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Low Albumin Determination as a Biomarker Cancer with Cyclic Voltammetry in Ag_{film}/ITO and $AgNO_{film}$/ITO Electrodes

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Abstract

Ag and AgNO film were fabricated through sputter deposition on indium tin oxide (ITO) conductive glass. Electrochemical detection using cyclic voltammetry (CV) was carried out for low albumin levels in blood as a parameter of cancer patients. This study aims to determinate low albumin level as a biomarker cancer. Albumin adsorption on the Ag_{film}/ITO and $AgNO_{film}$/ITO was also investigated to determine the level of sensitivity of both electrodes. Analysis of the CV measurements indicated that the Ag_{film}/ITO electrode was more sensitive compared to the $AgNO_{film}$/ITO electrode, with a sensitivity value of 4.564 μA $M^{-1}cm^{-2}$ for the Ag_{film}/ITO electrode and 2.123 μA $M^{-1}cm^{-2}$ for the $AgNO_{film}$/ITO electrode. The testing of albumin levels in blood within a range of low concentration levels between 10^{-1} and 10^{-8} g/dL indicated a detection limit of 10^{-9} g/mL. The selectivity of Ag electrodes was found to be very good for other interfering molecules such as urine, glucose, and lysozyme. The results of the modeling of electric field and magnetic field distribution showed that the Ag_{film}/ITO electrode possessed larger values than the $AgNO_{film}$/ITO electrode. From this analysis, it can be concluded that the electrode modified with micro-sized Ag achieved more effective results than the electrode modified with AgNO. This sensor can determine low albumin level as a blood cancer biomarker. This sensor holds great promise for use in real samples in the future.

Keywords: Albumin, Biomarker, Blood cancer, Cyclic voltammetry, Electrode, Electrochemical impedance spectroscopy, Silver

Introduction

Leukemia is a disease that originates from the bone marrow and mostly affects children. This disease is caused by the uncontrolled growth of abnormal white blood cells and, consequently, the functions of other blood cells. Based on the Global Burden of Cancer (GLOBOCAN) 2020 data, leukemia was ranked thirteenth in the estimated number of new cases among all types of cancer in 2020. While in Indonesia, leukemia was ranked ninth [1]. Leukemic cells are immature white blood cells that grow abnormally and uncontrollably in the bone marrow. These cells then enter the bloodstream. Generally, imaging tests such as x-rays are unable to detect leukemia because it does not form into a mass (tumor) [2]. Biomarkers can be used as an early detection method in the form of substances that can be measured. Biomarkers appear when biological processes in the human body are abnormal. Biomarkers can be used as prognostic indicators of response to therapy [3]. Cancer biomarkers can be found in tumor tissues or serum, which include various types of molecules such as deoxyribonucleic acid (DNA), mRNA, enzymes, metabolites, transcription factors, and cell surface receptors [4]. Early cancer detection using biomarkers can increase the chances for successful treatment. Furthermore, biomarkers can also be used for early detection of cancer recurrence after treatment. However, technological challenges in the accurate identification of cancer biomarkers are unavoidable. Cancer is a diverse disease, and a single biomarker does not have the ability to detect all types of cancer [5]. Besides specific biomarkers, measuring protein levels, particularly albumin level in blood, can be carried out for leukemia. A low level of albumin may be caused by leukemia. For adults, the normal range of serum albumin in blood is between 3.5 to 5.0 g/dL and the condition in which the level of serum albumin in blood is below 3.5 g/dL is called hypoalbuminemia. A low level of serum

albumin concentration may be caused by cytokines such as tumor necrosis factor (TNF). Cytokines are produced due to systematic inflammatory response to the tumor, in which these cytokines influence the production of albumin by hepatocytes. A chronic systematic inflammatory response causes the progression of the disease which in turn causes a significant decrease in the level of albumin [6]. Therefore, a low level of albumin is a good indicator for cancer prognosis. Bovine serum albumin can be used as a substitute to human serum albumin (HSA) because both have similar characteristics. BSA is often used in research due to it being easily obtainable [7]. Several researchers have developed methods for early detection of cancer using silver (Ag) such as the detection of prostate cancer, breast cancer, leukemia, and lung cancer using surface enhancement Raman spectroscopy (SERS) [8-10]. Besides using SERS, optical and electrochemical based cancer detection have also been carried out [11-12].

Ag possesses a higher electrical conductivity, thermal conductivity, and reflectivity value compared to other metals. The pure form of silver can be obtained from the crust of the earth [13]. Ag is used in various academic disciplines, including the field of medicine. Ag ions inhibit enzymes in bacteria that transport nutrients, form the structure, and synthesize the cell wall. Furthermore, Ag ions bind with the genetic material of bacteria [14]. Silver (Ag) and Gold (Au) are noble metals which are capable of strongly absorbing light in the local surface plasmon resonance (LSPR) wavelength due to the collective oscillation of free electrons with an illuminating light field [15]. However, Ag has an absorption peak higher than Au [16]. The selectivity and sensitivity of Ag as sensors is extensively used in analytical chemistry [17].

Electrochemical techniques have advantages for biosensors due to their fast cost, high accuracy, high precision, high sensitivity and low detection limit. Modification of the electrode surface with Ag and AgNO films can increase the sensor's sensitivity and detection limit. By increasing the sensitivity of the electrode with the addition of Ag and AgNO films can increase the current signal in electrochemical analysis. Therefore, this work was made by deposition of Ag and AgNO films on the surface of ITO electrodes for albumin detection as a biomarker of blood cancer. The modified electrode has high sensitivity and low detection limit. Sensor selectivity was also investigated with other molecules such as glucose, urea and lyzosime. In addition, a test was also carried out at a very low albumin concentration to test the sensitivity of the electrode.

Materials and methods

Materials

Deionized water (DI-H_2O), acetone (C_3H_6O) (99.8 %), and methanol (CH_3OH) (99.8 %) were obtained from SIP. Ag (99.99 %) and AgNO were obtained from Sigma Aldrich (Darmstadt, Germany). BSA was purchased from Sigma Aldrich, Phospate buffered saline (PBS) was purchased from MaxLab (Tangerang, Indonesia). Indium thin oxide (ITO) glass with a thickness of 1.1 mm, transmitivity 86 % and resistance 30 - 40 was purchased from Ali Laboratory and mechanic (Surabaya, Indonesia).

Ag and AgNO sputter deposition

The Ag and AgNO film were deposited using the DC sputtering method in BATAN Yogyakarta, Indonesia. The Ag and AgNO were placed at the anode, while the substrate ITO was placed at the cathode in distance 50 mm. The deposition was carried out with a discharge power of 7.5 W, an argon (Ar) gas flow rate of 0.3 I/s, and a pressure of 5 Pa. The deposition time was 4 seconds. At constant pressure, pure Argon Gas (99.99 %) is introduced through the valve.

Electrode characterization

The Thermo Scientific ProX-G6 scanning electron microscope (SEM) with energy-dispersive X-ray spectroscopy (EDS) was used for morphological analysis. The phase composition and structure of the Ag and AgNO thin films were analyzed using the X'pert Pro X-ray diffractometer. Optical analysis was performed using a Thermo Scientific GENESYS 10S UV-Vis spectrometer. The electrochemical detection medium was measured using the CorrTest CS2350 Potentiostat.

Electrochemical detection

The basic investigation of electrode modification was conducted using cyclic voltammetry (CV) with a potential range of -0.6 to 0.6 V. Each standard albumin solution was prepared by combining a 10 mM buffer solution of pH 7.2 with the appropriate amount of BSA. The selectivity of the electrodes for detecting albumin level was tested against competing molecules in the blood such as glucose, urea, and lyzosime. In the CV measurements, platinum was used as the counter electrode, Ag/AgCl was used as the reference electrode, and thin films of Ag and AgNO were used as the working electrode.

Results and discussion

Characterization of Ag_{film}/ITO and $AgNO_{film}$/ITO Electrodes

The Ag_{film}/ITO and AgNO/ITO electrodes were characterized using SEM, SEM-EDS, XRD to ensure the material was deposited on the surface of the substrate.

SEM

Figure 1(a) shows the top view of the SEM characterization and the side view of the SEM characterization. From the SEM image, the thickness of Ag and AgNO film was 10.13 µm.

XRD

Figure 1(b) shows the XRD diffraction of the Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes. In the $AgNO_{film}$/ITO, apart from the ITO diffraction peaks, AgNO diffraction peaks were also found at 37.34° and 63.51 ° with Miller index (111) and (220) respectively. Furthermore, for Ag_{film}/ITO, apart from ITO diffraction peaks, Ag diffraction peaks were also found at 35.5, 51.0 and 60.50 ° with Miller index (222), (400), (440) and (622). The appearance of the diffraction characteristic peaks of AgNO and Ag on the ITO electrode indicates that the AgNO and Ag films have successfully deposited on the electrode surface. Furthermore, SEM-EDS testing was carried out to strengthen this claim.

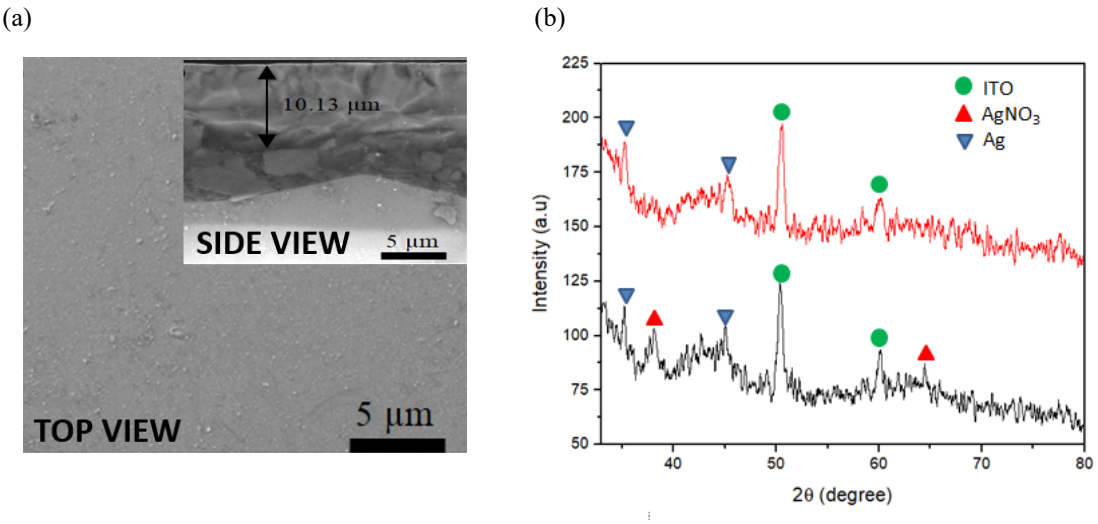

Figure 1 SEM characterization of the electrodes (a) top view and side view and (b) Ag X-ray diffraction of the AgNO/ITO and Ag/ITO electrodes.

SEM-EDS

The results of SEM-EDS for Ag and AgNO are shown in **Figures 2(a)** and **2(b)**, respectively. For $AgNO_{film}$/ITO electrodes, 3 atoms were found, namely Ag, N, O. In this test, Ag atoms had the largest proportion, namely 85.75 %. While the N and O atoms have atomic proportions of 13.21 and 1.04 %, respectively. For the Ag_{film}/ITO electrode, 2 atoms appeared, namely Ag and O, with the proportion of Ag atoms being 98.08 %. The appearance of O atoms on the electrode with Ag film deposition can be caused due to oxidation during the testing process. The results on SEM-EDS also show that the deposition of Ag and AgNO films has been successfully carried out.

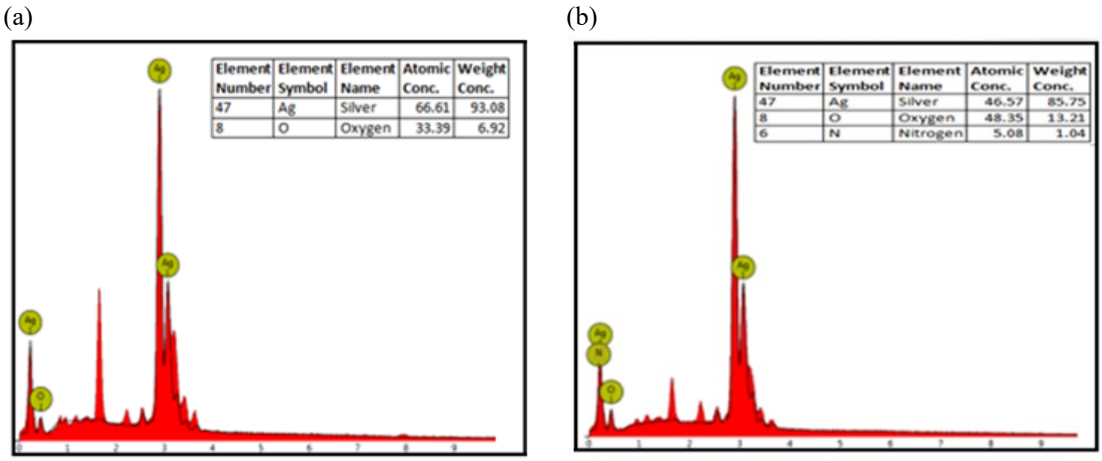

Figure 2 SEM-EDS results of the (a) Ag, and (b) AgNO.

Electrochemical albumin detection using the Ag and AgNO electrodes

In the presence of a permeable redox couple, CV is the most commonly used method to assess the degree of insulation of the electrode surface. CV was also used to detect BSA in real time. Measurements at very low concentrations and a wide range are needed to determine the performance of electrodes on BSA. In the running buffer, standard BSA solutions with concentrations ranging from 10^{-8} to 10^{-1} g/dL were prepared (0.1 M phosphate, pH 7.4). The $AgNO_{film}$/ITO electrode was used to compare the occurrence of other materials with pure Ag. In this study, the ability of the electrode to determine the BSA concentration was analyzed. Phosphate was used in this study due to it being a water-based salt solution that is able to maintain a constant pH of 7.4 in certain environments. Furthermore, the osmolarity and ion concentrations of the solution are in accordance to the human body [18]. While the use of BSA was due to its similarity with human albumin.

Figure 3(a) shows the CV measurement comparison of the Ag_{film}/ITO electrode with BSA and without BSA. The CV was conducted using 0.1 M PBS and PBS+BSA with a concentration of 0.2 g/mL and a scan rate of 75 mV/s. From **Figure 3(a)**, we can see that the peak current increased with the addition of BSA. When BSA was added to PBS, the current changed due to the interaction between proteins and surface molecules and electron transfer. Protein conformation causes a change in the surface density of the adsorption protein [19]. The addition of BSA to the PBS solution changes the voltammetric response and increases the current. This can occur due to the interaction between the protein molecules and the electrode surface. The strong interaction between Ag oxidation current and Ag ions and BSA increases the activity of Ag ions with the addition of BSA. Without the addition of BSA, the activity of oxidizable Ag is reduced due to the strong binding to the surface. This effect can be called BSA-induced surface-enhancing Ag activity [20]. The same response was shown at the $AgNO_{film}$/ITO electrode **Figure 2(b)**. The CV graph increases with the addition of BSA to PBS. However, the $AgNO_{film}$/ITO electrode with bare PBS has a higher value than the Ag_{film}/ITO electrode. PBS has a larger peak at the $AgNO_{flim}$/ITO electrode because the PBS reduction and oxidation reaction at the $AgNO_{film}$/ITO electrode is greater than that at Agfilm/ITO.

Figures 3(c) and **3(d)** show the response of the Ag_{film}/ITO electrode and the $AgNO_{film}$/ITO electrode towards various low concentrations of BSA. The concentration of BSA was varied from 4.10^{-3} to 4.10^{-8} g/dL. The response of the Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes indicates that the peak current increased linearly with increasing BSA concentration. The R2 values for the calibration curves of the Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes are 0.9635 and 0.9083, respectively. These values provide good sensitivity with the use of a practical and easy electrochemical method. The sensitivity values for the Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes are 4.564 µA $M^{-1}cm^{-2}$ and 2.123 µA $M^{-1}cm^{-2}$, respectively. It was determined that the Ag_{film}/ITO electrode was more sensitive compared to the $AgNO_{film}$/ITO electrode. The redox response of $AgNO_{film}$/ITO is smaller than that of the Ag_{film}/ITO electrode. This is because AgNO consists of a material (eg: amorphous) and one more additive. At a pH of 7.4, the $AgNO_{film}$/ITO electrode may be more negatively charged. While BSA is also negatively charged in PBS solution pH 7.4. The anisoelectric point of BSA is 4.5 so it will be more negative in the PBS solution. The redox peak at the Ag_{film}/ITO electrode is more visible and more sensitive because the Ag_{film}/ITO electrode is more positively charged in the PBS solution and at the applied potential. The amount of albumin adsorbed on the electrode surface is proportional to the amount of positive charge Ag. BSA formed more films on Ag_{film}/ITO electrodes than

$AgNO_{film}$/ITO. The formation of this film is predicted to result in increased charge transfer [21]. Overall, both electrodes have excellent sensitivity to albumin measurement at very low concentrations and over a wide range. This can also be proven by calculating the Limit of Detection.

Limit of Detection (LOD) at various scan rates can be determined after calculating the standard deviation by IUPAC as follows [22]:

$$\text{LOD} = \frac{3\times\text{SD}}{\text{S}}$$

In addition, the detection limit of the modified sensor is as low as 1 pg/mL for Ag_{film}/ITO electrode and 10 pg/mL for $AgNO_{film}$/ITO electrode.

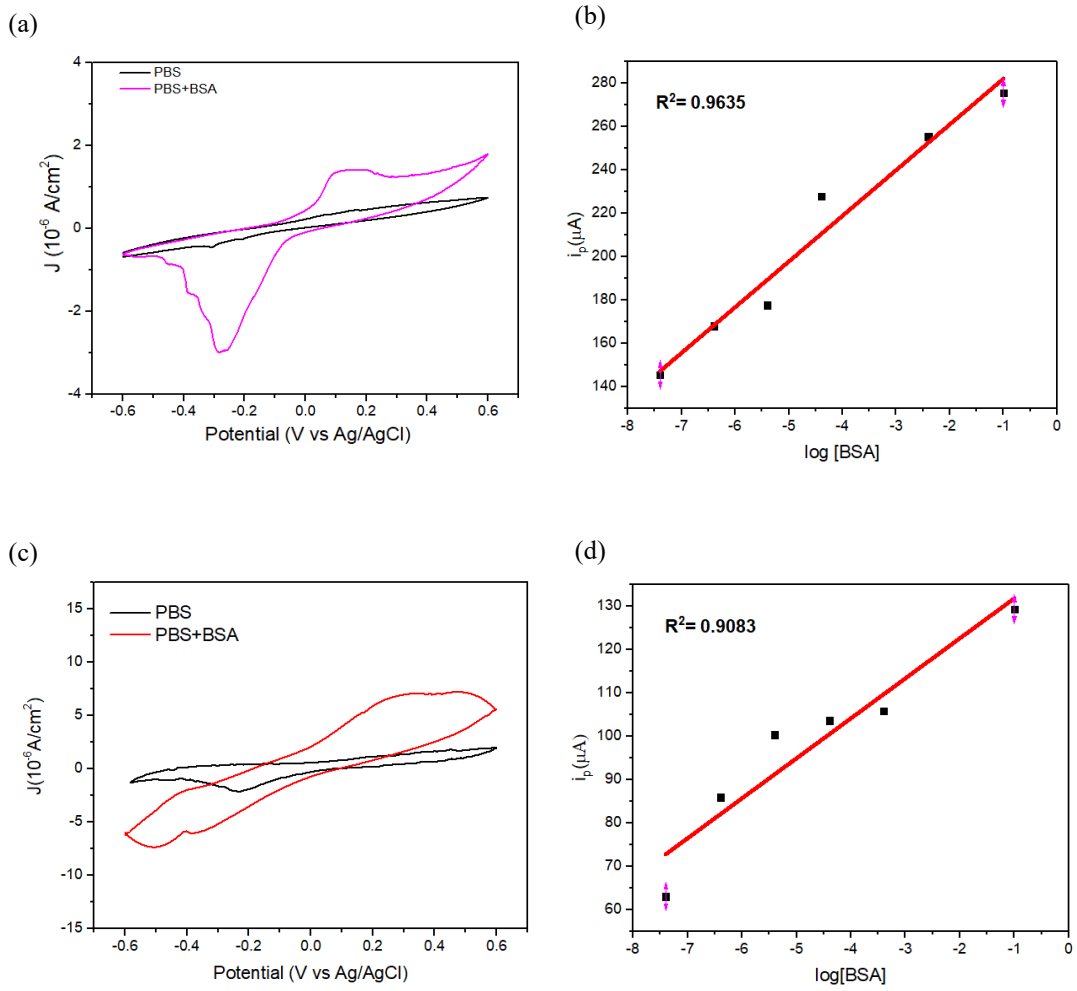

Figure 3 CV measurement of the electrodes, (a) Response of Ag_{film} towards PBS and PBS+BSA, (b) Response of Ag_{film} towards PBS and PBS+BSA, (c) Response of Ag_{film} towards low BSA concentration, and (d) Response of $AgNO_{film}$ towards low BSA concentration.

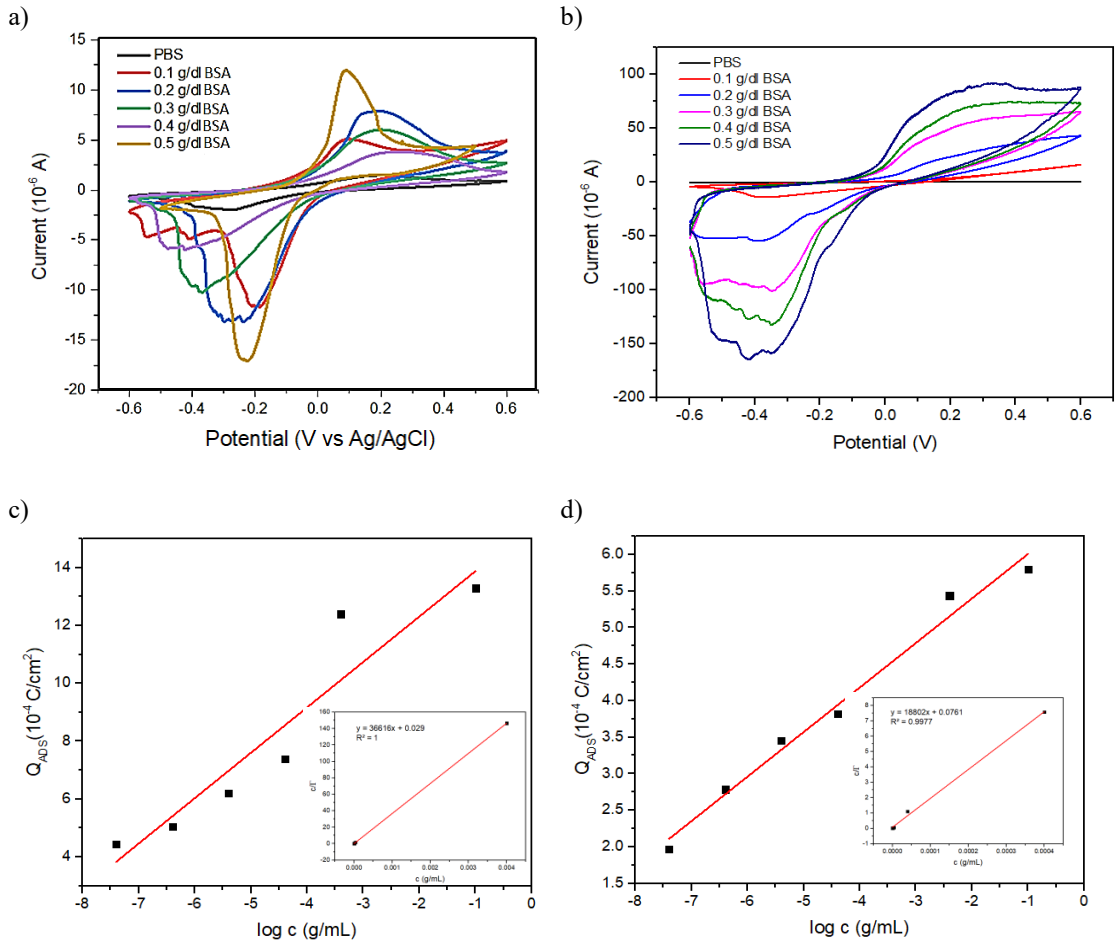

Figure 4 CV measurements of the a) Ag_{film}/ITO electrode and b) $AgNO_{film}$/ITO electrode. The influence of concentration towards the charge density Q_{ADS} of the c) Ag_{film}/ITO electrode and d) $AgNO_{film}$/ITO electrode.

Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes were also compared with the use of CV measurements using various albumin concentrations that occurred in cancer patients. **Figures 4(a)** and **4(b)** show the response of Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes to albumin concentrations in the range of 0.1 to 0.5 g/mL. **Figures 4(a)** and **4(b)** show CV graphs for Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes. The CV graph increases with increasing concentration at both electrodes. However, it was found that the CV value at the $AgNOg_{film}$/ITO electrode was smaller than the Ag_{film}/ITO electrode. This will also be studied further by analyzing the interactions of BSA, Ag ions, and AgNO.

The surface adsorption of the 2 electrodes was also studied to determine how much albumin can be adsorbed on the surface. This is also related to the performance of the electrode to bind albumin without additional treatment. The deposition of Ag on the surface of the electrode can increase the sensitivity of the electrode due to the more efficient electron transfer in oxidation and reduction [23]. The CV of the Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes are similar. However it was found that the current increased rapidly. This is due to the interaction between protein molecules and the surface of the electrode. The change in anodic and cathodic peaks are in accordance to the concentration. To prove this, the charge density Q_{ADS} value must be obtained. The value can be obtained by integrating the time and current graphs of CV. The influence of concentration towards the charge density can analyzed by plotting a graph of concentration against charge density. **Figures 4(c)** and **4(d)** indicate the dependency of charge density Q_{ADS} towards the protein concentration of the PBS+BSA solution. The surface charge density of both electrodes reached a plateau at a concentration of 0.1 g/mL with values of 13.3 10^{-4} and 5.79 10^{-4} C cm^{-2} for the Ag_{film}/ITO and the $AgNOg_{film}$/ITO electrode, respectively. The energy density value of pure Ag is higher than that of AgNO. By obtaining the charge density value, the surface concentration of protein may then be calculated Γ (mg m^{-2}) as follows [24]:

$$\Gamma = \frac{Q_{ADS} M_r}{nF}$$

where Q_{ADS} is the charge density (C cm^{-2}), Mr is the molecular weight of albumin (g mol^{-1}), and F is the Faraday constant (C mol^{-1}).

The inset of **Figures 4(c)** and **4(d)** show that the plot of c against c/Γ produces a straight line. From this line, the Γ_{MAX} value is obtained from the intercept and the B_{ADS} value is obtained from the slope. The surface charge density caused by protein adsorption was discovered to be sensitive to the protein's structural characteristics. To describe BSA adsorption on Ag_{film}/ITO electrodes, saturated surface coverage and thermodynamic parameters of BSA adsorption on Ag were investigated in this work. The Langmuir equation may be used to represent the connection between the protein concentration in solution c and the quantity of adsorbent on the surface [25]:

$$\frac{c}{\Gamma} = \frac{1}{B_{ADS}} \Gamma_{MAX} + \frac{c}{\Gamma_{MAX}}$$

B_{ADS} is the adsorption factor, reflecting the affinity of adsorbed molecules at a constant temperature at the adsorbent. The greatest amount of material that may be adsorbed to the surface is given by Γ_{MAX}. Complete BSA coverage of the Ag_{film}/ITO electrode was obtained from an analysis of albumin adsorption. This indicates that the negatively charged BSA will spontaneously adsorb the positively charged Ag. The total mass of BSA absorbed for Ag_{film}/ITO electrodes was 281 mg m^{-2}, which is larger than the theoretical monolayer surface coverage of BSA (6.7 mg m^{-2}) [26]. Ag_{film}/ITO electrodes showed excellent performance for binding albumin without additional treatment. This is related to the relationship between Ag ions and BSA which has been described previously. Ag electrodes were found to perform better in albumin absorption than AgNO. With this it was found that the Ag electrode is more sensitive than AgNO in the detection of albumin. In addition, the low detection limit indicates that the 2 electrodes have good performance in albumin detection.

Table 1 BSA absorption data on Ag electrodes.

Parameter	Data
Mr BSA (kDa)	66.000
B_{ADS} (L/mol)	360.000
Γ_{max} (mg/m^2)	281

Selectivity of albumin detection using Ag_{film}/ITO and $AgNO_{film}$/ITO electrode

Urea and glucose were used as competing molecules in the blood to assess the selectivity of BSA in the Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes. In addition, we utilize lysozyme as a competitive pepsin. These compounds were studied at their highest physiological concentrations. The interactions between aqueous solutions of BSA, urea, glucose, and lysozyme molecules and pre-mixed molecular solutions with BSA+glucose, BSA+urea, BSA+lysozyme, and BSA+glucose+urea were studied for this purpose.

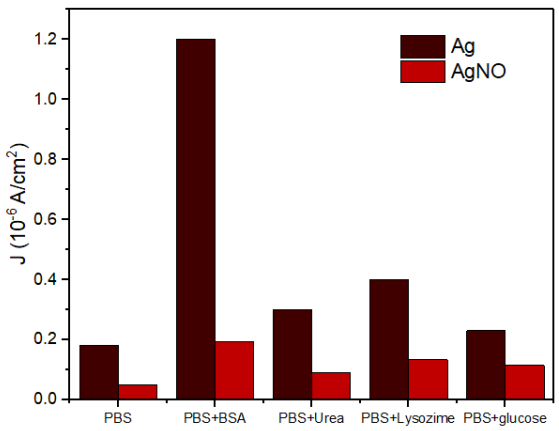

Figure 5 Selectivity of Ag_{film}/ITO and $AgNO_{film}$/ITO toward BSA and other interferences.

Figure 5 shows the CV response of the Ag_{film}/ITO electrode to BSA and other molecules. The greatest charge value is obtained when the electrode interacts with BSA. For other molecules such as glucose, urea, and lysozyme, the current density values were found to be lower. PBS was used as a buffer solution for all molecules in all measurements. Furthermore, **Figure 5** shows the CV response of the $AgNO_{film}$/ITO electrode to BSA and other molecules. At this electrode, the largest current density value also occurs in BSA. However, the lysozyme current density value also tends to be large. The smallest value occurs in the reaction between the $AgNO_{film}$/ITO electrode and urea. Based on the CV graph it was found that the Ag electrode interacted more easily with BSA. The value of the current density at the Ag electrode was found to be almost twice that of the $AgNO_{film}$/ITO electrode. The large increase in current for the addition of BSA to PBS and a not too large increase for the addition of other interferences (glucose, lysozime, and urea) indicates that the sensor is selective towards other interferences.

Reproducibility

The reproducibility of Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes also has proximity to changes in current density at the same concentration 35 times. The figure shows good reproducibility at both electrodes. Over 5 months, 100 tests have been carried out with good electrode performance.

Conclusions

In this study, we developed Ag_{film}/ITO and $AgNO_{film}$/ITO electrodes for low albumin detection based on the electrochemical method. The testing of albumin levels in the blood within a wide range of low concentration levels (10^{-1} - 10^{-8}) indicated a detection limit of 10^{-9} g/mL. This shows that the Ag_{film}/ITO electrode has a very good detection limit. The electrode modified with micro-sized Ag_{film}/ITO achieved more effective results than the electrode modified with $AgNO_{film}$/ITO electrode. The Ag_{film}/ITO electrode possessed an electron transfer rate constant higher than that of the $AgNO_{film}$/ITO electrode. The results of the modeling of the electric field and magnetic field distribution showed that the Ag_{film}/ITO electrode possessed larger values than the $AgNO_{film}$/ITO electrode. From this analysis, it can be concluded that the electrode modified with micro-sized Ag achieved more effective results than the electrode modified with AgNO electrode. This method can be used for the detection of a wide range of low protein levels as a parameter for early detection. The Ag_{film}/ITO electrode exhibited high sensitivity, selectivity, reproductivity, and low detection limit. This sensor can determine low albumin level as a blood cancer biomarker. This sensor holds great promise for use in real samples in the future.

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