

Design of Bentonite Acid Modified Electrodes in Cyanide Biosensors

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To cite this article:

Catherina Bijang, Abd.Wahid Wahab, Maming, Ahyar Ahmad, Paulina Taba. Design of Bentonite Acid Modified Electrodes in Cyanide Biosensors. *International Journal of Materials Science and Applications*. Vol. 4, No. 2, 2015, pp. 115-119.

doi: 10.11648/j.ijmsa.20150402.17

Abstract: A research has been done on the influence of sulfuric acid activation on the performance of bentonite as a cyanide biosensor. The concentration of sulfuric acid used was 0.5; 1; 1.5 and 2 M. Activation with 2 M sulfuric acid resulted in an increase in surface area and basal spacing. The membrane biosensor made from 2M acid activated bentonite had better performance than the untreated bentonite membrane biosensor. A study on the effect of membrane thickness on the performance of the cyanide biosensor has also been conducted. The result showed that the performance of membrane which 5 mg/mL of thickness did not differ significantly.

Keywords: Cyanide, Biosensor, Bentonite

1. Introduction

Biosensor is a chemical sensors using a biological elements in their recognition system. Biosensors commonly used are enzyme electrodes. Immobilization of enzyme on the electrode surface, thoroughly retention of biological activity and good diffusional properties of the substrate, are the main reasons for developing commercial biosensors. Two aspects which determine the immobilization of biomolecules are the appropriate matrix and analyte diffusion limitations in this matrix.

Among all the organic and inorganic matrices reported in the literature, Clays attracted the attention of many researches because of their interesting properties, such as, the hydrophilic nature, fluffy and porous, which can improve the activity and stability of enzyme immobilization. Shan, et al (2003) observed that the matrix of laponit improve analytical characteristics and long-term stability of the biosensor compared to a simple biosensor obtained from chemical cross-linking between glucose oxidase (GOD) or polyphenol oxidase (PPO) on the electrode surface. Biosensors PPO /laponit after being left for 2 weeks showed 93% stability and after 3 weeks of 47%. This initial activity greater than the electrode PPO/GA with stability, respectively 63%

and 36%. (Shan, 2002).

Making glucose biosensor with entrapment enzyme molecules in a layer of clay, increases the sensitivity of clay electrode from 1.4 to 130 $\text{mA} \cdot \text{cm}^{-2}$ (Cosnier, et al, 1999). –

The surface area and the pore volume of clay can be improved by treatment with mineral acids (Gogoy, 2007). This treatment is highly dependent on acids strength, time and temperature of the system (Rozic, 2008). Chemical changes that occur in the structure of clays during the treatment with acid generate octahedral vacancy in the crystal lattice, therefore Lewis acid sites increase. Proton of the hydroxyl group which is located at the corner of the octahedron becomes more unstable as a result of structural deformation caused by acid activation, thereby increasing the Bronsted acidity (Alemdaroglu, 2003; Rozic, et al. 2008).

2. Method

Material. Tyrosinase (EC. 1.14.18.1) from Mushroom (17.600 units mg^{-1}) was purchased from Sigma Chemical Co. (USA)., Bentonite, cathecol, KCN, Bentonite, H_2SO_4 , $\text{AlCl}_3 \cdot 6 \text{H}_2\text{O}$, glutaraldehyde (25%), Na_2CO_3 anhydride and all other chemicals were purchased from Merck.

Apparatus. All the experiment were carried out in a conventional thermostated three- electrode cell (10 ml) at

room temperature. The amperometric measurement was performed with a eDAQ potentiostat in conjunction with a recorder. The working electrode was a glassy carbon electrode. An Ag/AgCl electrode saturated with NaCl solution was used as reference electrode, and a Pt wire was placed in a separate compartment containing the supporting electrolyte as a counter electrode.

Activation of Clay. One hundred grams of clay was dispersed into 300 mL of 2 M sulfuric acid solution and stirring with a magnetic stirrer for 6 hours. The mixture was allowed for 24 hours and then filtered with a vacuum filter and washed with hot distilled water until free of sulfate ions. Clay was then dried in an oven at a temperature of 100°C. Dried clay was crushed into powder and then sieved using a 100 mesh sieve size. The powder sample was characterized by FT-IR, XRD, and Surface Area Analyzer.

2.1. Catechol Substrate Current Measurement and Cyanide Inhibition by Cyclic Voltammetry

Biosensor electrode was used at a speed of 400mV/sec to

measure the flow of catechol at concentrations of 10^{-9} - 10^{-3} M on the operational potential of -0.2V to 0.2 V. Then the electrodes were dipped in 10^{-5} M cyanide solution for 5 minutes and then used again to measure the concentration of catechol. The experiment was repeated for each solution of catechol, from 10^{-9} - 10^{-3} M. I% (the inhibition percent or the degree of inhibition of cyanide on the activity of the enzyme tyrosinase) was determined by the following equation:

$$I\% = (E_0 - E_1) / E_0 \times 100\%$$

2.2. Current Measurement of Substrate and Cyanide

The catechol substrate solution with the maximum percentage of inhibition was used. Each cyanide standard solution with concentrations of 10^{-6} – 1 M was added in to the substrate solution. The current of solution was measured directly after the addition

3. Result and Discuss

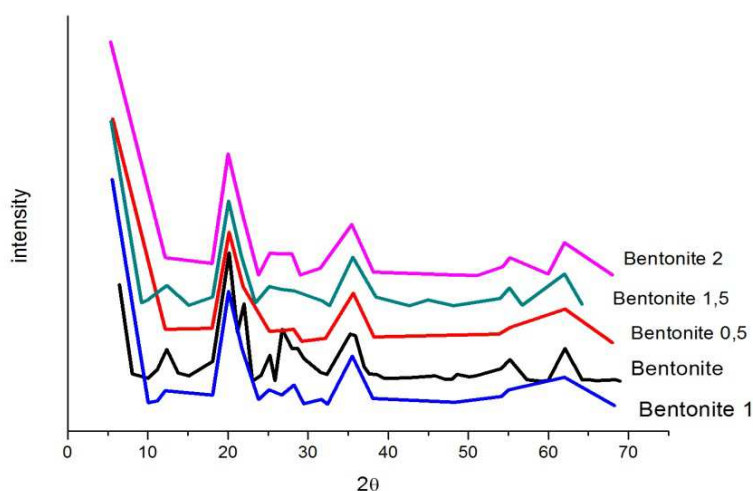


Figure 1. Diffractogram of Bentonite and Acid Bentonites

3.1. The Result of X-Ray Characterization

Figure 1 is the XRD diffractogram of bentonite at different acid concentrations. The peak that appears at 2θ of 6.450 indicates the type of clay minerals 2:1 ($D_{001} = 13.678 \text{ \AA}$) (Manohar, 2006). Treatment with acid resulted in a shift to a smaller 2θ angle stating the increase in the distance between the layers. Treatment with sulfuric acid at concentrations; 0.5; 1; 1.5; 2 M resulted in the distance between layers of 15.72; 15.9; 16.36; 16.54 Å, respectively. The greater the concentration of acid used (0.5 M - 2 M) the higher the distance between the layers of clay minerals was observed. If the distance between the layers increases the surface area also increases.

It also indicates that the crystallinity of clays increases (Noyan *et al.*, 2007). Wijaya *et al.* (2002) stated that treatment with sulfuric acid to 3 M did not damage the structure of bentonite. The peak at 2θ of 20.114 ($d = 4.411 \text{ \AA}$) declared non-clay minerals (Noyan *et al.*, 2007). This

type of mineral is dominated by silica structure.

3.2. The results of FTIR Characterization

Two groups that normally characterizes the frequency region of infrared spectrum of most clay minerals are: (1) the area between $4000 - 3000 \text{ cm}^{-1}$, which is caused by the stretching vibration of adsorbed water and/or octahedral OH groups, called the functional group region and (2) the region between $1400 - 800 \text{ cm}^{-1}$, which is caused by the bending vibration of Al-OH and/or Si-O-called fingerprint region.

From Figure 2, there has been several changes in absorption bands caused by addition of sulfuric acid with concentrations of 0.5 ; 1; 1.5 and 2M. The existence of absorption at 3695.6 cm^{-1} and 3624.25 cm^{-1} indicates the presence of -OH stretching vibration. According to Madejova (Soleman, 2004), the evidence of a peak at wave numbers 3669; 3653 and 3620 cm^{-1} indicates the presence of -OH stretching vibration from silanol group or from -OH found in the tetrahedral to octahedral sheets.

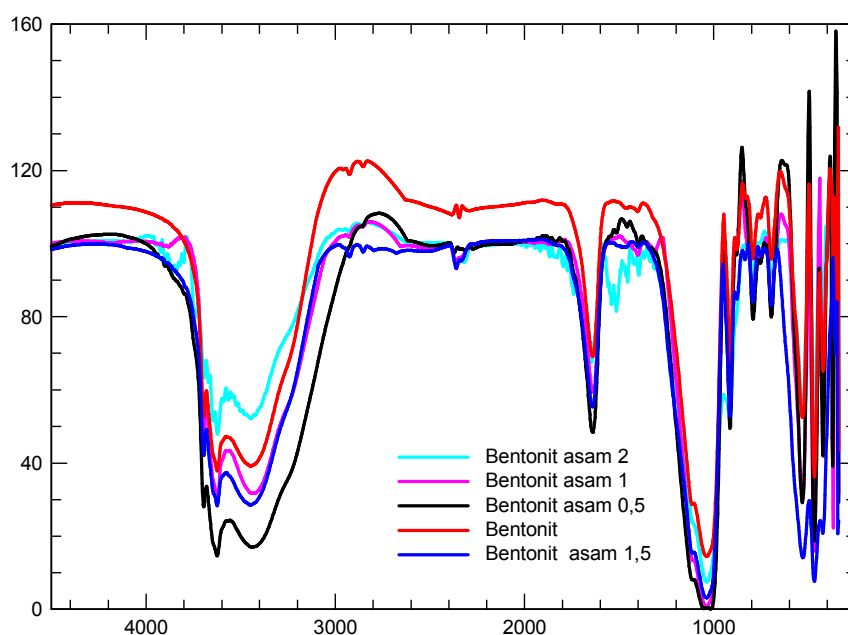


Figure 2. FTIR spectrum of Bentonite and Acid Bentonite

This peak has not changed in 1.5 M sulfuric acid treatment. However, the addition of intensity significantly occurred in the treatment with 2 M sulfuric acid. This is consistent with the absorption of the OH bending vibration that appears at 1639.49 cm^{-1} region. There is a significant increase of the intensity with the treatment of 2 M sulfuric acid. The HOH vibrational absorption band that form hydrogen bonds with water, which appears at 3446.79 cm^{-1} region, also experienced an increase in intensity, with 2 M sulfuric acid treatment.

With the addition of 0.5M sulfuric acid, stretching absorption band of Si-O-Si that appears in the 1037.7 cm^{-1} on bentonite, has shifted to 1053.3 cm^{-1} and the intensity decreased. On treatment with sulfuric acid, 1; 1.5 and 2M absorption band position remains at 1037.7 cm^{-1} . Treatment with 1 and 1.5M sulfuric acid lowered the intensity but treatment with 2M sulfuric acid greatly increases the intensity of peak. Increased absorption intensity indicates successful treatment with acid to remove organic and inorganic impurities. Reduction in the intensity of the disturbance indicates the occurrence of bond Si-O-Si.

Treatment with sulfuric acid also affects the -OH group on the octahedral structure. The addition of sulfuric acid also resulted in a change in absorbance of Al-Al-OH at 914.2 cm^{-1}

region. On addition of 0.5 M and 1M sulfuric acid, there is no shift, but the addition of 1.5 and 2M sulfuric acid causes the shift of absorption band to 916.19 cm^{-1} . This fact indicates that the addition of sulfuric acid with increasing concentrations up to 2M resulted in the growing strength of Al-O bond aluminosilicate.

Vibrational spectra of Si-O or Al-O which appears on 833.2 cm^{-1} region are also experiencing a shift to the larger wave numbers which, in accordance with the increase in the concentration of sulfuric acid. Treatment with 2M sulfuric acid produces absorption band at 839.03 cm^{-1} with an intensity which is also increasing. Bending vibrations of Si-O-Al (Al octahedral) appeared at 532.35 cm^{-1} wave number. This tape is very sensitive to the presence of Al in the octahedral layer. The intensity of uptake is greatly increased by treatment with sulfuric acid 2 M.

Similarly, the pore volume (Table 1), increased with of the acid used. This confirms that treatment with sulfuric acid resulted in a more porous clay. The surface area and pore volume can be increased by treatment with mineral acids. This treatment is highly dependent on acid strength, time and temperature of the system (Gogoy, 2008; Rozic et al, 2008). This is consistent with the results shown in Table 1.

Table 1. Analysis of Surface Area Characterization

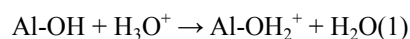
Sampel	Surface Area (m^2/g)	Pore Volume (cc/g)	Pore Diameter (nm)
Bentonit	76,4	$1,38 \text{ e-}01$	7,2
Bentonit 0,5 M	99,2	$1,548 \text{ e-}01$	6,2
Bentonit 1 M	146,0	$1,873 \text{ e-}01$	5,13
Bentonit 1,5 M	139,5	$1,753 \text{ e-}01$	5,02
Bentonit 2 M	178,5	$1,977 \text{ e-}01$	4,43

Treatment with a high concentration of acid will dissolve inorganic and organic material contained in the bentonite. It can be assumed that the chemical changes that occur in the bentonite structure during the activation process produces a vacancy on the octet structure. This resulted in the increase of Lewis acid site. It is known that during the activation process, to achieve equilibrium charge, protons of sulfuric acid will replace Na^+ and Ca^{2+} , which is located between the 2 layers. These protons do not contribute substantially to the surface acidity. On the other hand, the proton of sulfuric acid can not occupy the empty space left by Al^{3+} and Mg^{2+} in the octahedron center. This resulted in a void octets. Vacancy octet is a major cause of the increase in acidity. There is another possibility that the proton of the hydroxyl group at the corner of the octahedron becomes more unstable due to the deformation of the structure which caused by acid activation. It will also increase the acidity of Bronsted site (Alemdaro & Akkus, 2003; Hart & Brown, 2004; Babaki *et al.*, 2008)

Measurement of the maximum current on the catechol concentration of 10^{-9} - 10^{-3} M using bentonite (without treatment) membrane electrode get linear range at 10^{-9} - 10^{-5} M with a linearity of 0.958. Performance of bentonite acid 2 membrane electrode is the linearity of 0.986 obtained in the range of 10^{-9} - 10^{-5} M. An increase in the electrode performance was characterized by the increase in the linearity.

This can be explained by the results of characterization of 2 M bentonite acid. There is an increase in the surface area and the spacing between layers compared to the initial bentonite. This indicates that more open pore structure in acidic bentonite is experienced so the penetration of electroactive species was accelerated.

Treatment of Bentonite with acid also affects the performance of the modified bentonite electrode. The maximal inhibition percentage of bentonite membrane electrode is 10.5% while the maximum inhibition percentage of acid bentonite electrodes is 21.9%. Falaras (1998), expressed high Bronsted acidity on acid-activated bentonite can be considered as a source of electrocatalytic efficiency. Hydroxyl groups on the surface of the fracture alumina sheet can cause the adsorption of anions according to the reaction:



The detection limit of cyanide using bentonite membrane electrode was 1.48×10^{-6} M. Range Nernstian linear was at concentrations of 10^{-6} - 10^{-3} M with a linearity of 0.987 and a sensitivity of $3.994 \text{ mA M}^{-1} \text{ cm}^{-2}$. The sensitivity to cyanide for Acid 2 bentonite membrane electrode was $3.604 \text{ mA M}^{-1} \text{ cm}^{-2}$. Range Nernstian linear was at concentrations of 10^{-7} - 10^{-3} M with a linearity of 0.962 and a detection limit of 2.01×10^{-7} M cyanide. The formation of the protonated bentonite framework, caused by acid treatment (equation 1), may also lead to Coulomb force interaction with anionic species. These conditions explained the slight decrease in sensitivity of acid bentonite membrane. Acid activation treatment also caused

the change in the physical appearance in which the edge of the clay layers became irregular, which facilitates penetration of the anion species (Falaras, 1998). This condition explains the lower detection limit of the bentonite acid.

Membrane bentonite acid 2 clay 5 electrode showed the performance which did not differ significantly with the electrode of bentonite acid 2 membrane. The linearity cyanide was 0.977 on a linear concentration range of 10^{-7} - 10^{-3} M. The detection limit was 1.59×10^{-7} M. Sensitivity of membrane bentonite acid 2 clay 5 electrode was $2.208 \text{ mA M}^{-1} \text{ cm}^{-2}$.

The decrease in the sensitivity as a consequence of the increase in the thickness of the membrane layer is caused by the limited number of immobilized enzyme, which can be achieved by the substrate. At the same time the increase in the thickness of the membrane layer also caused a small increase in the diffusion path of the substrate from bentonite-solution interface into the enzyme active site in the electrode layer. These conditions minimized the detection limit value.

4. Conclusion

Measurement of the maximum current on the catechol concentration of 10^{-9} - 10^{-3} M using bentonite (without treatment) membrane electrode get linear range at 10^{-9} - 10^{-5} M with a linearity of 0.958. Performance of bentonite acid 2 membrane electrode is the linearity of 0.986 obtained in the range of 10^{-9} - 10^{-5} M. The maximal inhibition percentage of bentonite membrane electrode is 10.5% while the maximum inhibition percentage of acid bentonite electrodes is 21.9%. Membrane bentonite acid 2 clay 5 electrode showed the performance which did not differ significantly with the electrode of bentonite acid 2 membrane. The linearity cyanide was 0.977 on a linear concentration range of 10^{-7} - 10^{-3} M. The detection limit was 1.59×10^{-7} M. Sensitivity of membrane bentonite acid 2 clay 5 electrode was $2.208 \text{ mA M}^{-1} \text{ cm}^{-2}$.

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