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Study of Calcination Temperatures of Pillared Bentonite to the Performance of Cyanide Biosensor and its Application to Determine Cyanide in Cassava

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Abstract: *Amperometric biosensors for determining cyanide which are accurate, cheap and practical have been made. The biosensor used a modified bentonite as a electerode membrane. Current measured by a potensiostat with a Cyclic Voltametry method. Study of calcination temperature on pillarization of bentonite and thickness of bentonite membrane was conducted to get electrode with the best performance. The performance was tested using : Cathecol linearity, maximum inhibition percentage, cyanide linearity, detection limit and sensitivity. The data showed that electrode with membrane of bentonite pillarized at 250 C and the thickness is 5 mg/mL had the best performance.*

Keywords: Biosensor, Cyanide, Pillared-Bentonite, Tyrosinase

1. INTRODUCTION

Utilization of inorganic host matrix is one alternative use of organic polymer commonly utilized as immobilizing enzyme molecule. By comparison with polymers inorganic matrix has several advantages, for example, high chemical inert properties, biocompatibility, lattice crystals and crystal size that can be formed, an open pore structure, physical properties and chemical stability, and low production costs. Among all the organic and inorganic matrices reported in the literature, bentonite occupies a special place because of the hydrophilic nature, fluffy and porous which can increase the activity and stability of enzyme immobilization [1,2].

Some plants may contain cyanogenic glycosides which can release hydrogen cyanide during digestion processor biodegradation. Cassava is a staple food in some regions containing cyanogenic glycosides. These compounds are highly toxic, with threshold doses that can cause death of 5mg/kg of human body weight.

Cyanide can be detected by the inhibition action of the enzyme tyrosinase oxidation process at phenolic compounds. The tyrosinase activityis inhibited by cyanide in the form of a competition binding oxygen, not on the substrate [3,4]. The enzyme tyrosinase is immobilized by entrapment to the layer of pillared clay. Acid activated clay has a dominant positive charge [5,6] ,and treatment with Al pillarization increase the surface area and form a stable lattice [7]. Positively charged layer of clay can be an attractive point for negative analyte preconcentration. Immobilization matrix inhibitors can reduce sharply the detection limit. Immobilization of the tyrosinase enzyme to the positively charged clay layers can enhance the inhibitory effects of cyanide because of cyanide accumulation in the clay [8].

2. EXPERIMENTAL

2.1. Material

Tyrosinase (EC. 1.14.18.1) from Mushroom (17.600 units mg^{-1}) was purchased from Sigma Chemical Co. (USA). , cathecol, KCN, Bentonite, H_2SO_4, $AlCl_3.6\ H_2O$, Na_2CO_3 anhidrate. glutaraldehyde (25%), and all other chemicals were purchased from Merck.

2.2. Apparatus

The amperometric measurement was performed with a eDAQ potentiostat in conjunction with a recorder. All the experiment were carried out in a conventional thermostated three- electrode cell (10 ml) at room temperature. 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. The working electrode was a glassy carbon electrode.

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2.3. Pillarization of Clay

Al pillared clay(Al-PILC) were synthesized as follows: 1 M Na_2C0_3 solution of 300 mL was added drop wise into 500mL of 0.5 M $AlCl_3.6H_2O$ at 60ºC . The solution was stirred for 2 hours and then left over night at 60ºC. With rapid stirring 50g of acid activated clay incorporated into the solution mixture was then stirred continuously for 5 hours at the same temperature, and cooled to room temperature. After 24 hours, the precipitate was separated by filtration and washed with demineralized water four times. Clay obtained was dried at 110ºC for 24 hours,crushed into powder and sieved at 100 mesh size. Calcination temperature studies were conducted at a temperature of 200ºC, 250ºC, 300ºC. Characterization was conducted by FT-IR XRD,Surface Area Analyzer and SEM EDX.

2.4. Enzyme Immobilization

The clay colloidal suspension (2 mg mL^{-1}) was sonicated about 15 minutes. Tyrosinase was dissolved in water with at a concentration of 4 mg mL^{-1}. A defined amount of aqueous mixtures (2.5 µL clay and 2.5 µL of enzyme) was spread on the surface of the glassy carbon electrode. The coating was dried in the air at room temperature. The resulting electrode was placed in saturated gluteraldehyde vapor for 20 minutes for cross-linking of the membrane. Finally, the Tyrosinase/clay biomembrane was rehydrated for 20 minutes into 0.05 M phosphate buffer solution (pH 6). Composition studies were performed using a colloidal suspensionof bentonite (5 mgmL-1).

2.5. Catechol Substrate Current Measurement and Measurement of Cyanide Inhibition by Cyclic Voltammetry

Biosensor electrode was used to measure the flow of catechol at concentrations of 10^{-9}-10^{-3}M on the operational potential of -0.2Volt-0.2Volt, a speed of 400mV/sec. The electrodes were then dipped in cyanide solution 10^{-5} Mfor 5 minutes and then used again to measure the concentration of catechol. The experiment was repeated for each catechol solution with concentration of 10^{-9}-10^{-3}M. I% is determined by the equation: I% =(E 0-E1) /E0x100%. I% is the Percentage of inhibition or the inhibition degree of cyanide on the activity of the enzyme tyrosinase.

2.6. Current measurementof substrateandcyanide

Catechol substrate solution used was a solution of a substrate that having maximal inhibition percentage. The cyanide solutions with the concentration of 10^{-6} – 1 M were added separately into several substrate solutions. Current was measured after each addition of standard solutionof cyanide. Current measurements on a samples of cassava that have been macerated in a solution of 0.5M phosphate buffer pH6 were also conducted. Determination of cyanide content in the sample was based on the regressi online equation of cyanide standard solution.

3. RESULT AND DISCUSSION

Figure 1 shows the diffractograms of pillared bentonite calcined at a temperature of 200, 250 and 300ºC. Bentonite activated with 2 M sulfuric acid, was subsequently pillared with Al and calcined at a temperature of 200, 250 and 300ºC. At Acid Bentonite 2Θ = 5.3383 (d =16.541), with application at 250 C calcinations temperature, the

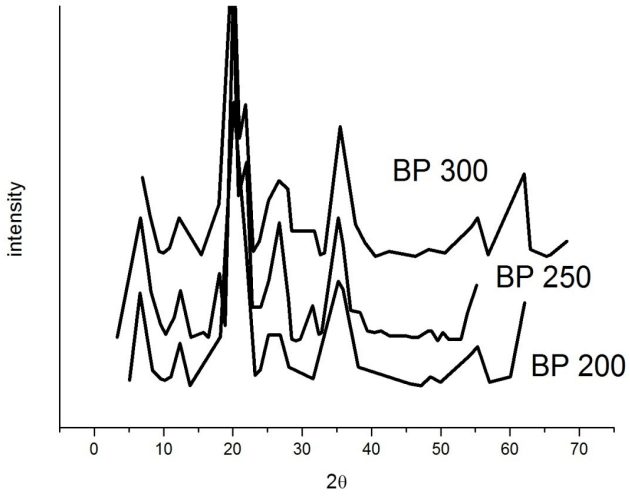

Figure 1. Diffractogram of Pillared Bentonite

distance between layers increased significantly (d =26.792) but on the calcinations temperature of 300ºC, there is no peak, which may be indicated as a breakdown of the tetrahedral lattice Si and Al. Affter pillarization, a new peak at 2Θ of 6-10 region is observed. The intensity of the new peak at the calcination temperature 250ºC increases, whereas that at the calcination temperature of 300ºC decreases.

BET surface areas, pore sizes and pore radii of pillared bentonites are given in Table 1.This indicates a reduction in the mesoporous BP250, compared to the mesoporous BP200. BP300 indicates the formation of more mesopori particles . This is supported by the pore volume and pore radius, which is larger than the other pillars of bentonite.

SEM results (Figure 2) show the presence of flakes on bentonite pillared structure compared to the structure of the agglomerated bentonite acid. Pillarization process also resulted in a more porous structure of bentonite. From SEM images, it appears that the B250 has more pore particles than B200 has.This is supported by the diffractogram in which the distance between layer in B250 is greater than that of B200. In the SEM image of B300, there are many small-sized particles distributed. This can be attributed to the XRD data-showing that there is the damage of the lattice and there are new peaks with small intensity.

EDX results showed that the ratio of Al/Si pillared Bentonite at 200C;250C;and 300ºC were 0.54; 0.6; and0.63, respectively.There is an increase in the aluminum content of pillared bentonite with increasing the calcination temperature. There is also a new peak in

Table 1. BET surface areas, pore sizes and pore radii of pillared bentonites

Sample	Surface area (m^2/g)	Pore volume (cc/g)	Pore radius (^0A)
BP 200	14,65	2,55 e-02	3,45 e +01
BP 250	15,15	2,11 e-02	2,796 e+01
BP 300	21,192	3,19 e-02	3,015 e+01

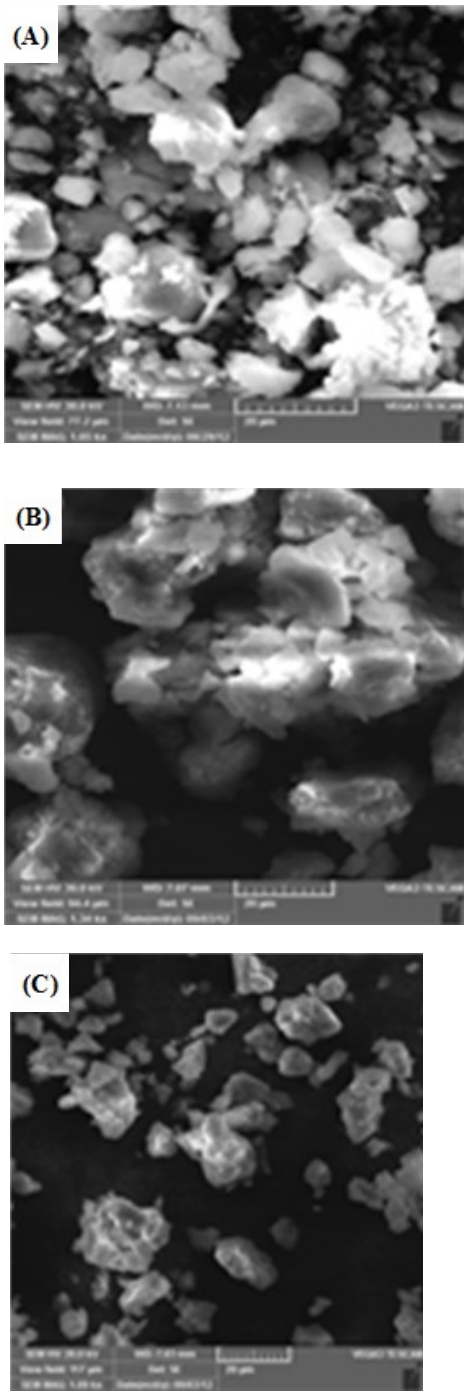

Figure 2. SEM of Pillared Bentonit at 200 (A),250 (B) and 3000 C (C).

addition to the basal spacing observed in the pillared bentonite with higher ratio of Al / Si. This new peak may be indicated as a space interpilar. The increase in the ratio of Al/Clay resulted in the increase of surface area and pore volume [9]. This is consistent with the ratio of the surface area of BP200 to BP250(Table 1), but the smaller pore volume of BP250 can be associated with the diffractogram data in which the basal spacing of BP 250 significantly increased.

Table 2 shows that there is no significant difference in the linearity of the standard concentration of catechol caused by the use of the electrode membrane of acidbentonite, bentonite P200, and P250 with the thicness of 2 mg/L. This is incontrasts with the increasing distance between the spaces (in the diffractogram) and the increasing content ofA l(EDX) with pillarization treatment.An explanation of these results is that the high the density of Al causes the formation of mesoporous fraction in the internal cavity so that the display on the pore structure of pillared bentonite is more dispersed (SEM image). These conditions affect the rate of penetration of electroactive species, therefore the performance of the pillared bentonite membrane electrode on catechol tends to be the sameas that of the acid bentonite membrane electrode. Treatment pillarization calcinations at the temperature of 200C increased the maximum inhibition by31.1%. The increase in the calcinations temperature to 250 and 300 lowered the maximum inihibition by 21.1% and22%, respectively. The Al lower density of 200 pillared bentonite membrane (ie 0.54) indicates the lower hydrophobic propertiy of the membrane. As a result, anion species have a large electroactivity.

The addition in the thicknessof P250 bentonite membrane greatly enhanced the inhibition ability of the electrode. Acid activated and pillared bentonite has a Bronsted and Lewis acid side. Bronsted acid is in the frameworkof bentonite, whereas Lewis acid is on alumina pillars [10]. Based on the more open structure and amphoteric nature of alumina pillars, the penetration of anionic species takes place more easily and allows the buildup of anionic species.These conditions explain the increase in the maximum percentage of inhibition of membrane BP250C5. The high sensitivity and low detection limit of the electrode membrane BP250 bentonite is caused by the larger surface area and smaller pore sizes the mebrane material (Table 1). Most of the area is formed on the internal cavity, which supports electrical activity of anion species [11] .The high content of Al in the bentonite p300 cannot support the performance of the electrodes because based on the results of XRD characterization, structural damage has occurred in bentonite P300 as shown by the loss of peaks in the basal spacing.

Delamination of micropore size to the higher size has occurred.Therefore, the enzyme was not evenly distributed. The decrease in sensitivity due to the increase in the thickness of the membrane layer is caused by the limited number of immobilized

Table 2. Analytical characteristic of bentonite-electrode towards cyanide determination

Electrode	Linierity of Cathecol	Inhibition Max (%)	Linierity of cyanide	Limit Detection	Sensitivity ($\mu A\ M^{-1}cm^{-2}$)
BP 200	0.981	31.1	0.976	2.51×10^{-6}	2.892
BP 250	0.982	21.1	0.976	1.61×10^{-6}	3.656
BP 300	0.991	22	0.874	6.87×10^{-5}	0.652
BP 250C5	0.980	41.4	0.975	1.5×10^{-6}	2.864

enzymes which can be reached by the substrate [12].

At the same time, the increase in the thickness of the membrane layer also led to a small increase in the diffusion path of substrate, from bentonite-solution interface to the active site of the enzyme in the electrode layer. These conditions led to increasingly smaller detection limit value. The highest percentage of the maximum inhibition given by the electrode BP250C5, linearity of cyanide which was quite good, and the sensitivity which did not change significantly on the increase of the amount of membrane and the low detection limit are the reason for choosing a BP250C5 electrode as the electrode with optimum capabilities.

Selectivity to the NO_3-value is-2.738 and repeatability of 87.3% allows the electrode BP250c5-to be used as electrodes in the determination of cyanide in cassava. The result showed that the concentrationof cyanide in the cassava sample was 9.1x10-6M. By converting the value, it was obtained that there was 2.457 mg of cyanide in 1 gram of cassava. Determination of cyanide content by titrimetric method,-gave a value of 0.1242 mg cyanide in 1 gram sample of cassava whereas the result obtained using a UV spectrophotometer based on a picric method was 1.134 mg in 1gram cassava.

4. CONCLUSION

The electrochemical biosensor designed from the acidic and pillared bentonite membrane calcined at a temperature of 2500C and a thickness of 5 mg/mL can be used to analyze the amount of cyanide in cassava. This biosensor has higher efficiency and accuracy than the method of titrimetric and UV-Vis spectrophotometer.

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