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Research paper



Milkfish (Chanos Chanos) Gelatin as Biosensor Material for Chromium (III) Detection

T.P.H. Hutapea[1], Rukisah[2], Mulyadi[3], K.A. Madurani[4], Suprapto[4], F. Kurniawan[4,5,*]

[1]*Fisheries Product Technology Department, Faculty of Fishery and Marine Science, University of Borneo Tarakan, North Borneo, Indonesia*
[2]*Aquaculture Department, Faculty of Fishery and Marine Science, University of Borneo Tarakan, North Borneo, Indonesia*
[3]*Elctrical Engineering Department, Faculty of Engineering, University of Borneo Tarakan, North Borneo, Indonesia*
[4]*Chemistry Department, Faculty of Science, Institut Teknologi Sepuluh Nopember, Surabaya, East Java, Indonesia*
[5]*ITS Halal Center, Institute of Research and Community Service, Institut Teknologi Sepuluh Nopember, Surabaya, East Java, Indonesia*
**Corresponding author E-mail: fredy @chem.its.ac.id*

Abstract

Milkfish (*Chanos chanos*) gelatin was successfully developed as biosensor material. The milkfish bone was obtained from local restaurants in Tarakan, North Borneo, Indonesia. Gelatin was extracted from milkfish bone using acid method at 55°C. Characterization by FTIR showed that milkfish gelatin had similar functional group with commercial gelatin. The gelatin was used as biosensor material for detecting chromium. The gelatin was mixed with carbon in 1:1 ratio to form gelatin/carbon paste modified silver electrode. Electrochemical impedance spectroscopy (EIS) analysis of the gelatin/carbon paste modified silver electrode showed a better conductivity than paraffin/carbon paste modified silver electrode. Performance of the gelatin/carbon paste modified silver electrode in chromium (III) solution was conducted using cyclic voltammetry technique. Measurement was carried out at -1 V to +1 V with scan rate of 100 mV/s in acid and base condition. The best result was shown by gelatin/carbon paste modified silver electrode. It can detect chromium (III) ions at reduction potential of -0.78 V in alkaline condition. Unspecific responses were observed from silver electrode, paraffin/silver electrode, carbon/silver electrode, gelatin/silver electrode and paraffin/carbon paste modified silver electrode. This result can be concluded that the milkfish gelatin obtained have a potential to be developed as chromium (III) biosensor.

Keywords: *Biosensor; chanos chanos; chromium (III), cyclic voltammetry; gelatin; tarakan.*

1. Introduction

Biosensor is one of electrochemical studies that used natural product as based material for sensor [1]–[6]. Biosensor has good selectivity and sensitivity because its specific material that modified on the electrode [4], [7], [8]. Most of the modified electrodes are platinum, silver and gold [3], [9]. Several scientists were developed biosensor by modification electrode for many purpose [1], [4], [7], [10]–[17]. Fitriyana and Kurniawan has been reported that polyaniline-invertase-gold nanoparticles modified gold electrode can be used as sucrose detection [18]. Hutapea et al. (2015) was utilized maja pulp extract for detecting glucose at -0.22 mV by cyclic voltammetry [19]. Maja leaves was shown a good performance to urea determination [20], [21]. Insulin sensor was also developed by chitosan modification [2], [22]. Utilization of natural product for biosensor material is very interesting because the active site of natural product can give a good selectivity toward target compound. Another natural product that has several active sites is gelatin.

Gelatin is a heterogeneous mixture of polypeptides [23], [24]. Gelatin contains a high amount of amino acid with a repeated of tripeptide unit [25]–[28]. This unit consists of glycine, proline, and 4-hydroxyproline, so that it has a similar properties to collagen [25]. Gelatin is material with a relatively low cost and easy to get from bones, hide and skins waste of slaughter by acidic or alkaline treatment. Today, gelatin is derived from pigskin which is about 45.8%, while 28.4% derived from cattle skin, 24.2% from cow bones, and 1.6% derived from the other raw material [29]. Active site of gelatin will be obtained when it reaction with acid or alkaline solution. Based on the origin of the raw material and hydrolysis process, gelatin is classified as type A gelatin and type B gelatin. Acid treatment can produce Type A gelatin with isoionic point of 7 to 9. This acidic hydrolysis is mainly used for pigskin, marine fish skin, and sometimes bone raw material treatment. Whereas, alkaline treatment was resulted type B gelatin with isoionic point of 4 to 5 [23], [24], [30]. This active site is possible to react with another compound and useful as biosensor material.

Gelatin as biosensor material has been reported by Periasamy et al. (2011). The results show that modified sensors by gelatin have a good sensitivity and selectivity [15]. Gelatin was also modified by carboxymethylcellulose and TiO_2 for oxygen determination. The biosensor exhibits high analytical performance with a wide linear range (1.5 nM to 2 mM), low detection limit (1.5 nM), high sensitivity and fast response time (1.8 s) [31]. Another modification by gelatin was reported by Zheng et al. (2015). Zheng et al. was fabricated and developed an acetylcholinesterase biosensor based on ionic liquid functionalized graphene-gelatin-modified electrode for sensitive detection of pesticides. The biosensor can detect the pesticides such as carbaryl and monocrotophos. The detection limit for carbaryl and monocrotophos were $5.3x10^{-15}$ M and $4.6x10^{-14}$ M, respectively [14]. Gelatin can also be used as mediator for electrochemical DNA biosensor for DNA hybridization. DNA hybridization can be detected up to 10^{-12} mol by this

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biosensor [32]. Sharma et al. (2017) was reported the modification of homogeneous gelatin organogel-based nanocomposited (GA-NC) as urea biosensor. This GA-NC was prepared on indium tin oxide (ITO) coated glass plate. The GA-NC/ITO electrode shows specific response for urea with sensitivity of 32.7 and 5.56 μA mM^{-2} cm^{-2} in two concentration ranges of 0.1 to 2 mM and 2 to 20 mM, respectively [33]. All studies show that developed biosensor from gelatin has many advantages, e.g. high sensitivity, good selectivity, acceptable stability and low cost [14], [15], [31], [33].

Trivalen chromium, Cr (III), is involved in some biochemical process at cellular level [34], [35]. Cr (III) belongs to essential trace element in human nutrition which has great impact on the metabolism of carbohydrates, fats, proteins and nucleic acids [35], [36]. Cr (III) can be also reacted with insulin to reduce blood sugar. Its deficiency in human body would lead to variety of diseases such as diabetes and cardiovascular disease. In the other hand, high level of Cr (III) can react with DNA and cause negatively affecting cellular structures and damaging the cellular components [34], [35], [37]. Therefore, a fast, sensitive and selective method to determine Cr (III) is needed.

In this present study, we used milkfish (*Chanos chanos*) gelatin as biosensor material to detect chromium (III) ions. Gelatin was extracted from milkfish bone that obtained from Tarakan, North Borneo, Indonesia. This milkfish bone can be found easily in Tarakan. The production of milkfish in Tarakan reached 136 tons at 2014 [38]. In the addition, most people in Tarakan only consume the meat of milkfish so that the milkfish bone became abundance waste. This abundance waste can be used to obtain gelatin and can be utilized as biosensor material. Gelatin was mixed by carbon to form paste and will be used as modification material on silver electrode. The performance of gelatin/carbon paste modified silver electrode for chromium (III) detection was observed by cyclic voltammetry in acidic and alkaline solutions. Comparison study was conducted using paraffin/carbon paste modified silver electrode.

2. Materials and Method

2.1. Chemicals

Bones of milkfish (*Chanos chanos*) were bought from local restaurants in Tarakan, North Borneo, Indonesia. The milkfish bones from Tarakan are displayed in Fig. 1. Sodium hydroxide (NaOH), chloride acid (HCl, 37%), chromium (III) nitrate ($Cr(NO_3)_3 \cdot 9H_2O$) and paraffin were purchased from Merck. All chemicals was used without any purification. Silver wire was bought from a local market (99.999% with diameter 1 mm). Demineralized water was used for cleaning and chemical preparation.



Fig. 1: Milkfish bone from local restaurant in Tarakan, North Borneo, Indonesia.

2.2. Instrumentation

Characterization of gelatin from milkfish (*chanos chanos*) bone was carried out using Fourier transform infrared (Shimadzu-FTIR-8400S). Electrochemical measurements were conducted by Autolab PGSTAT128N which is equipped with Nova 1.1 software. A three electrode cell system, i.e. platinum as the counter electrode (CE), Ag/AgCl (3 M KCl) as the reference electrode (RE) and paraffin/carbon paste modified silver electrode or gelatin/carbon paste modified silver electrode as working electrode, were used in all measurements.

2.3. Extraction and characterization of gelatin from milkfish (*Chanos chanos*) bone

Milkfish bone was cleaned and then boiled in demineralized water until the bone becomes a bit pale and fluffy. The bone was then immersed in 0.1 N NaOH solution for three days and continued immersion in 0.1 N HCl for two days. Each immersion treatment, the bone was washed to achieve neutral condition. The bone was extracted in demineralized water with composition 3:1 for 4 hours at 55ºC. Gelatin from milkfish bone would be obtained after filtering, concentrating and heating (50ºC) processes. The functional group of gelatin from milkfish bone was analysed by FTIR using KBr-disk technique.

2.4. Fabrication of paraffin/carbon paste modified silver electrode and gelatin/carbon paste modified silver electrode

The fabrication of the modified silver electrode is shown in Fig. 2. It was prepared by mixed paraffin and carbon or mixed milkfish gelatin and carbon in 1:1 ratio. The mixture was stirred to form paste. Modification of silver electrode was conducted by inserting the paste and flattening it onto the silver electrode. The surface of paraffin/carbon paste modified silver electrode (Fig. 2a) and gelatin/carbon paste modified silver electrode (Fig. 2b) were abraded using emery paper grades 1000. In addition, silver electrode with and without modification by carbon or paraffin or milkfish gelatin were also prepared for comparison studies. The electrodes were stored prior to use in desiccator.

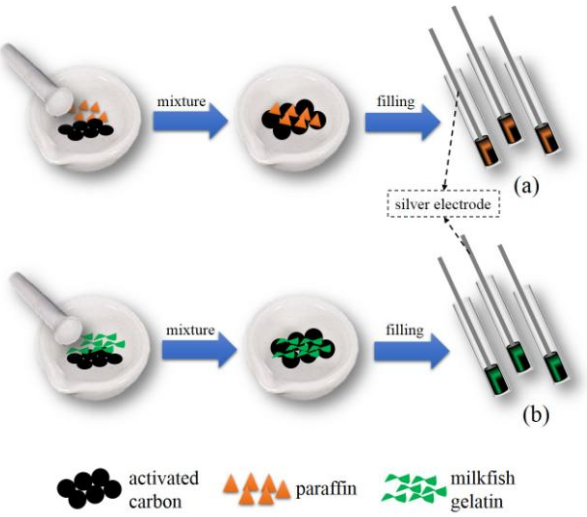

Fig. 2: Fabrication of paraffin/carbon paste modified silver electrode (a) and gelatin/carbon paste modified silver electrode (b).

2.5. Electrochemical measurement

Conductivity of paraffin/carbon paste modified silver electrode and gelatin/carbon paste modified silver electrode were measured by electrochemical impedance spectroscopy (EIS) method. The measurement was conducted at range frequency of 1 MHz – 1 KHz with amplitude 0.1 A. It was done in alkaline solution at room temperature.

The electrochemical performances of paraffin/carbon paste modified silver electrode and gelatin/carbon paste modified silver electrode in chromium (III) solution were performed by cyclic voltammetry. The potential was swept from -1.0 V to +1.0 V with scan rate of 100 mV/s at room temperature. The test solution of 40 ppm chromium (III) nitrate solution. The electrochemical performances of silver electrode, paraffin/silver electrode and car-

bon/silver electrode for chromium (III) detection were also investigated.

3. Results and Discussion

3.1. Characterization of milkfish gelatin

Gelatin from milkfish was characterized using FTIR. The result was compared by commercial gelatin (pure gelatin). This characterization aims to identify the functional group of both gelatin. The pattern of the FTIR spectra from milkfish gelatin is needed for knowing to confirm gelatin characteristic functional groups. FTIR characterization was also reported by several scientists [27], [30], [39], [40].

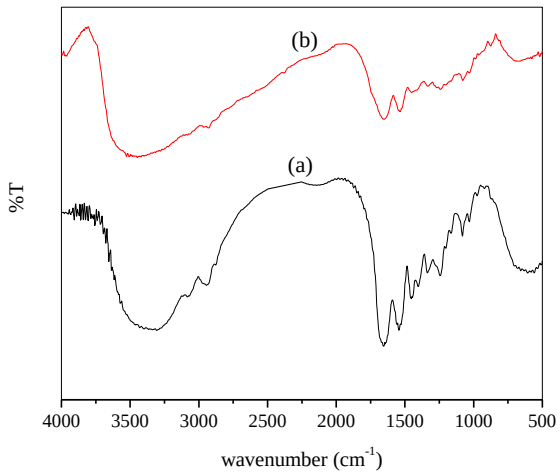

Fig. 3: FTIR spectra of commercial gelatin (a) and milkfish gelatin (b).

The FTIR spectra of milkfish gelatin and commercial gelatin is shown in Fig. 3. We can see that milkfish gelatin (Fig. 3b) has a similar functional groups with commercial gelatin (pure gelatin) (Fig. 3a). The FTIR spectra was shown the amide A region at 3285 cm^{-1} which related to N-H bonding and free O-H. Amide I region was displayed by carbonyl group (C=O) at 1628 cm^{-1}. The amide II region was appeared from N-H bending at 1530 cm^{-1}. We also found the amide III region at 1238 cm^{-1}. Those functional groups are in a good agreement by Hanani et al. [39], Cebi et al. [40] and Pradini et al. [30] studies.

3.2. Conductivity measurement

Conductivity of paraffin/carbon paste modified silver electrode and gelatin/carbon paste modified silver electrode were characterized by electrochemical impedance spectroscopy (EIS) technique. The impedance response (Nyquist plot) of paraffin/carbon paste modified silver electrode is represented at Fig. 4A. While, Nyquist plot of gelatin/carbon paste modified silver electrode is shown in Fig. 4B.

The resistance value of both modified silver electrode were obtained from Nyquist plot extrapolation. The resistance value are 4.19 MΩ and 2.04 kΩ for paraffin/carbon paste modified silver electrode and gelatin/carbon paste modified silver electrode, respectively. Conductivity of both electrode were measured by Eq. 1.

$$\sigma = \frac{L}{R \times A}, \quad (1)$$

where σ is conductivity value (S/cm), L is length of the electrode (5 cm), R is resistance of the electrode (Ω) and A is cross-sectional area of the electrode (0.0314 cm^2). The result shows that the conductivity value is 3.800x10^{-2} S/cm for paraffin/carbon paste modified silver electrode, whereas for gelatin/carbon paste modified silver electrode is 7.806x10^{-2} S/cm. This result was confirmed that gelatin/carbon paste modified silver electrode has a better conductivity than paraffin/carbon paste modified silver electrode. Good conductivity is the important and initial properties needed for biosensor [41]–[44].

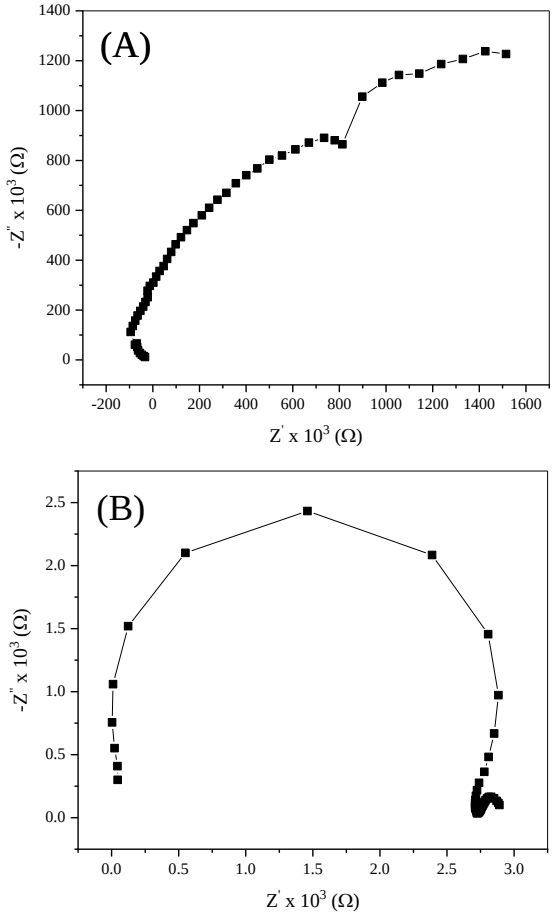

Fig. 4: Nyquist plot for paraffin/carbon (A) and gelatin/carbon (B) paste modified silver electrode.

3.3. Performance of silver electrode with and without modification

The performance of silver electrode with and without modification was shown in Fig. 5. Fig. 5A is shows the testing result for silver electrode in chromium (III) solution would be given a similar pattern both in acidic and alkaline condition. Similar results were also obtained in measurement of chromium (III) solution by carbon/silver electrode (Fig. 5B), paraffin/silver electrode (Fig. 5C) and gelatin/silver electrode (Fig. 5D). Even those three electrodes show a noise responses. It means that ions of chromium (III) cannot be determined by silver electrode, carbon/silver electrode, paraffin/silver electrode or gelatin/silver electrode. Thus, in our opinion, we need to mixture the modification material, i.e. paraffin/carbon and/or gelatin/carbon to improve the signal of chromium (III) ions.

The response in the form of cyclic voltammograms from chromium (III) solution by paraffin/carbon paste modified silver electrode and gelatin/carbon paste modified silver electrode at acidic and alkaline condition are shown in Fig. 5E and 5F, respectively. Both electrodes have no response to chromium (III) ions in acidic condition. It was proved by disappearing of redox peak at voltammograms. In alkaline condition, the paraffin/carbon paste modified silver electrode has also no response for chromium (III) ions. Whereas, the gelatin/carbon paste modified silver electrode gives a better response to chromium (III) ions at alkaline condition.

The gelatin/carbon paste modified silver electrode can detect chromium (III) ions at potential of -0.78 V for reduction peak. This result indicate that the addition of milkfish gelatin can increase on the electrode performance to chromium (III) detection.

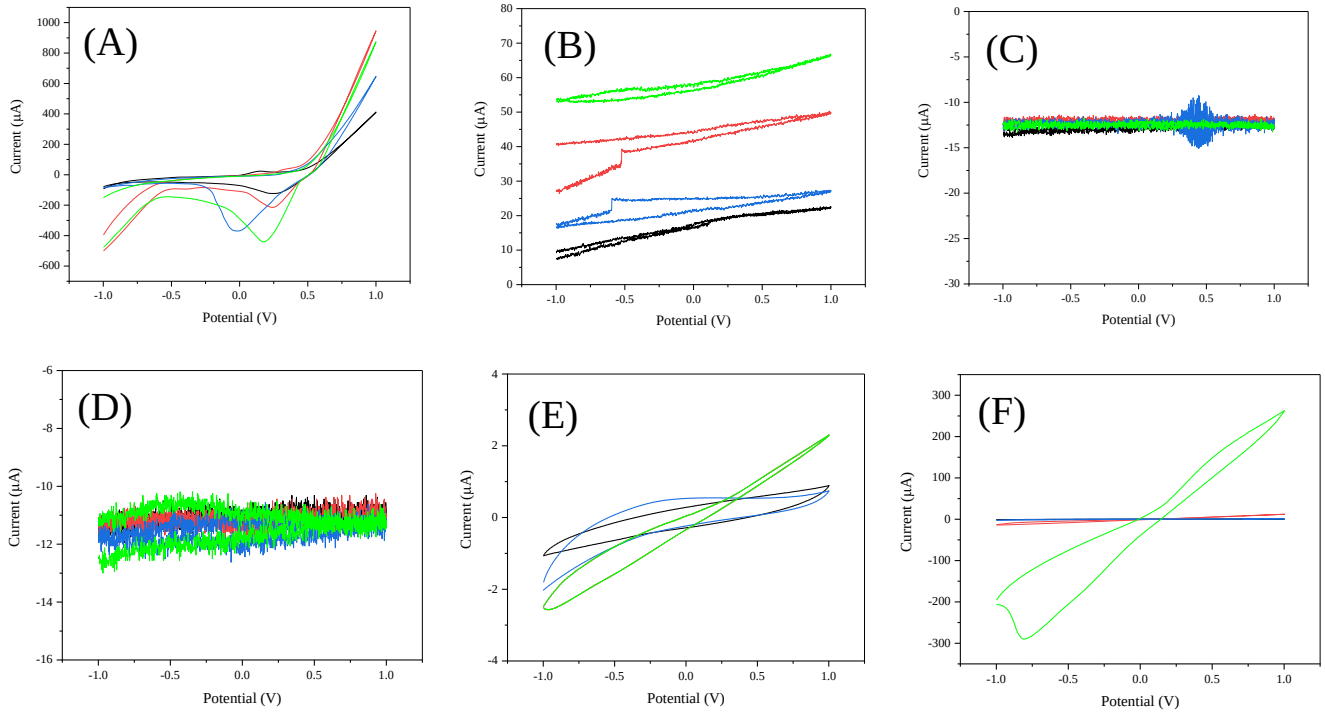

Fig. 5: Cyclic voltammograms obtained for silver electrode (A) in blank solution at acidic condition (black) and alkaline condition (blue) and 40 ppm chromium (III) solution at acidic condition (red) and alkaline condition (green). Cyclic voltammograms obtained for carbon/silver electrode (B) in blank solution at acidic condition (black) and alkaline condition (blue) and 40 ppm chromium (III) solution at acidic condition (red) and alkaline condition (green). Cyclic voltammograms obtained for paraffin/silver electrode (C) in blank solution at acidic condition (black) and alkaline condition (blue) and 40 ppm chromium (III) solution at acidic condition (red) and alkaline condition (green). Cyclic voltammograms obtained for gelatin/silver electrode (D) in blank solution at acidic condition (black) and alkaline condition (blue) and 40 ppm chromium (III) solution at acidic condition (red) and alkaline condition (green). Cyclic voltammograms obtained for paraffin/carbon paste modified silver electrode (E) in blank solution at acidic condition (black) and alkaline condition (blue) and 40 ppm chromium (III) solution at acidic condition (red) and alkaline condition (green). Cyclic voltammograms obtained for gelatin/carbon paste modified silver electrode (F) in blank solution at acidic condition (black) and alkaline condition (blue) and 40 ppm chromium (III) solution at acidic condition (red) and alkaline condition (green)All measurement were conducted in room temperature at scan rate of 100 mVs^{-1}.

A possible mechanism of the chromium reaction and interaction between the gelatin/carbon paste modified silver electrodes with chromium are presented in Fig. 6.

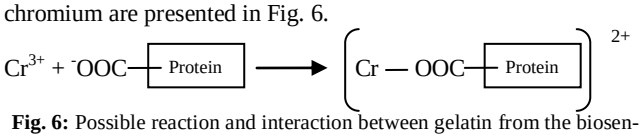

Fig. 6: Possible reaction and interaction between gelatin from the biosensor and chromium (III)

Gelatin as protein has carbonyl groups that can react with chromium (III) to form coordinate bond and carbonyl group required that the latter be ionized (Fig. 6). In alkaline conditions, the formation of coordinate bond stronger than in acidic conditions [45]. Consequently, the reaction can be observed as current values so that it will appear as a peak on the voltammogram (Fig. 5F).

4. Conclusion and Suggestion

The potential of milkfish (*Chanos chanos*) gelatin from Tarakan, North Borneo, Indonesia was successfully develop as biosensor material. The performance of gelatin/carbon paste modified silver electrode demonstrates that it is a good biosensor to chromium detection. A 40 ppm chromium (III) solution can detect at reduction potential of -0.78 V in alkaline condition. Comparison studies were also showed that silver electrode, carbon/silver electrode, paraffin/silver electrode, gelatin/silver electrode and paraffin/carbon paste modified silver electrode have insignificantly responses for chromium (III) ions both in acidic and alkaline condition. This result has been proved that gelatin from milkfish can be used as chromium (III) biosensor.

According to the results, we will do some improvement in the next work. The detail characterization such as sensitivity, selectivity, linier range of measurement, repeatability, reproducibility and limit of detection (LOD) of the electrode were needed.

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