The Characterization of Chitosan-ZnO Nanoparticles Modified Screen-Printed Copper Electrodes as the Analytical Sensor

I Nainggolan¹, Saisa², H Agusnar³ and Z Alfian⁴

¹,²,³,⁴Chemistry Department, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara, Medan, Indonesia
²Chemical Engineering Department, Faculty of Engineering, Universitas Serambi Mekkah, Aceh, Indonesia

Email: irwana@usu.ac.id

Abstract. Characteristics of chitosan-ZnO-modified screen-printed copper electrodes showed good sensitivity as a sensor which characterized by using cyclic voltammetry. The copper electrode was prepared for the sensitivity testing before being used as a sensor substrate material, the tests carried out in sodium phosphate buffer solution (BSP), the use of BSP as a standard test in this study.

1. Introduction

Copper (Cu) is a material that is very suitable and economical to be used as a sensor, copper is a compound of transition element groups, and its availability very abundant in nature that found in soil, rock, water, and air. Copper has excellent electrical properties and often used as electrical conductor [1]. The sources of copper ore are from mining, and have been widely used in electricity, wire, and to make alloys, bronze, and silver [2]. Various technologies and research results have applied the use of copper metal as an electric current conducting substrate that is able to provide fast and highly selective responses in the presence of chitosan as a sensitive thin film sensor media [3], chitosan has also been widely used as a sensor in the detection of heavy metals where chitosan provide good and sensitive response [4]. Addition of metal oxide as a filler for chitosan is very important to improve sensitivity, response and selectivity of chitosan film-based sensor.

2. Materials and Method

Materials are chitosan (medium molecular weight, Sigma Aldrich), ZnO (~ 99%, Sigma Aldrich), Acetic Acid (Merck), Copper Substrate, Di-Sodium hydrogen phosphate (Merck), Sodium dihydrogen phosphate (Merck), Sodium Hydroxide (Merck).

2.1 Fabrication Process of Copper Substrate (Cu/SPE)

The stages of making copper-screen-printed electrode (Cu/SPE) substrate, begin with preparing the circuit board which known as PCB (printed circuit board) [5], [6], the size of PCB strip is 3.0 cm x 1.0 cm x 0.1 cm. In this case, copper is a working electrode prepared by coating method on the surface of PCB board, with diameter of working surface circle area is 5 mm. Copper coating is formed with length and width of stem at 10 mm x 1.5 mm, and has a hook measuring 2.5 mm x 5.0 mm, the hook function as a conductor of electrical current.
2.2 Process of Making Chitosan-ZnO Nanoparticles (CS-ZnO NPs)
Chitosan nanoparticles (CS NPs) 1.5% was dissolved in acetic acid solvent 2% in a volume of 100 mL, chitosan dissolved for 24 hours. The same was carried out for dissolving of Zinc Oxide nanoparticles (ZnO NPs) at different concentrations (0.2; 0.4; 0.6; 0.8; 1.0%), with the same solvent was used (acetic acid 2%) each of it made in 50 mL volume and stirred for 12 hours. Stirring was carried out with magnetic stirrer [7]. After both solutions are prepared, then mixing chitosan with zinc oxide at various concentrations for 24 hours using magnetic stirrer to obtain a solution of zinc oxide nanoparticles (CS-ZnO NPs) [8]. 20 µL mixture of CS-ZnO NPs was placed on a copper screen-printed electrode (Cu/SPEs) then heated for 30 minutes until the CS-ZnO NPs film was formed and ready to be tested for sensitivity with cyclic voltammetry method. Characterization tests of CS-ZnO NPs films were performed using Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR), and X-Ray Diffraction (XRD).

2.3 Process of Making Sodium Phosphate Buffer Solution
The process of making buffer solution of sodium phosphate [9]–[12], the initial stage of treatment by preparing beaker glass. Sodium phosphate buffer solution (BSP) made by mixing a solution of Na$_2$HPO$_4$ and NaH$_2$PO$_4$ concentration of 1 M (molar), to get a sodium phosphate buffer solution of 0.1 M concentration in 50 mL pH 7.0 at a temperature of 25°C is by dissolving an amount of 2, 89 mL Na$_2$HPO$_4$ and 2.12 mL NaH$_2$PO$_4$ and put in a 50 mL volumetric flask carefully and carefully then add aquadest water to the volumetric flask to get the appropriate amount of volume. Re-measure the pH of the solution for compliance, if the pH measured is below the desired value then add sodium hydroxide (NaOH) solution to increase the right pH, and if the measured pH is above the desired value then add phosphoric acid solution (H$_3$PO$_4$) to reduce to the desired pH [13]–[15].

2.4 Analysis of Copper Substrate using Cyclic Voltammetry
Analysis of copper substrate is carried out by preparing Cu-SPE that has been made on circuit board, copper still has a printing layer is removed by using sandpaper, so the copper electrode is clearly visible and then copper rod coated with the purpose of coating on the copper rod to keep only the copper circle used as the sensor area. Copper substrate analysis is performed to know the sensitivity of the electrode as a sensor. The cyclic voltammetry analysis system has three voltammetry cell electrodes, consisting of working electrode, reference electrode, and counter electrode [16].

2.5 Process of Electrochemical Oxidation and Reduction Process by Cyclic Voltammetry
SPE copper electrode performance was measured using a buffer solution of sodium phosphate (BSP), the purpose of the measurement was to qualitatively observe anodic peak currents (Ip$^a$) and cathodic peak currents (Ip$^c$). Based on the results of anodic measurements (oxidation) and cathodic (reduction) peak currents, so that the number of electrons (n) can be determined by the Nernst equation, the potential change can be determined from potential difference at the anodic peak with potential at cathodic peak.

\[
\frac{\Delta E_p}{n} = 0,0592 V \quad \text{or} \quad \frac{\Delta E_p}{n} = 59,2 \text{ mV}
\]

Explanation:
\[
\Delta E_p = |E_{p^a} - E_{p^c}|
\]

3. Result and Discussion
3.1 The Process of Making Copper Substrate (Cu-SPE)
The copper substrate is electrode that used as sensor in this study. The making of copper substrate is carried out by screen printing process of electrode on circuit board [17]–[21]. PCB size 30x10 mm is prepared as substrate, in which copper is coated according to size that shown in Figure 1. The first step
of making copper substrate is designing electrode, then screen-printing for copper coating on substrate is cleaned by using sandpaper until copper surface is clearly visible, so that copper electrode media can be used as sensor to be coated with chitosan and zinc oxide (ZnO) which the sensor matrix in this study.

![Figure 1](image.png)

**Figure 1.** The process of making copper substrate (a) Cu electrode design; (b) Copper SPE (Cu-SPE) process; (c) Cleaning with sandpaper; (d) Coating of sensor area.

Copper screen-printed electrode (Cu/SPE) substrate as medium to conduct current that used as working electrode (WE) in this study. Sensor area with 5 mm diameter was prepared for placement of chitosan-ZnO thin film nanoparticles (CS-ZnO NPs). The selection of copper as working electrode because it has good electrical conductivity, high selectivity, high sensitivity, and strong responsivity [22], [23], and the availability of materials are easily obtainable and economical in terms of making copper screen-printed electrode substrate.

### 3.2 Characterization of CS-ZnO NPs Sensor Film

Surface morphology and microstructure characteristics of chitosan and ZnO were determined using SEM (Scanning electron microscopy), and the surface morphology results are shown in Fig. 2. SEM analysis was carried out to test the surface morphology of CS-ZnO composition compounds obtained under process conditions, the surface shape of chitosan film was smooth is showed excellently distance between particles because it arranged tightly. The condition of chitosan ratio to zinc oxide in Fig. 1(a) showed the surface is lower smoothness and pores, while Fig. 1(b) showed the surface is higher fineness and pores. The smooth surface and large pores indicate that chitosan is capable of providing sensitive and selective sensor reactions [24][25].
Figure 2. SEM morphology analysis of CS-ZnO conditions (a) ratio of CS: 1%, (b) ratio of CS: 1.5%

Analysis of ZnO chitosan compound functional group using Fourier transform infrared (FT-IR) instrument. The results of FT-IR spectra analysis of chitosan ZnO composition are shown in Fig. 3 below:

Figure 3. FT-IR characterization results of Chitosan-ZnO compounds

Based on FT-IR spectra analysis results, Fig. 3 shown absorption band at wave number 3261.37 cm\(^{-1}\) which stretching vibration of primary N-H amine group from chitosan. Hydrogen bonds cause the peaks to widen and there is a shift towards short wave number. Aliphatic C-H bond vibrations occurred in the range 3000-2850 cm\(^{-1}\) as seen by C-H vibrations at wavelengths of 2924.09 cm\(^{-1}\) and 2862.36 cm\(^{-1}\) which causes peak shifted to toward the maximum [26].

FT-IR results for chitosan-ZnO composites showed in absorption band with wave number 1554.13 cm\(^{-1}\) indicate the existence of Zn-O-Zn vibrations. Furthermore, in region of wave numbers at 1402.88 cm\(^{-1}\) and 1019.03 cm\(^{-1}\) are O-H stretching vibrations and O-H bending vibrations of water molecules. Zn-O group stretching absorption and N-Zn group vibration absorption are at 523.16 cm\(^{-1}\) and 574.45 cm\(^{-1}\) wave numbers. The reason for this is to provide information on formation of
hydrogen bonds between chitosan and ZnO and interaction between them to form chitosan-ZnO composite [27].

Figure 4. Results of X-ray diffraction (XRD) (a) chitosan-ZnO diffraction spectra compound (b) Standard chitosan diffraction spectra and ZnO nanoparticles

Fig. 4 is the result of chitosan-ZnO characterization using X-Ray diffraction (XRD) analysis. XRD observation is to observe the ZnO Crystal structure which is bound to Chitosan. The results of the analysis of Fig. 4(a) show that the ZnO crystal structure is bound to chitosan as evidenced by a peak characteristic of chitosan at a value of \( \theta = 20^\circ \) and can be proven correlation with the semi crystalline nature of chitosan [28]. The crystal structure of ZnO NPs shows strong bonds in chitosan as evidenced by the diffraction peak values of 20 \(^\circ\) respectively at 34.47\(^\circ\) (002), 36.87\(^\circ\) (101), 47.89\(^\circ\) (102), 57.25\(^\circ\) (110), 64.38\(^\circ\) (103), 70.01\(^\circ\) (201), and 73.47\(^\circ\) (202) are very suitable and close to the hexagonal wurtzite structure of ZnO crystals and proven by data from JCPDS (card no. 36-1451) [29], the same results are shown in the standard spectra analysis shown in Fig. 4(b) which is an analysis of chitosan, ZnO NPs, and ZnO-Chitosan [30], [31]. The intensity of each peak ZnO showed that ZnO interact strongly on chitosan, only the intensity of the peak value (101) which provides a very high intensity values with standard intensity value. However, the ZnO crystal structure remains stable interact with chitosan that can be affirmed that the formation of ZnO on chitosan successful [32].

3.3 Test Performance of Cu-SPE Substrates with Cyclic Voltammetry

In cyclic voltammetry process, there are three types of voltammetry cells, the working electrode (WE), the reference electrode (RE), and the assist electrode (counter electrode/CE), the three types of voltammetry cells are showed in Fig. 5.
Figure 5. Voltammetry Cells

The working electrode (WE) is electrode where redox reactions occur to solution test and depended on potential given. The potential variations are giving different currents depending on solution test being analyzed, the current values can be known from peak of voltammogram. The working electrodes are copper electrodes coated on circuit board, known as copper screen printed electrodes (Cu-SPE). The second electrode is comparison electrode (RE). This electrode is electrode which potential value fixed during measurement and its value does not depend on type and composition of solution being measured. Comparative electrodes are usually used Ag/AgCl electrodes, in this study used calomel electrodes. The third type of electrode is auxiliary electrode (CE). These electrodes are usually made of materials such as Platinum (Pt). This supporting electrode is used to flow current between working electrode and assist electrode, so that the current can be measured [33][34].

3.4 Sensitivity of Cu-SPE Substrate in the Sodium Phosphate (BSP) Buffer
Characteristics of copper screen-printed electrodes visits sensitivity capabilities substrate by using cyclic voltammetry. Some of the copper electrode is prepared for the sensitivity performance test before being used as a sensor substrate material, the tests carried out in sodium phosphate buffer solution (BSP), the use of BSP as a standard test in this study. Sensor performance test results in a BPS solution as shown in Fig. 6 below.
Figure 6. Electrochemical process of copper-SPE substrate in sodium phosphate buffer solution (a) Variation of Cu-SPE electrode; (b) Linear ring electrode Cu-SPE.

Observations from the results of cyclic voltammetry showed copper electrode responds well be used as a sensor substrate electrode where oxidation and reduction reactions which occur very stable. The test results linearity of the electrode was also conducted in which the R-square value obtained at 0.98867 showed that copper is the proper electrodes are used and the economic value in the process of preparation of copper screen-printed into a very appropriate choice. Respectively electrode current strength value is shown in Table 1.

| Cu Electrodes | Current (mA) | R-Square |
|---------------|-------------|----------|
| 1,0           | 0,0588      |          |
| 2,0           | 0,0667      |          |
| 3,0           | 0,0773      |          |
| 4,0           | 0,0902      |          |
Figure 7. Oxidation reaction and reduction of voltammetry cell (a) Oxidation; (b) Reduction.

4. Conclusion
The chitosan-ZnO-modified screen-printed copper electrodes characteristic showed good sensitivity as a sensor which characterized by using cyclic voltammetry. The copper electrode was prepared for the sensitivity testing before being used as a sensor substrate material, the tests carried out in sodium phosphate buffer solution (BSP), the use of BSP as a standard test in this study.

5. References
[1] X. Zhang, Z. Tang, D. Tian, K. Liu, and W. Wu, “A self-healing flexible transparent conductor made of copper nanowires and polyurethane,” Mater. Res. Bull., vol. 90, pp. 175–181, 2017.
[2] L. K. Kumawat, N. Mergu, A. K. Singh, and V. K. Gupta, “A novel optical sensor for copper ions based on phthalocyanine tetrasulfonic acid,” Sensors Actuators B Chem., vol. 212, pp. 389–394, 2015.
[3] A. Eljali, I. Nainggolan, S. Hashim, and T. I. Nasution, “Highly Responsive and Sensitive Copper Sensors Based on Chitosan Films,” Adv. Mater. Res., vol. 1125, pp. 255–259, 2015.
[4] R. Ravikumar, L. H. Chen, M. M. X. Hui, and C. C. Chan, “Ion-Imprinted Chitosan-Based Interferometric Sensor for Selective Detection of Heavy Metal Ions,” J. Light. Technol., vol. 37, no. 11, pp. 2778–2783, 2019.
[5] S. Bhunia and M. Tehranipoor, “Chapter 4 - Printed Circuit Board (PCB): Design and Test,” in Hardware Security, S. Bhunia and M. B. T.-H. S. Tehranipoor, Eds. Morgan Kaufmann, 2019, pp. 81–105.
[6] F. T. C. Moreira, M. J. M. S. Ferreira, J. R. T. Puga, and M. G. F. Sales, “Screen-printed electrode produced by printed-circuit board technology. Application to cancer biomarker detection by means of plastic antibody as sensing material,” Sensors Actuators B Chem., vol. 223, pp. 927–935, 2016.
[7] M. R. P, K. Muraleedaran, and V. M. A. Mujeeb, “Applications of chitosan powder with in situ
synthesized nano ZnO particles as an antimicrobial agent,” *Int. J. Biol. Macromol.*, vol. 77, pp. 266–272, 2015.

[8] X. Hu, X. Jia, C. Zhi, Z. Jin, and M. Miao, “Improving the properties of starch-based antimicrobial composite films using ZnO-chitosan nanoparticles,” *Carbohydr. Polym.*, vol. 210, pp. 204–209, 2019.

[9] K. Ganesh, R. Soumen, Y. Ravichandran, and Janarathan, “Dynamic approach to predict pH profiles of biologically relevant buffers,” *Biochem. Biophys. reports*, vol. 9, pp. 121–127, Dec. 2016.

[10] O. S. Marković, M. P. Pešić, A. V Shah, A. T. M. Serajuddin, T. Ž. Verbić, and A. Avdeef, “Solubility-pH profile of desipramine hydrochloride in saline phosphate buffer: Enhanced solubility due to drug-buffer aggregates,” *Eur. J. Pharm. Sci.*, vol. 133, pp. 264–274, 2019.

[11] P. Patyar, K. Kaur, and G. Singh, “Interactions of glycine with aqueous solutions of sodium phosphate buffer at T=288.15, 293.15, 298.15, 303.15, 308.15, 310.15, 313.15, 318.15, 323.15 and 328.15 K: Volumetric and UV absorption studies,” *J. Mol. Liq.*, vol. 251, pp. 70–76, 2018.

[12] S. Ondaral, E. Çelik, O. Ç. Kurtuluş, E. Aşıkuzun, and İ. Yakın, “Chitosan adsorption on nanofibrillated cellulose with different aldehyde content and interaction with phosphate buffered saline,” *Carbohydr. Polym.*, vol. 186, pp. 192–199, 2018.

[13] G. He and L. Wang, “One-step preparation of ultra-thin copper oxide nanowire arrays/copper wire electrode for non-enzymatic glucose sensor,” *Ions (Kiel).* vol. 24, no. 10, pp. 3167–3175, 2018.

[14] G. Ackermann, Y. J. Tang, J. P. Henderson, A. C. Rodloff, J. Silva Jr, and S. H. Cohen, “Electroporation of DNA sequences from the pathogenicity locus (PaLoc) of toxigenic Clostridium difficile into a non-toxigenic strain,” *Mol. Cell. Probes*, vol. 15, no. 5, pp. 301–306, 2001.

[15] M. R. Green and J. Sambrook, “Molecular cloning: A Laboratory Manual 4th,” *Cold Spring Harb. Lab. Press*, vol. 3, 2012.

[16] H. V Tran, C. D. Huynh, H. V Tran, and B. Piro, “Cyclic voltammetry, square wave voltammetry, electrochemical impedance spectroscopy and colorimetric method for hydrogen peroxide detection based on chitosan/silver nanocomposite,” *Arab. J. Chem.*, vol. 11, no. 4, pp. 453–459, 2018.

[17] S. A. Tukur, N. A. Yusof, and R. Hajian, “Gold Nanoparticles-Modified Screen-Printed Electrode for Determination of Pb(II) Ion Using Linear Sweep Anodic Stripping Voltammetry,” *IEEE Sens. J.*, vol. 15, no. 5, pp. 2780–2784, 2015.

[18] M. F. Kabir, M. T. Rahman, A. Gurung, and Q. Qiao, “Electrochemical Phosphate Sensors Using Silver Nanowires Treated Screen Printed Electrodes,” *IEEE Sens. J.*, vol. 18, no. 9, pp. 3480–3485, 2018.

[19] L. Manjakkal, A. Vilouras, and R. Dahiya, “Screen Printed Thick Film Reference Electrodes for Electrochemical Sensing,” *IEEE Sens. J.*, vol. 18, no. 19, pp. 7779–7785, 2018.

[20] K. Layek, Nagahanumaiah, and K. K. Mistry, “A Study of Optimization of Various Parameters in the Fabrication of Screen-Printed Electrodes,” *IEEE Sens. J.*, vol. 18, no. 19, pp. 7917–7923, 2018.

[21] A. Cranny, N. R. Harris, M. Nie, J. A. Wharton, R. J. K. Wood, and K. R. Stokes, “Sensors for Corrosion Detection: Measurement of Copper Ions in 3.5% Sodium Chloride Using Screen-
Printed Platinum Electrodes,” *IEEE Sens. J.*, vol. 12, no. 6, pp. 2091–2099, 2012.

[22] M. R. Ganjali, A. Ghafarloo, F. Faridbod, and P. Norouzi, “Copper-selective PVC membrane sensor,” *Int J Electrochem Sci*, vol. 7, pp. 3706–3716, 2012.

[23] J. Schwarz, K. Trommer, and M. Mertig, “Novel Screen-Printed All-Solid-State Copper (II)-Selective Electrode for Mobile Environmental Analysis,” *Am. J. Anal. Chem.*, vol. 7, no. 07, p. 525, 2016.

[24] T. I. Nasution, I. Nainggolan, D. Dalimunthe, M. Balyan, R. Cuana, and S. Khanifah, “Humidity detection using chitosan film based sensor,” in *IOP Conference Series: Materials Science and Engineering*, 2018, vol. 309, no. 1, p. 12080.

[25] L. Al-Naamani, S. Dobretsov, and J. Dutta, “Chitosan-zinc oxide nanoparticle composite coating for active food packaging applications,” *Innov. Food Sci. Emerg. Technol.*, vol. 38, pp. 231–237, 2016.

[26] M. Figiela, M. Wysokowski, M. Galinski, T. Jesionowski, and I. Stepniak, “Synthesis and characterization of novel copper oxide-chitosan nanocomposites for non-enzymatic glucose sensing,” *Sensors Actuators B Chem.*, vol. 272, pp. 296–307, 2018.

[27] L.-H. Li, J.-C. Deng, H.-R. Deng, Z.-L. Liu, and L. Xin, “Synthesis and characterization of chitosan/ZnO nanoparticle composite membranes,” *Carbohydr. Res.*, vol. 345, no. 8, pp. 994–998, 2010.

[28] P. Mujeeb Rahman, V. M. Abdul Mujeeb, K. Muraleedharan, and S. K. Thomas, “Chitosan/nano ZnO composite films: Enhanced mechanical, antimicrobial and dielectric properties,” *Arab. J. Chem.*, vol. 11, no. 1, pp. 120–127, 2018.

[29] S. H. S. Dananjaya, R. S. Kumar, M. Yang, C. Nikapitiya, J. Lee, and M. De Zoysa, “Synthesis, characterization of ZnO-chitosan nanocomposites and evaluation of its antifungal activity against pathogenic Candida albicans,” *Int. J. Biol. Macromol.*, vol. 108, pp. 1281–1288, 2018.

[30] F. Wahid et al., “Synthesis and characterization of antibacterial carboxymethyl Chitosan/ZnO nanocomposite hydrogels,” *Int. J. Biol. Macromol.*, vol. 88, pp. 273–279, 2016.

[31] D. Bharathi, R. Ranjithkumar, B. Chandarshaker, and V. Bhuvaneshwari, “Preparation of chitosan coated zinc oxide nanocomposite for enhanced antibacterial and photocatalytic activity: As a bionanocomposite,” *Int. J. Biol. Macromol.*, vol. 129, pp. 989–996, 2019.

[32] M.-B. Bouzourâa et al., “Effects of silicon porosity on physical properties of ZnO films,” *Mater. Chem. Phys.*, vol. 175, pp. 233–240, 2016.

[33] V. Climent and J. M. Feliu, “Cyclic Voltammetry,” in *Encyclopedia of Interfacial Chemistry*, K. B. T.-E. of I. C. Wandelt, Ed. Oxford: Elsevier, 2018, pp. 48–74.

[34] R. J. Forster and L. R. Cumba, “Voltammetry | Cyclic Voltammetry of Organic Compounds☆,” in *Encyclopedia of Analytical Science (Third Edition)*, P. Worsfold, C. Poole, A. Townshend, and M. B. T.-E. of A. S. (Third E. Miró, Eds. Oxford: Academic Press, 2019, pp. 197–208.

**Acknowledgments**

The author would like to thank to Universitas Sumatera Utara.