Optical Sensor for the Determination of Pb^{2+} Based On Immobilization of Dithizone onto Chitosan-Silica Membrane

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ABSTRACT

Optical sensor based on immobilization of dithizone onto chitosan-silica for the determination of Pb^{2+} has been prepared. The sensor was made of the composite membrane of chitosan and silica in the ratio of volume 2:1. Sol-gel technique has been chosen to prepare the membrane. The fabricated sensor indicated a good selectivity at pH 5 with response time at ± 180 s. Linearity response was obtained with concentration ranged from 0.2 to 1.1 ppm with r^2 = 0.9921. The percentage of relative standard deviation (%RSD) on precision and accuracy as recovery percentage (% recovery) were 1.46 and 100.96%, respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were 0.11 and 0.37 ppm, respectively.

Keywords: *optical sensor; chitosan-silica membrane; dithizone; Pb^{2+}*

ABSTRAK

Sensor optik berdasarkan imobilisasi dithizon pada kitosan-silika untuk penentuan Pb^{2+} telah disiapkan. Sensor optik dibuat dari membran komposit kitosan dan silika dengan rasio volume 2:1. Teknik sol gel telah dipilih untuk pembuatan membran. Sensor yang telah dibuat menunjukan selektivitas yang baik pada pH 5 dengan waktu respon pada ± 180 s. Linearitas diperoleh pada kisaran konsentrasi 0,2-1,1 ppm dengan r^2 = 0,9921. Persen simpangan baku relatif melalui uji presisi dan akurasi (persen perolehan kembali) berturut-turut yaitu 1,46 dan 100,96%. Batas deteksi (LOD) dan batas kuantifikasi (LOQ) berturut-turut adalah 0,11 dan 0,37 ppm.

Kata Kunci: *sensor optik; membran kitosan-silika; ditizon; Pb^{2+}*

INTRODUCTION

The contamination of lead (Pb) in the environment has become a concern due to its effects on health. Lead poisoning can cause brain damage and take down the IQ, damage the organ function, decrease the kidney function and anaemia. The main sources of lead contamination are industrial waste and transportation facilities which both occur on land, in rivers, and in the sea. Realizing the danger of lead contamination, the government enacts the regulation on the Pb^{2+} level in water intended for public consumption. The maximum tolerated concentration level of Pb^{2+} approved by Indonesian National Standard (SNI) 01-3553-2006 is 0.005 ppm. Regulation of the Minister of Health of the Republic of Indonesia Number 492/Menkes/PER/IV/2010 and World Health Organization (WHO) underlined that the level of lead concentration in drinking water should not exceed 0.01 ppm.

The level of heavy metal ions, especially Pb^{2+} can be determined using various methods. According to the SNI number 06-6989.8-2004 [1], the level of Pb^{2+} can be measured by Atomic absorption spectrophotometry. Isotope dilution inductively coupled plasma mass spectrometry [2], inductively coupled plasma optical emission spectrometry [3] as well as voltammetry method [4], can also be used to determine the levels of heavy metal in samples. Although some of these techniques are very sensitive and selective, the operation procedures used are quite complicated, the analyzing time is quite long, and the instruments are quite expensive [5].

In recent years, optical sensor (optode) and biosensor have emerged as alternative tools to analyze the heavy metal contamination, particularly Pb contamination in the environment. Both sensors have low costs, rapid detection time and good performance. The study of sensors in the determination of Pb^{2+} has been reported. Aksuner [6] developed fluorescent

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sensor using triazolo-thiadiazin derivative which was immobilized onto polyvinyl chloride membrane to detect Pb^{2+} metal ions. The sensor was able to detect Pb^{2+} metal ions with a limit of detection at a concentration of 2.2×10^{-8} M. Zargoosh and Babadi [7] made optical sensor based on immobilization of dithizone on agarose membrane for determination of Pb^{2+} and Hg^{2+} metal ions. The limit of detection which was obtained for Hg^{2+} and Pb^{2+} were 2×10^{-9} M and 4×10^{-9} M for, respectively.

The membrane that was made of a mixture of chitosan-silica can be an alternative in the fabrication of optical sensors. The fragile and low biocompatibility characteristics of silica can be solved by mixing the silica with chitosan [8]. In addition, silica is also able to increase the stability of membranes and pores of chitosan membrane [9]. This parameter is influential for optode membrane as pores in the membrane are needed as metal adsorption media [10]. Chitosan-silica membrane has been applied in biomedical, pharmaceutical, biosensors, and adsorbents. Moreover, the membrane has porosity and mechanical stability that it can be used as biosensors for dopamine and uric acid detection [11].

The sol-gel method is an alternative method for preparing optodes membranes which are performed in low temperatures for the synthesis of inorganic materials in nature or mixing of inorganic and organic materials. The method provides several advantages as it has low chemical reactivity, high mechanical stability, good compatibility with various substrates and also does not require high temperatures [12].

Dithizone is one of the reagents used for analysis of some metal ions. The dithizone can react with some metals and later forms specific complexes of metal dithizonate. Dithizone has a good selectivity against Pb^{2+} ion in alkaline medium and has the sensitivity to detect the Pb^{2+} metal up to 0.0035 ppm as well [13].

In this study, a sensor to be used to detect Pb^{2+} metal sensitively and selectively was made based on the immobilization of dithizone onto the chitosan-silica membrane. The sensor response was observed according to the discoloration. Validation of method was carried out to measure the performance of the sensor.

EXPERIMENTAL SECTION

Materials

All chemicals were purchased as analytical grade and used without further purification. Commercial chitosan with a degree of deacetylation of 80-85% had a molecular weight in the range of 100,000 to 250,000 g/mol. Tetraethyl orthosilicate (TEOS), absolute ethanol (EtOH), hydrochloric acid (HCl), glacial acetic acid (CH_3COOH), sodium hydroxide (NaOH), dithizone, and standard solution of $Pb(NO_3)_2$; $Fe(NO_3)_3$; $Zn(NO_3)_2$; $Cd(NO_3)_2$ were purchased from Merck.

Instrumentation

The pH of the solution was measured by pH meter (Hanna HI 2211). The absorbance of the membrane was determined by UV-Vis (Ocean Optics) Spectrophotometer. Characterization of membrane morphology was observed by using the Scanning Electron Microscope (SEM) (Carl Zeis EVO MA 10) and Fourier Transform Infrared (FTIR) (Bruker Tensor 3) for analysis of functional group in the membranes. Atomic absorption spectrophotometer (Shimadzu AA-6800) was used to determine the concentration of metal ions.

Procedure

Fabrication of chitosan-silica membrane and immobilization of dithizone

Fabrication of chitosan-silica membrane was conducted in several steps. Silica sol solution was prepared by stirring the mixture solution of tetraethyl orthosilicate (TEOS) (22.2 mL), ethanol (22.2 mL), distilled water (88.8 mL) and HCl (0.1 M, 5 mL) for 24 h using a magnetic stirrer. A total of 3 g of chitosan was dissolved in 100 mL of 2% CH_3COOH and stirred for 3 h (3% w/v of chitosan solution). Furthermore, the silica sol solution was added to the chitosan solution with a variation of the volume ratio of chitosan:silica were 3:7, 1:1, 2:1, and 3:1 (v/v) respectively, and was stirred for 2 h. Later, the homogeneous solution was poured into a glass plate and dried at room temperature to obtain a chitosan-silica dried membrane. The membrane was soaked in NaOH (1%) to ensure that the membrane can be removed from the mold. Then, the membrane was treated with distilled water until it became neutral [9].

The chitosan-silica membrane was treated by a solution of dithizone (5×10^{-4} M) in ethanol for 6 h. This step was intended to immobilize dithizone onto the chitosan-silica membrane. Afterward, the membrane was treated with the distilled water for 12 h [7].

Characterization of chitosan-silica membrane

The membrane was characterized by FTIR and SEM. FTIR was used to analyze the functional groups of the membrane while SEM was used to analyze the morphology of membrane surface of chitosan and chitosan-silica. The membrane was firstly coated with gold for 40 min thus the surface membrane was able to be seen by SEM.

Fig 1. Membranes with various volume ratio of chitosan-silica of 3:7 (a), 1:1 (b), 2:1 (c), 3:1 (d), and chitosan-silica membrane ratio of 2:1 after immobilization of dithizone (e)

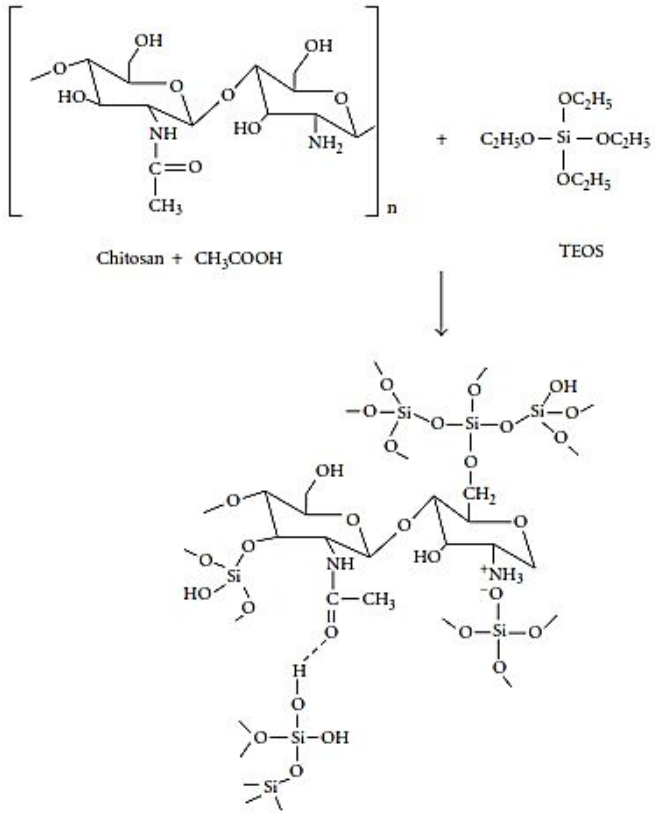

Fig 2. Schematic structure model of chitosan-silica membrane

Measurement of performance and validation of optode

Optode performance can be determined through measurements of maximum wavelength, optimum pH and response time of optode against Pb^{2+} metal ions. The measurement of optode performance was conducted by using UV-VIS Spectrophotometer Ocean Optics. Optode with the size of 1×1 cm was treated in Pb^{2+} solution until its color changed from orange to pink. Then, the optode was removed and dried. The dried optode was put in Cuvette holder Ocean Optics and its absorbance was measured at the range of visible wavelength of 450-500 nm to find the maximum value [10].

The optode was treated with a Pb^{2+} solution in various pH and time durations to measure the optimum pH and response time. The variation of pH used was from pH 3 to 9 and the variation of time set was from 60 to 300 s.

The validation test of optode included a test of linearity, limit of detection, limit of quantification, precision, accuracy and also selectivity [14-16]. The interfere metal ions used in selectivity test were: Cd^{2+}, Zn^{2+}, and Fe^{3+}.

RESULT AND DISCUSSION

Chitosan-Silica Membrane

The chitosan-silica membrane was made by sol-gel method with a various volume ratio of chitosan:silica (Fig. 1). The ratio of chitosan:silica, 3:7 (Fig. 1a) did not form a membrane (the membrane was broken). However, on ratio 1:1 (Fig. 1b), 2:1 (Fig. 1c) and 3:1 (Fig. 1d), membranes were formed. The amount of chitosan and silica addition affected the formation of the membrane, the higher the amount of silica, the more difficult to achieve the membrane formation as membrane will break due to the fragile characteristic of silica. Besides, the addition of excess chitosan affected the color of the membrane (more opaque) which also impacted on the optode measurement. The Precise composition of chitosan-silica resulted in optode membrane with porosity and mechanical stability. The ratio of chitosan and silica sol used in the fabrication of membrane for sensors was 2:1. Moreover, this ratio succeeded in producing a quite transparent membrane and did not shrink the sheet of the membrane (Fig. 1c).

Characteristics of Chitosan-Silica Membrane

The Interaction of chitosan-silica (Fig. 2) according to Al-Sagher and Salim [17], indicated hydrogen bonds formed between amide group of chitosan and silanol. Other interactions were ionic bonds between amino group and silanol and also covalent bonds which were possible to be formed due

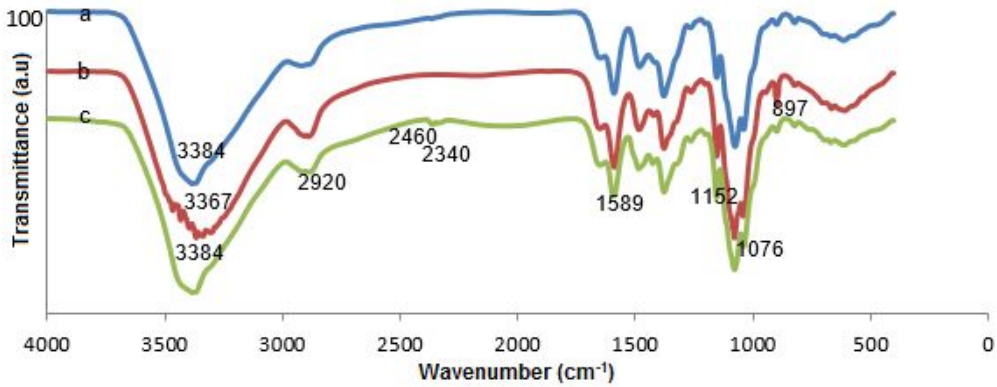

Fig 3. FT-IR spectra of chitosan (a), chitosan-silica membrane (b), and chitosan-silica-dithizone membrane (c)

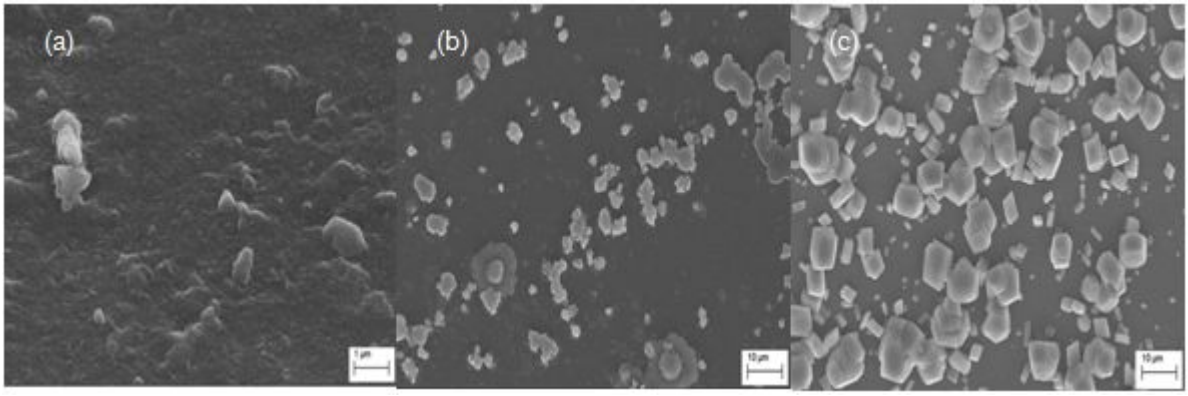

Fig 4. Electron microscopy image of chitosan membrane (a), chitosan-silica membrane (b), and chitosan-silica-dithizone membrane (c)

to esterification reaction between the hydroxyl group of chitosan and silanol group of silica.

The characterization of the membrane by FTIR (Fig. 3) showed a peak at 3384 cm^{-1} which indicated the presence of NH group that overlapped with the absorption of OH group [18]. Wave number at 2920 cm^{-1} was the absorption of CH stretch and peak at 1589 cm^{-1} was the C-N stretch. The absorption of C-O-C stretch appeared at 1152 cm^{-1} and the C-O stretch was found at 1074 cm^{-1}. According to Taba [19], the wave number obtained was the characteristic absorption of chitosan. The characteristic spectrum of the chitosan-silica membrane and chitosan-silica membrane immobilized by dithizone were not different from the spectrum of chitosan membrane as there was only a narrow shift of wave number and addition of new peak. In the spectrum of the chitosan-silica membrane, wave number shifted from 3384 cm^{-1} to 3367 cm^{-1} which indicated the interaction between OH groups of silica and N-H of chitosan [9]. The presence of absorption at 1076 cm^{-1} showed the vibration of Si-O-Si while at 897 cm^{-1}, Si-OH was found to be the reason [18]. In the spectrum of membranes immobilized by dithizone, new peaks appeared, those were at 2460 cm^{-1} (S-H group) and at 2340 cm^{-1} (C=N group of dithizone) [20].

The result of SEM analysis showed the dense and smooth surface of chitosan membrane (Fig. 4a) that the pores were not visible. The addition of silica into chitosan membrane (Fig. 4b) and the addition of dithizone into the chitosan-silica membrane (Fig. 4c) made its surface rough although it was not properly distributed (not homogeneous). The stirring time can affect the homogeneity of silica on the surface of chitosan. The shape of silica particles was not homogeneous since the precursor used in the composite was TEOS. Therefore, it caused the particles on the surface membrane seen in different forms (round, oval, rectangular and triangular) [21]. The addition of dithizone made the particle shape was larger because of the silica particle on the chitosan surface was covered by dithizone particle. Dithizone which was used as metal detector substance diffused onto the surface of the chitosan-silica membrane. As the membrane was produced by the sol-gel method, dithizone can be adsorbed onto chitosan-silica membrane [22].

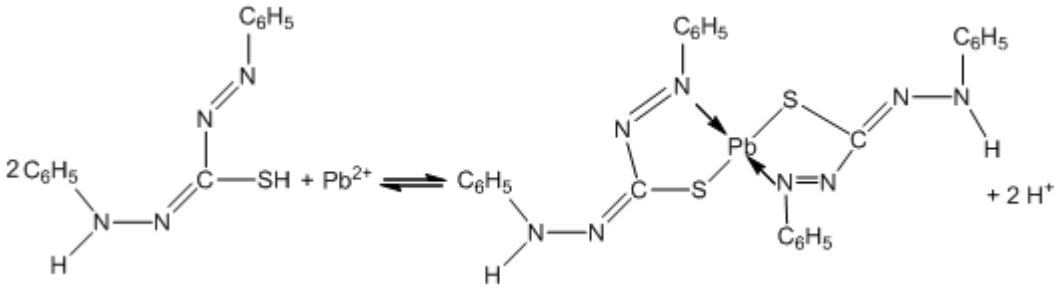

Fig 5. Reaction of complex formation of dithizone with Pb^{2+}

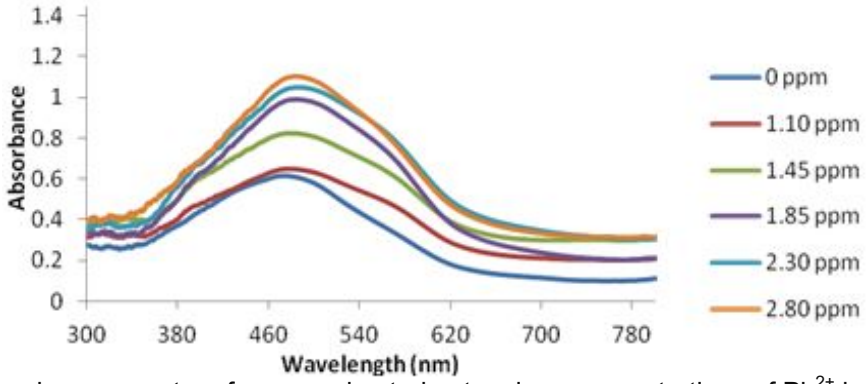

Fig 6. Absorbance spectra of proposed optode at various concentrations of Pb^{2+} ion solution

Immobilization of Dithizone on Chitosan-Silica Membrane

Immobilization of dithizone onto chitosan-silica membrane was conducted by soaking the membrane in dithizone solution with a concentration of 5×10^{-4} M. Dithizone immobilized onto the membrane resulted in orange color (Fig. 5). According to Zargoosh and Babadi [7], the color of dithizone solution in ethanol was dark green at high concentrations and orange at low concentrations. Dithizone has been used as metal detector because it can diffuse onto the surface of the chitosan-silica membrane, thus it is able to adsorb onto the pores of the chitosan-silica membrane.

Optical Sensor (Optode) of Pb^{2+}

The immobilized membrane was tested at various concentrations of Pb^{2+} solution. Dithizone formed complexes of Pb^{2+}-dithizonate which further led to color change, orange to pink, on the optode. Nitrogen and sulfur atoms of dithizone bound with Pb^{2+} metal ions (Fig. 5) [23].

Maximum absorbances at various concentration of Pb^{2+} were depicted (Fig. 6). The maximum wavelength of optode was obtained at 488 nm. However, this result was different from the previous research, i.e 486 nm [13], 425 nm [7], and 510 nm [24] due to the difference in membrane used to produce optode. Thus, it affected on the shifting of maximum wavelength.

The stability of dithizone complex with some metals was influenced by pH. In this study, the optimum pH of dithizone and Pb^{2+} metal ion complex was obtained at pH 5 (Fig. 7a). At pH 3 and 4, the reaction between dithizone and Pb^{2+} ion was not stable due to protonation of nitrogen atom and sulfur which reduced the donor-acceptor interaction between dithizone and Pb^{2+} ion. Whereas at pH 6 to 9, the reaction was not stable because Pb^{2+} ion formed hydroxyl complex and precipitate.

The contact time of optode with the Pb^{2+} solution was constant at 3 to 6 min (Fig. 7b). The optimum time was obtained at 3 min when the Pb^{2+} metal ions completely reacted with the dithizone that immobilized onto optode. The optode produced was found to have better response time than optode that was made using triacetyl cellulose membrane which obtained response time for 11 to 15 min [25].

Optode produced in this study had linearity at a concentration range of 0.2 to 1.1 ppm with an r^2 value of 0.9921 (Fig. 8). Limit of detection and quantitation were obtained at 0.11 and 0.37 ppm, respectively. Limit of detection obtained was quite low. However, optode produced using the agarose membrane was very low, those were 0.0008 ppm [7]. Comparison of performance between optodes fabricated in this study is listed in Table 1.

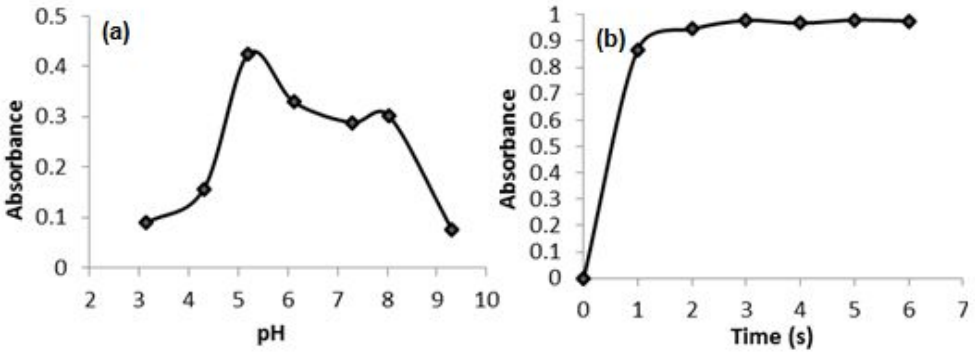

Fig 7. Effect of pH (a) and contact time of sensor (b) against the absorbance

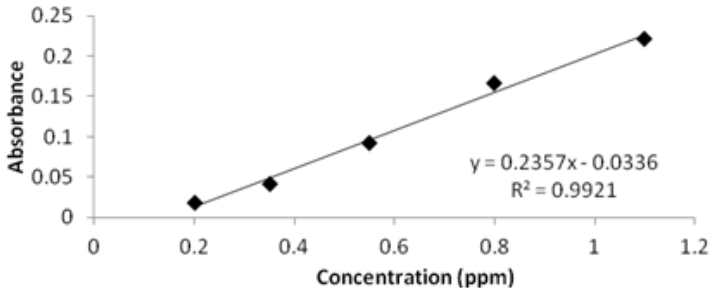

Fig 8. Graph of optode linearity

Table 1. Performance comparison of various membrane immobilized by dithizone as optodes

Membrane	Working range (ppm)	Limit of Detection (ppm)	Sources
Triacethylcellulose	0.5 – 5.5	0.15	[24]
Agarose	0.002 – 0.49	0.0008	[7]
Chitosan-silica	**0.2 – 1.1**	**0.11**	**Present work**

Table 2. Calculation result of accuracy optode, replicate n = 3

Replicates concentration (ppm)	Measured concentration (ppm)	Recovery (%)
0.20	0.219 ± 0.004	109.50 ± 2.00
0.35	0.319 ± 0.007	91.24 ± 1.91
0.55	0.533 ± 0.009	96.85 ± 1.55
0.80	0.848 ± 0.002	106.05 ± 0.29
1.10	1.080 ± 0.004	98.18 ± 0.37

The accuracy of a method is needed for the fabrication of optode which is also one of validation tests. Accuracy value is defined as the percentage of ratio value between concentrations obtained from the measurement and actual concentration [16]. Good accuracy values were in the range of 80 to 110% (for concentrations in ppm) [15]. Later, the analysis is performed by calculating the percentage of recovery. The value of % recovery for each concentration in this research (Table 2) was included in the range of good accuracy value.

The precision of an optode is also required in the determination of analyte concentration. The optode precision obtained is a repeatability value that can be seen from the percentage of relative standard deviation (%RSD). Repeatability is a precision result which is obtained if measurements are repeated [15]. Acceptance condition for %RSD according to the standard of AOAC [26] are as follows: very precise: %RSD < 1, precise: %RSD 1 to 2, medium: %RSD 2 to 5, and not precise: %RSD > 5. The analysis was conducted by detecting Pb^{2+} ions in solution at concentrations of 0.35, 0.55 and 5.00 ppm. %RSD obtained were 1.35, 1.61, and 1.34, respectively, therefore, optodes that had been made in this study had enough precise value and can be used as a detector of Pb^{2+} ions in solution.

Selectivity of Optode

The optode selectivity measurements were performed with the addition of interfering metal ions

into the test solution. The metal ions used in this study were: Fe^{3+}, Zn^{2+}, and Cd^{2+}. The three metals used can form complexes with dithizone, but they produced different colors of the complex for each metal. The complex colors can be measured at the maximum wavelength of each complex. According to Van Staden and Taljaard [27], the maximum wavelength for Dz-Cd^{2+}, Dz-Zn^{2+}, and Dz-Fe^{3+} were 435, 571, and 425 nm, respectively. These metals were also used as interfere metals in Pb metal test with dithizone method [24].

The concentrations of Pb metal ions against the three interfere metals were varied by two ratios of the concentration of Pb^{2+}: Fe^{3+}: Zn^{2+}: Cd^{2+} i.e 1:1:1:1 and 1:2:2:2 with pH of 5 in the mixed solution. Optode color that changed from orange to pink did not interfere, but absorbance value decreased at the maximum wavelength of 488 nm. The reduction of optode absorbance affected the selectivity because it had passed the limit of reduction tolerance of 5% [7].

The reduction of absorbance values obtained in Pb^{2+} metal detection was due to the interference of three metals. That occurrence indicated competition of complex formation with dithizone. The metal ions in the solution diffused onto optode and reacted with dithizone which later was adsorbed onto membrane [22]. Hence, the concentration of metal ions in solution decreased. Residue solution from the optode immersion process was tested with AAS to determine which metal ions diffused onto optode. The result of AAS test showed that concentration of Fe^{3+} and Pb^{2+} metal ions decreased, but the concentration of Zn^{2+} and Cd^{2+} did not. The concentration of Fe^{3+} metal ions decreased about 70%, higher than that of Pb^{2+} which was about 60%. In fact, this result disturbed the performance of sensor Pb^{2+}. Sensors made in this study were still selective against Pb^{2+} metal ions despite metal ions of Cd^{2+} and Zn^{2+} were found in the solution tested. However, the detection sensor was not selective with the Fe^{3+} metal ions.

CONCLUSION

An optical sensor (optode) for the determination of lead (II) can be prepared from the chitosan-silica membrane which immobilized by dithizone. Optode has good accuracy with average %recovery of 100.96% and precise precision with an average %relative standard deviation of 1.43 and 3 min of response time as well. Linearity was found at concentration ranged from 0.2 to 1.1 ppm with an r^2 value of 0.9921. Limit of detection and quantitation obtained were quite low i.e. 0.11 and 0.37 ppm, respectively. Optode that has been obtained was quite sensitive but not yet selective for Pb^{2+} ions, particularly in the presence of Fe^{3+} metal ions in the solution.

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REFERENCES

[1] Badan Standar Nasional, 2004, *Air dan air limbah-Bagian 8: Cara uji timbal (Pb) dengan spektrofotometer serapan atom (SSA)-nyala*, SNI 06-6989.8-2004, 1–5

[2] Chen, W.N., Jiang, S.J., Chen, Y.L., and Sahayam, A.S., 2015, Determination of Pb in lipsticks by flow injection chemical vapor generation isotope dilution inductively coupled plasma mass spectrometry, *Microchem. J*., 119, 128–132.

[3] Thangavel, S., Dash, K., Dhavile, S.M., and Sahayam, A.C., 2015, Determination of traces of As, B, Bi, Ga, Ge, P, Pb, Sb, Se, Si and Te in high-purity nickel using inductively coupled plasma-optical emission spectrometry (ICP-OES), *Talanta*, 131, 505–509.

[4] Zhu, L., Xu, L., Huang, B., Jia, N., Tan, L., and Yao S., 2014, Simultaneous determination of Cd(II) and Pb(II) using square wave anodic stripping voltammetry at a gold nanoparticle-graphene-cysteine composite modified bismuth film electrode, *Electrochim Acta*, 115, 471–477.

[5] Moyo, M., Okonkwo, J.O., and Agyei, N.M., 2014, An amperometric biosensor based on horseradish peroxidase immobilized onto maize tassel-multi-walled carbon nanotubes modified glassy carbon electrode for determination of heavy metal ions in aqueous solution, *Enzyme Microb. Technol.*, 56, 28–34.

[6] Aksuner, N., 2011, Development of a new fluorescent sensor based on a triazolo-thiadiazin derivative immobilized in polyvinyl chloride membrane for sensitive detection of lead(II) ions, *Sens. Actuators, B*, 157 (1), 162–168.

[7] Zargoosh, K., and Babadi, F.F., 2015, Highly selective and sensitive optical sensor for determination of Pb^{2+} and Hg^{2+} ions based on the covalent immobilization of dithizone on agarose membrane, *Spectrochim. Acta, Part A*, 137, 105–110.

[8] Repo, E., Warchoł, J.K., Bhatnagar, A., and Sillanpää, M., 2011, Heavy metals adsorption by novel EDTA-modified chitosan–silica hybrid materials, *J. Colloid Interface Sci.*, 358 (1), 261–267.

[9] Yunianti, S., and Maharani, D.K., 2012, Pemanfaatan membran kitosan-silika untuk

menurunkan kadar ion logam Pb(II) dalam larutan, *U. J. Chem.*, 1 (1), 108–115.

[10] Samadi-Maybodi, A., and Rezaei, V., 2014, A new sol–gel optical sensor with nonporous structure for determination of trace zinc, *Sens. Actuators, B*, 199, 418–423.

[11] Liu, X., Xie, L., and Li, H., 2012, Electrochemical biosensor based on reduced graphene oxide and Au nanoparticles entrapped in chitosan/silica sol–gel hybrid membranes for determination of dopamine and uric acid, *J. Electroanal Chem.*, 682, 158–163.

[12] Yari, A., and Abdoli, H.A., 2010, Sol–gel derived highly selective optical sensor for sensitive determination of the mercury(II) ion in solution, *J. Hazard. Mater.*, 178 (1-3), 713–717.

[13] Rajesh, N., and Manikandan, S., 2008, Spectrophotometric determination of lead after preconcentration of its diphenylthiocarbazone complex on an Amberlite XAD-1180 column, *Spectrochim. Acta, Part A*, 70 (4), 754–757.

[14] Scindia, Y.M., Pandey, A.K., Reddy, A.V.R., and Manohar, S.B., 2004, Chemically selective membrane optode for Cr(VI) determination in aqueous samples, *Anal. Chim. Acta*, 515 (2), 311–321.

[15] Harmita, 2004, Petunjuk pelaksanaan validasi metode dan cara perhitungannya, *Majalah Ilmu Kefarmasian,* 1 (3), 117–135.

[16] Purwanto, A., Supriyanto, C., and Samin, P., 2007, Validasi pengujian Cr, Cu, dan Pb dengan metode spektrometri serapan atom, *Proceeding PPI-PDIPTN*, Pustek Akselerator dan Proses Bahan-BATAN, Yogyakarta, 10 July 2007.

[17] Al-Sagheer, F., and Muslim, S., 2010, Thermal and mechanical properties of chitosan/SiO_2 hybrid composites, *J. Nanomater.*, 2010, 1–7.

[18] Lee, E.J., Shin, D.K., Kim, H.E., Kim, H.W., Koh, Y.H., and Jang, J.H., 2009, Membrane of hybrid chitosan–silica xerogel for guided bone regeneration, *Biomaterials*, 30 (5), 743–750.

[19] Taba, P., Natsir, H., Fauziah, St., and Ismail, M., 2010, Adsorpsi ion Cd(II) oleh kitosan-silika mesopori MCM-48, *Marina Chim. Acta*, 11 (1), 13–22.

[20] Mudasir, Raharjo, G., Tahir, I., and Wahyuni, T., 2008, Immobilization of dithizone onto chitin isolated from prawn seawater shells (*P. merguensis*) and its preliminary study for the adsorption of Cd(II) ion, *J. Phys. Sci*. 19 (1), 63–78.

[21] Rashidova, S.Sh., Shakarova, D.Sh., Ruzimuradov, O.N., Satubaldieva, D.T., Zalyalieva, S.V., Shpigun, O.A., Varlamov, V.P., and Kabulov, B.D., 2004, Bionanocompositional chitosan-silica sorbent for liquid chromatography, *J. Chromatogr. B*, 800 (1-2), 49–53.

[22] Jerónimo, P.C.A., Araújo, A.N., and Montenegro, M.C.B.S.M., 2007, Optical sensors and biosensors based on sol–gel films, *Talanta*, 72 (1), 13-27.

[23] Lang, L., Chiu, K., and Lang, Q., 2008, Spectrophotometric determination of lead, *Pharm. Technol.*, 32 (4), 74–83.

[24] Clesceri, L.S., Greenberg, A.E., and Eaton, A.D., *Standard Methods for the Examination of Water and Wastewater*, 20th ed., American Public Health Association, Washington DC.

[25] Tavallali, H., and Dorostghoal, L., 2014, Design and evaluation of a Lead(II) optical sensor based on immobilization of dithizone on triacetylcellulose membrane, *Int. J. ChemTech Res.*, 6 (5), 3179–3186.

[26] Feldsine, P., Abeyta, C., and Andrews, W.H., 2002, AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis, *J. AOAC Int*., 85 (5), 1187–1200.

[27] Van Staden, J.F., and Taljaard, R.E., 2007, Determination of Lead(II), Copper(II), Zinc(II), Cobalt(II), Cadmium(II), Iron(III), Mercury(II) using sequential injection extractions, *Talanta*, 64 (5), 1203–1212.