 5 minutes and 30 minutes of incubation time used to have the highest surface coverage and electron transfer rates. This concentration would be utilized in the subsequent experiment to evaluate the binding of various bacterial species. These findings suggest that the SAM in a modified screen-printed gold electrode may be functionalized to detect microorganisms.

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1. INTRODUCTION

The self-assembled monolayers (SAM) method is an easy-to-use tool for modification, and SAMs deposited on gold substrates or screen-printed gold electrodes have been used to investigate how features affect bacterial adhesion [1]. The stable platform for immobilization is another component in developing an immunosensor, in addition to the working electrode's excellent conductivity [2]. Proper immobilization on the functionalized surface area can still enhance all of this. A SAM is a single layer of molecules that spontaneously arrange themselves and covalently bind to a substrate to produce a highly organized,

homogeneous, and densely packed structure. By increasing attractive contacts between molecules and their substrate and decreasing repulsive interactions within the molecules, one may decrease free energy, which is what drives SAM formation. The two basic steps in the formation of SAMs are adsorption and organization. The molecules of interest are first brought into contact with a solid substrate through a solution. These molecules have two ends: one forms the exposed surface of the monolayer, while the other binds to the substrate. There are two distinct functional groups in these compounds [3]. SAMs can serve as protective coatings to guard surfaces from corrosion, chemical degradation, and environmental impact. Due to their potential to change a surface's electrical properties, SAMs are crucial for the development of electronic devices and sensors [4]. All the biological identifications such as protein adsorption, antibody-antigen interaction, and deoxyribonucleic acid (DNA) can all be performed on the SAM using sensitivity instruments such as differential pulse voltammetry (DPV) [5], [6].

DPV is a method for examining the electrochemical behavior of a system. DPV is the most used technique for gathering qualitative data about electrochemical processes. When analyzing numerous electron transfers in electrochemical reactions as well as the oxidation and reduction processes, the DPV approach has grown in popularity for electrochemical investigations [7]. These systems were employed in fundamental studies of electron transfer, electrochemical sensors, and electrochemical surface science in general [8], [9]. According to the paper, there was no evidence of hydrogen generation in other experiments. This problem is called surface functionalization, which needs to be effective in altering the surface properties of a material to achieve the desired bio response of inhibiting a potential adverse reaction between the screen-printed gold electrode, alkanethiols, and bacteria sample. The purpose of this experiment is to establish the optimum conditions for the binding of the substrates N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and 16-mercaptohexadecanoic acid (16-MDA) to the screen-printed gold electrode. When the substrate is already established then it can be used to bind with the bacteria for the next experiment. To describe the optimized condition in this work, we used an electrochemical approach. By using DPV to evaluate and optimize the functionalized surface working electrode, 16-MDA, EDC, and NHS were measured. The following experiment will be conducted under these ideal circumstances. Overall, investigations of nanomaterials with modified surfaces have some limitations despite improved analytical technologies [10]–[12].

2. RESEARCH METHOD

2.1. Experimental method

Figure 1 shows the modifications of 16-MDA with different concentrations. The concentrations of 16-MDA are 0.4 mM, 0.8 mM, 1 mM, 1.4 mM, and 1.8 mM. The screen-printed gold electrode was purchased from the Metrohm Company in Switzerland. The 16-MDA powder, 1.0 mg/ml in a 5% solution in methanol or ethanol, was purchased from Sigma Aldrich (St. Louis, MO, USA). After dropping the 16-MDA on the screen-printed gold electrode and inserting the electrode into the DPV, put the potassium hexacyanoferrate ~99%, potassium hexacyanoferrate (II) trihydrate 98.5–102.0% (3 aminopropyl) triethoxysilane to ensure the activation of the 16-MDA reaction. After optimization of the 16-MDA, the exact value will be used with the second and third substrates. The concentrations of EDC are 0.4 M, 0.8 M, 1 M, 1.4 M, and 1.8 M. This concentration will be optimized for the next experiment with different substrates. The EDC of 98.5 with a 191.7 molar weight was purchased from Sigma Aldrich (St. Louis, MO, USA). The optimized 1.8 mM 16-MDA, 1 M EDC, and NHS concentrations on a screen-printed gold electrode. The concentrations of NHS are 0.4 M, 0.8 M, 1 M, 1.4 M, and 1.8 M NHS, with a purity of 98% and a molar weight of 111.59, was also obtained from Sigma Aldrich (St. Louis, MO, USA).

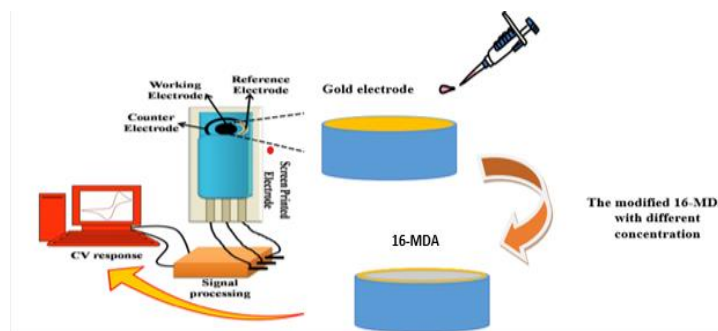


Figure 1. The modified 16-MDA with different concentrations on screen printed gold electrode

2.2. Experiment setup

Electrochemical measurements and DPV were performed to run the screen-printed gold electrode. Performed different concentrations for 16-MDA, EDC, and NHS. With a sequence of regular voltage pulses superimposed on the potential linear sweep or staircase DPV, a voltammetry technique used to produce electrochemical measurements is a derivative of linear sweep voltammetry or staircase voltammetry [13]. Due to the following two characteristics, such as the ability to minimize the effect of the charging current and achieve high sensitivity while only extracting faradaic current, these measurements can be used to study the redox properties of extremely low concentrations of chemicals [14]. Then, selectivity is a result of the fact that DPV makes it possible to alter the working electrode surface or incorporate chemical agents to allow for the selective detection of analytes. When target compounds and interferents can be separated using selected electrodes or modified surfaces, analyses are trustworthy and accurate. DPV is a versatile electrochemical technique that is commonly used in analytical chemistry [15]–[17]. DPV was run between a potential of -0.2 to +0.2 with a fixed scan rate of 50 mVs⁻¹. The 16 MDA, EDC, and NHS solution was synthesized according to a previous approach with slight modifications. To generate more functional groups which, provide the site for further binding of bacteria in the next experiment. 2.888 mg 16-MDA powder was prepared by dissolving in different concentrations in 10 mL ethanol and mixing the solution with the mixer equipment. The solution was drop cast onto the surface screen-printed gold electrode and left for 5 minutes. The fabricated electrode was then rinsed with the distilled water (DI). The EDC and NHS were prepared by dissolving in different concentrations for each EDC and NHS in a buffer solution and mixing the solution with the mixer equipment. The solution was drop cast onto the surface screen-printed gold electrode and left for 30 minutes. The 16-MDA were diluted using 10 mL ethanol. The EDC and NHS were diluted with a 5 mL buffer solution. In this experiment, will optimize the 16-MDA before using it for the next experiment with different substrates. All the electrodes will be incubated for 30 minutes at room temperature. The functionalized working electrode was then rinsed with the DI. functional groups that may enhance selectivity, increase biocompatibility, or make it simpler to immobilize biomolecules [17], [18].

3. RESULTS AND DISCUSSION

3.1. The optimization of modified

According to Figure 2, gold nanoparticles have a large surface area, great electrical conductivity, and help accelerate electron transfer. Due to the presence of the substrate's 16-MDA layer on the surface of the screen-printed gold electrode, the current peak for the functionalization electrode was decreased. The screen-printed gold electrode formed a connection with the 16-MDA film layer, which caused the current peak to further decrease after the electrode was modified and incubated at 5 minutes. The lowest concentration was 1.8 mM, and the present peak for 16-MDA was 23.91 nA the strong blocking action of 16-MDA SAM on $K_3Fe(CN)_6$ is said to be the source of the peak decrease at the modified electrode. Since the 16-MDA is hydrophobic, the dense monolayer that formed when it was assembled at the bare gold electrode prevented hydrophilic $K_3Fe(CN)_6$ from reaching the electrode surface [19], [20].

Figure 3 showed due to the presence of the substrate's 1.8 mM 16-MDA layer on the EDC surface of the screen-printed gold electrode. The different concentrations of EDC were incubated on the modified electrode for 30 minutes to ensure that the EDC was attached to the carboxylic groups. Figure 4 showed due to the presence of the substrate's 1.8 mM 16-MDA layer on the EDC surface of the screen-printed gold electrode. The current peak should grow more than the peak current of EDC due to the reaction between them, and the peak current of 1 NHS increased at a rate of 418 nA, which was the optimum.

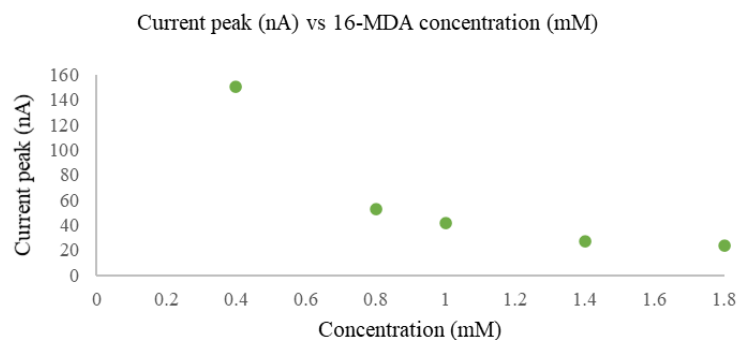


Figure 2. Different concentration 16-MDA with screen-printed gold electrode

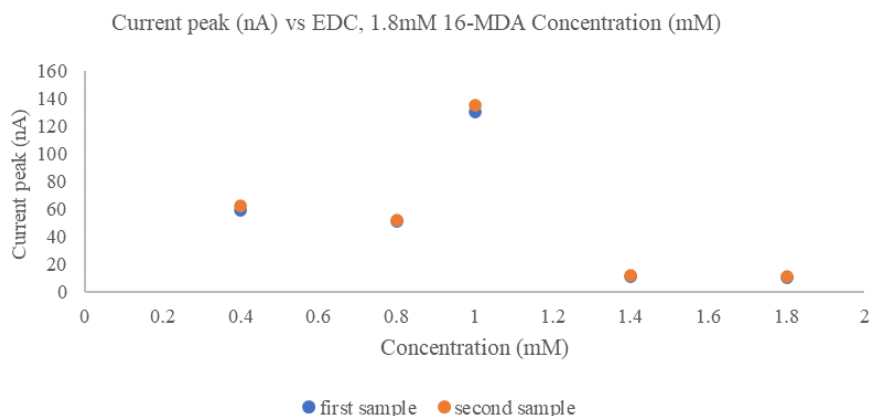


Figure 3. Different concentration EDC with 1.8 mM 16-MDA

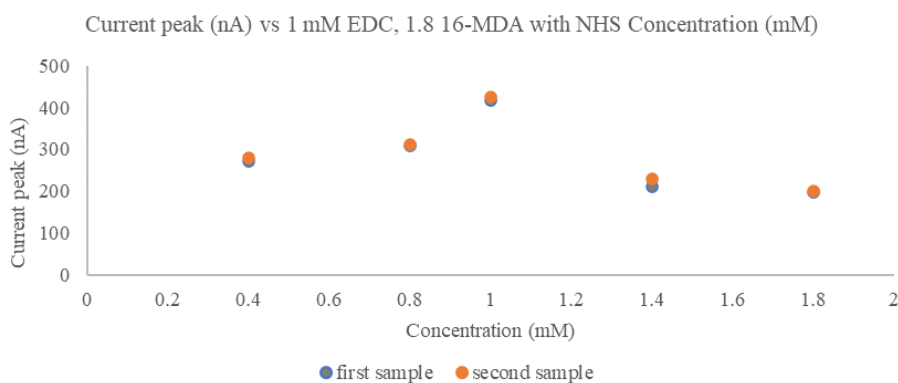


Figure 4. Different concentration NHS with 1 mM EDC and 1.8 mM 16-MDA

Tables 1-3 showed the peak for each result for 16-MDA, EDC, and NHS on DPV. In Table 1, it can be concluded that for 16-MDA, the lowest current peak, which is 23.91 nA, is the best because of the blocking action between the screen-printed gold electrode and the 16-MDA. The incubation times within 5 minutes for each concentration were analyzed and compared to efficiently achieve the optimal conditions for 16-MDA. Table 2 shows that the higher current peak of 130.0 nA will be chosen due to the reaction between EDC and 16-MDA. Lastly, Table 3 shows that the current peak at 418 nA is the optimum that has already been chosen because the current peak should be growing more than the peak current EDC.

Table 1. Differential DPV results for 16-MDA

Concentrations 16-MDA (mM)	Current peak (nA)	Time (s)
0.4	149.91	5 min
0.8	52.52	5 min
1.0	41.72	5 min
1.4	27.05	5 min
1.8	23.91	5 min

Table 2. Differential DPV results for EDC with 1.8 mM 16-MDA

Concentrations EDC (M)	Current peak (nA)	Time (s)
0.4	59.1	30 min
0.8	51.1	30 min
1.0	130.0	30 min
1.4	11.5	30 min
1.8	10.51	30 min

Table 3. Differential DPV results for NHS with 1 mM EDC and 1.8 mM 16-MDA

Concentrations NHS (M)	Current peak (nA)	Time (s)
0.4	217	30 min
0.8	309	30 min
1.0	418	30 min
1.4	210	30 min
1.8	197	30 min

3.2. Optimization of the experimental conditions

In contrast to the modified electrode's current peak, the electron transfer impedance was reduced for a 1.8 mM 16-MDA concentration as shown in Figure 5. For concentration in EDC and NHS, 1.8 mM of 16-MDA will serve as the fixed value. According to a prior study, electrodes with low values have superior analytical properties because the charge transfer rate is enhanced by the increased surface conducting area. The hydrophilicity and hydrophobicity, charge, coverage, structure, incubation duration, and other surface properties of SAMs such as 16-MDA likely influenced the interaction's strength. This may be caused by the potential for multilayer formation after 16-MDA, EDC, and NHS, which will increasingly serve as a barrier for the transfer of electrons between the electrode and the electrolyte. The optimal concentrations were determined to be 1 M EDC and 1 M NHS. The EDC-NHS chemistry-based immobilization would activate the carboxyl group of gold resulting in NHS ester as an intermediate that finally leads to covalent attachment [20].

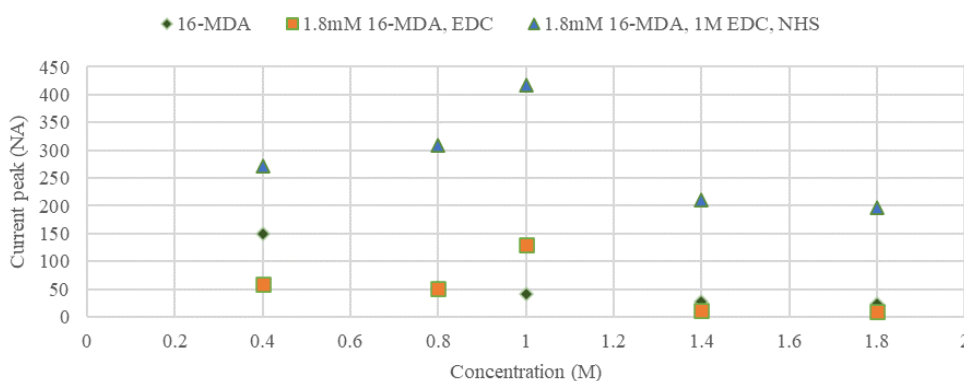


Figure 5. The mixture of 16-MDA, EDC, and NHS

4. CONCLUSION

The screen-printed gold electrode was effectively applied to the modified substrate with a different concentration. Under optimal conditions, the peak current, scan rate, DPV, and substrate amounts were analyzed. Different concentrations were applied to the 16-MDA, EDC, and NHS, and incubation periods between 5 and 30 minutes were used. In terms of efficiency, speed, and sensitivity, this immunosensor for the substrate is comparable with other affinity electrochemical biosensors. The suggested platform has a good analytical response in terms of optimal binding capacity under the optimized operating circumstances. The improved electrical conductivity and surface area may be responsible for the successful performance. All these characteristics can be used for additional surface functionalization research. It can be concluded that SAMs are particularly suitable for formation on gold surfaces because the Gold does not readily react with most substances, including several solvents and reagents, due to its chemical inertness. SAMs may form without being inhibited by interactions between the gold surface and the molecules that make up the monolayer because of their inert nature.

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