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IMPLICATION OF CLAY MODIFIED ELECTRODE ON CYANIDE BIOSENSOR

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ABSTRACT

A novel, inexpensive and simple amperometric biosensor based on immobilization of tyrosinase enzyme onto bentonite and modified bentonite is applied for determination of cyanide. The determination of cyanide was performed via its inhibiting action on the tyrosinase electrode. Measurement was carried out using cathecol as substrate. The enzymatically generated quinoid products were electroreduced at 0.1 V vs Ag/AgCl. An extremely sensitive detection limit (2 x 10^{-7} M) was obtained for cyanide. Enzyme immobilitation onto an modified bentonite seems to cause an increase in cyanide inhibition because of the greater surface area.

KEYWORDS : Biosensor, Cyanide, Bentonite, Tyrosinase

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INTRODUCTION

Toxic nature of cyanide can cause death. Examples of cyanide compounds are hydrogen cyanide gas and cyanide salts such as sodium cyanide, potassium cyanide. Some bacteria, fungi and algae can produce cyanide. Cyanide can also be produced by certain plant foods including almonds, five beans, soybeans, spinach, bamboo shoots, and cassava roots (which is the main food of tropical countries). Edible parts of plants including cassava starch from roots may contain small amounts of cyanide. There are more than 2500 species of plants and some insects contain cyanogenic glycosides, necessitating the development of a method that is both fast and accurate. Several conventional methods to determine cyanide such as titrimetric, colorimetric, spectrometric procedures, and chromatographic methods require several steps such as sample pretreatment. There are weaknesses in these methods includings the use of carcinogenic compounds in the colorimeter method, they also need long and unspecific results. The use of ESI as a chemical sensor on cyanide can not overcome interfering substances such as S, Cl^-, I^-, Br^-, Cd, Ag, Zn, Cu, Ni and Ag. Hence they are not applicable for on-site cyanide monitoring[1]. An attractive alternative way consists of the design of portable cyanide biosensors. Electrochemical biosensors have been increasingly developed for continuous monitoring in environmental and health care applicants. Such devices contain a biological sensing element intimately associated with a transducer that converts the specific biorecognition event to an electrical signal. The advantages of biosensor are the simple measurement procedure, short response time and highly selectivity by substrate spesificity. These devices were applied to the direct monitoring of cyanide [2].

The stable immobilization of an enzyme on an electrode surface, with complete retention of its biological activity and good diffusional properties for substrates, is a crucial problem for biosensor development [3]. Solids with a 2D structural arrangement have an open structure favourable for interchalation a large variety of organic molecules, macromolecules, and biomolecules. Therefore, clay minerals and related layered structures can be advantageously exploited to improve analytical characteristics of biosensors [4,5,6].

MATERIALS AND METHODS

Materials

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

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.

Clay Activation

A total of 100 grams of clay was dispersed into 300 mL of 2 M sulfuric acid solution while stirring with a magnetic stirrer for 6 hours. Allowed for 24 hours and then filtered with a vacuum filter and washed with hot distilled water until free of sulfate ions. This is indicated by a negative test for $BaCl_2$. Clay is then dried in an oven at a temperature of 100C.Dried clay was crushed into powder and then sieved using a 100 mesh sieve size. It was characterized by FT-IR, XRD Surface Area Analyzer and SEM EDX.

Clay Pillarization

Al pillared clay (Al-PILC) was synthesized by means of: 300 mL of 1 M Na_2C0_3 solution was added dropwise into 500 mL of 0.5 M $AlCl_3$ .6 H_2O at 60°C. The mixture was stirred for 2 hours and then left overnight at 60°C. With rapid stirring 50 g 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 mixture was separated by filtration and washed with demineralized water four times. The Clay was then dried at 110°C for 24 hours and crushed into powder and sieved at 100 mesh size then calcined at a temperature of 250°C. Finally, it was characterized by FT-IR, XRD Surface Area Analyzer and SEM EDX.

Characterization

A Shimadzu 7000 diffractometer utilizing Cu*Ka* radiation was used for obtaining powder X-ray diffraction (XRD) patterns. Specific surface area, pore volume, and pore size distributions were obtained using a Quantachrome NOVA 10.01 surface area analyzer. The samples were degassed for 7 hours at 300°C prior to analysis. Materials were examined using a JEOL JSM-840A scanning electron microscope and an energy-dispersive X-ray analysis system (EDX).

Enzyme Immobilization

The clay colloidal suspension (2 mg mL^{-1}) was sonificated about 15 minutes. Tyrosinase was dissolved in water with at a concentration of 4 mg mL^{-1}. A defined amount of aqueous mixtures (25 µL of clay and 25 µL of enzyme) was spread on the surface of the glassy carbon electrode. The coating was dried in air at room temperature. The resulting electrode was placed in saturated gluteraldehyde vapor for 20 minutes for cross-linking of the membrane. Finally, the complex Tyrosinase/clay biomembrane was rehydrated for 20 minutes into 0.05 M phosphate buffer solution (pH 6).

RESULTS AND DISCUSSION

Surface Area Characterized

Surface area and pore volume of clay can be increased by treatment with mineral acids [7]. This treatment is highly dependent on acid strength, time, and temperature of the system [8,9]. This is consistent with the data shown in Table 1.

Table 1
Surface Area of Clay

Clay	Surface Area (m^2/g)	Pore Diameter (A)
Bentonite	76.423	7.2274
Acid Bentonite	178.450	4.4300

The addition of highly concentrated acid will dissolve inorganic and organic materials in clays. We can pressume that the chemical changes that take place in the structure during acid activation lead to octet vacancies in the crystal lattice, thus leading to an increase in Lewis acidity. It is known that during acid activation, in order to provide charge equilibrium, the protons of sulfuric acid primarily the exchangeable cations such as Na^+ and Ca^{2+} that are between the layers. These protons do not contribute much to surface acidity. On the other hand, the protons of sulfuric acid cannot fill in the empty spaces left by ions such as Al^{3+} and Mg^{2+} that occupied the octahedron centers before being carried away by progressing activation, and hence, octet vacancies occur. It is also possible that the protons of the hydroxyl groups at the corners of the octahedron may become more labile as a result of structural deformation due to acid activation and this may increase the Bronsted acidity, but the main reason for the increased acidity seems to be octet vacancies.The qualitative analysis results of surface acidity by FTIR spectrophotometer showed that the acidification process generate Lewis acid sites on the wave number 1539.2 cm-1 and Bronsted acid sites on the wave number 1419.5 cm^{-1} (Fig 1).[10,11,12]

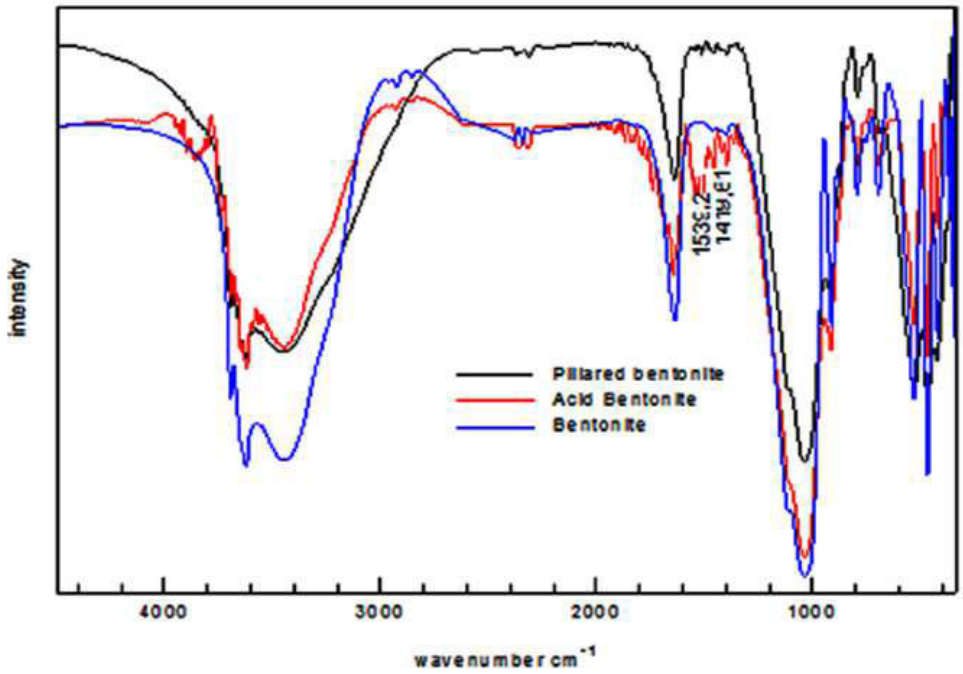

Figure 1
FTIR Spectra of Bentonite, Acid Bentonite and Pillared Bentonite

Characterization of Basal Spacing

Performed using XRD method, the results obtained can be used to determine the magnitude of the shift distance between the silicate layers of clay. If the distance between the layers increases the surface area also increased [13]. The data in Table 2 indicate that the modification of bentonite by acid causes a shift 2 Θ becomes smaller and the larger the distanced. This is consistent with the data of modificated acid in Table 1.

Table 2
Data of Angels Change and Distance between Layer Change

2 Θ (deg)		d (A)	
Bentonite	Acid bentonite	Bentonite	Acid bentonite
22.0314	21. 8400	4.031	4.066
20.1140	20.0255	4.411	4. 430
6.4560	5.3383	13. 678	16.541

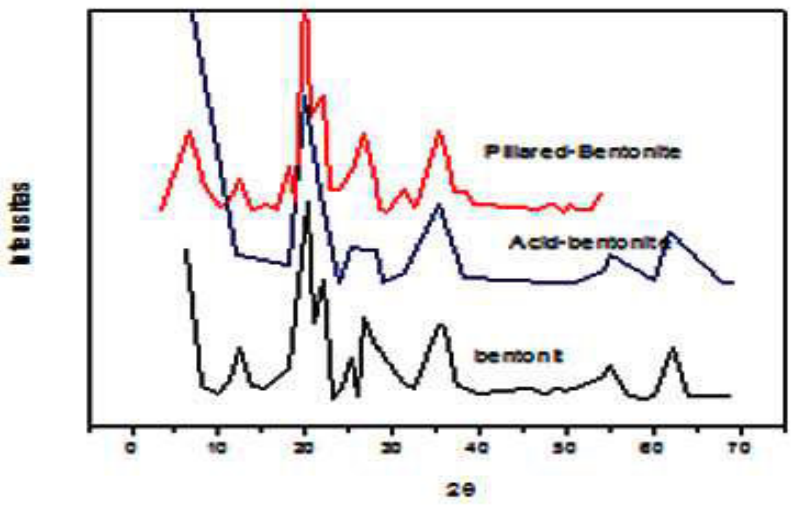

Figure 2
Diffractograme Bentonite, Acid Bentonite and Pillared Bentonite

The technique is by far the most widely used in the characterization of PILC: X-ray diffraction. This provides immediate information about the succes of the pillaring process, evidenced by the shifting of the basal spacing to higher values; i.e. lower angles in the diffractogram [14,15]. By comparing the diffractogram bentonite and bentonite acid, bentonite peaks are missing in the bentonite acid (2 Θ = 10-15). This indicates that there is damage to Si and Al tetrahedral lattice. At pillared-bentonite diffractogram there is a new peak at 2 Θ = 10-20^0

SEM EDX CHARACTERIZED

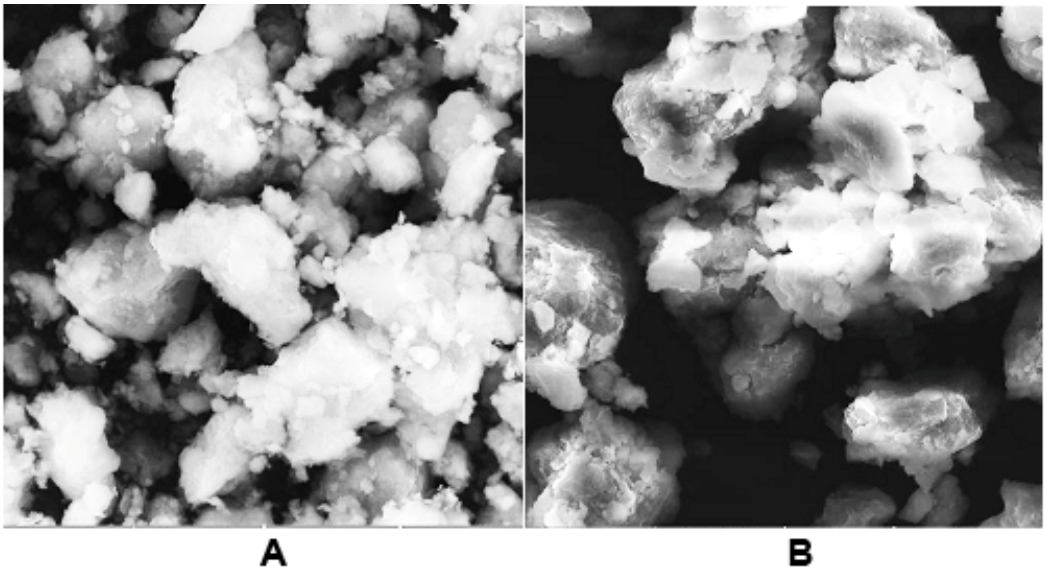

Figure 3
SEM EDX Photograph of Acid Bentonite (A) and Pillared Bentonite (B)

The scanning electron micrographs of acid bentonite and pillared bentonite are shown in Fig 3. The modified clay particles are irregular in shape and size. The parent clay (Acid –Bentonite) are agglomerated while the alumina pillared clay shows flake structures [16].

Table 3
The EDX Analysis for acid-bentonite and pillared bentonite

Element (%)	AB	PB
Al	23.32	32.64
Si	65.30	54.08

The EDX data for the acid bentonite and pillared bentonite are presented in Table 3. The Al/Si ratio is about 1.7 times higher in the case of Pillared Bentonite compared to that of Acid Bentonite due to the insertion of Al_{13} oxide clusters into clay interlayers by pillaring process[17].

Biosensor Cyanide

Electrodes are used to detect cyanide is an electrode that has a good performance for cathecol. The determination of cyanide was thus carried out through its inhibitory effect on the oxydase activity of tyrosinase toward cathecol[18,.19]. The role of the host matrix (on the improvement of the biosensor performance) was investigated by comparison among Bentonite, Acid Bentonite and Pillared Bentonite. The data in Table 4 show that the performance of the acid bentonite as a host matrix has a better performance than that of bentonite, proved by the lower detection limit. It can be connected with the greater surface area and the greater distance between layers of acid bentonite than those of Bentonite.

Table 4
Analytical Characteristic of Bentonite-electrode towards Cyanide determination

Electrode	Linier range Cathecol (M)	Inhibition Max (%)	Linier Range Cyanide(M)	R^2	Limite Detection	Sensitivity ($\mu A\ M^{-1}cm^{-2}$)
Bentonite	$10^{-9} – 10^{-5}$	10.5	$10^{-6} – 10^{-3}$	0.974	1.479×10^{-6}	1.997
Acid-Bentonite	$10^{-9} – 10^{-5}$	21.9	$10^{-7} – 10^{-3}$	0.925	2×10^{-7}	1.,802
Pillared-Bentonite	$10^{-9} – 10^{-5}$	21.1	$10^{-6} – 10^{-3}$	0.953	1.61×10^{-6}	1.828

The acidity of a clay matrix can be improved by acid activation. The acid activation process increases the number of Bronstec acid sites on the clay due, partly, to the introduction of lattice protons [20]. Chemical changes that occur in the structure of clays during treatment with acid generating vacancies in the crystal lattice octets so that increase lewis acid. This condition allows the accumulation of cyanide in the host matrix [20].Performance of Pillared bentonite electrode lower than acid-bentonite electrode can be explained by formation of Pillar that may cause inhibition to immobilization of enzyme tyrosinase.

CONCLUSION

Acid activation of the clay can improve the performance of the cyanide biosensor. Pilaritation the acid-activated clays is ineffective toward the biosensor performance.

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