A New Method for Heavy Metals and Aluminium Detection Using Biopolymer-Based Optical Biosensor

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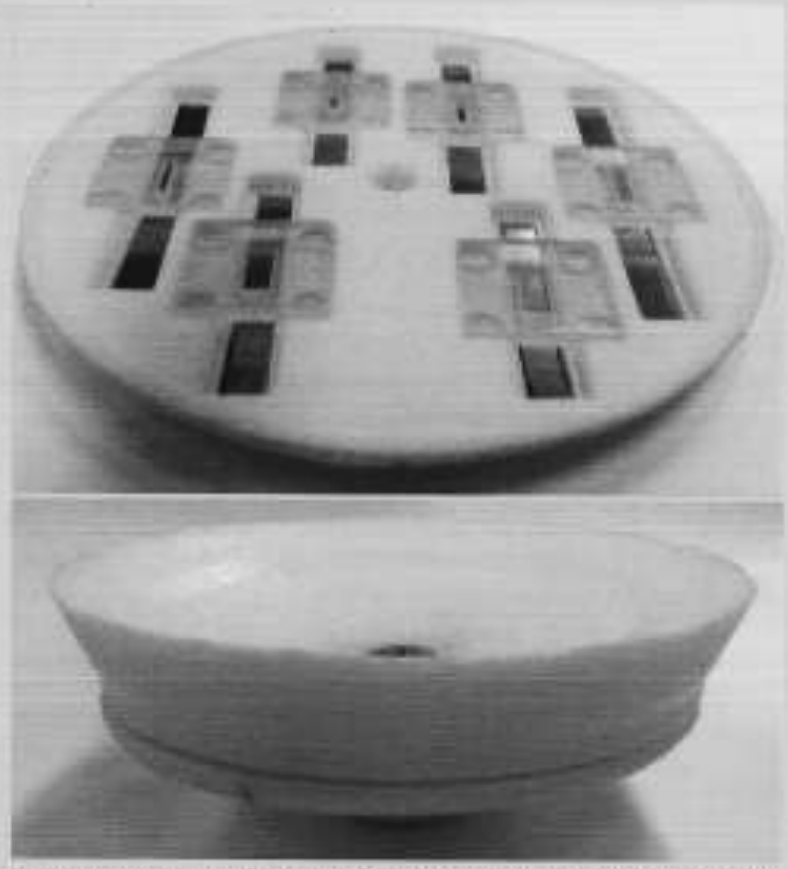

The lock-in portion of a lower limb prosthetic knee apparatus, showing magnetostrictive sensor strips and load applicators (see page 596).

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A New Method for Heavy Metals and Aluminium Detection using Biopolymer-Based Optical Biosensor

Ling Shing Wong, Chee Sien Wong

***Abstract*—A biopolymer-based biosensor for heavy metals and aluminium (Al) detection was constructed with naturally occurring β-carotene in palm kernel oil used as the biological reporter. The biosensor was designed with β-carotene entrapped by polyurethane- a polymer formed through prepolymerization of palm kernel oil. The presence of copper (Cu), lead (Pb), zinc (Zn), and Al was detected through the emulsification of β-carotene, which caused the change of optical density (OD) at λ = 450 nm. The results showed the OD increased with the presence of heavy metals and Al within 0.1 mg/L – 10.0 mg/L. The biosensor was constructed without extra steps to immobilize the biological component and it was simple to use with one step detection. Together with high reproducibility and fast response to Cu within 15 minutes, the biosensor showed good potential to be developed as a method to detect the presence of heavy metals and Al.**

***Index Terms*—Chemical and biological sensor, β-carotene, heavy metals, spectrophotometry**

I. Introduction

HEAVY METALS and Al are widely available in the environment. Although the detection of these metals previously relied heavily on conventional analytical equipment e.g. atomic absorption spectrometer and inductively coupled plasma mass spectrometer [1-3], biosensors are increasingly reported as alternative instruments for the detection of these metals.

A biosensor is an analytical tool that couples a biological component with an electronic device, in which the response from the biological component can be captured and transduced into readable electronic signal. To date, several sensitive enzyme-based biosensors have been developed for the detection of heavy metals and Al, e.g. urease conductometry biosensor [4], α-chymotrypsin amperometric biosensor [5], acetycholinesterase optical biosensor [6], and invertase-mutarotase-glucose oxidase-based conductometry biosensor [7]. Organic compound and DNA have been utilized in biosensors for heavy metal and Al detection as well [8-10].

Cells are highly favoured biological components in biosensors for heavy metal detection due to the ability of cells to respond to wide range of heavy metals [11, 12]. To date, many types of cells have been reported to be good candidates for heavy metals detection, for example cyanobacteria [12], algae [13-15], plant cells [16], and bacteria [17, 18]. The biological responses of certain photosynthetic pigments, the change in the concentration of oxygen, and the change of enzymes' activities in cells are some of the parameters which have been measured to detect the presence of heavy metals.

Chlorophyll is a photosynthetic pigment which has been widely used as the reporter group in whole cell biosensors [12, 15, 19]. A few biosensors based on carotenoids as the reporter have been reported as well [16, 20]. However, the utilization of carotenoids as a reporter in biosensors has been limited to cell-bound carotenoids. To date, the application of non-cell-bound carotenoids in biosensor application has never been reported.

In this paper, a biopolymer-based biosensor with immobilized β-carotene on biopolymer is reported. β-carotene contained in the kernel of oil palm [21] was immobilized by the polymer synthesized through the polymerization of palm kernel oil. The emulsification of β-carotene with the heavy metals and Al solutions was detected using a spectrophotometer. This biosensor is novel as it uses non-cell-bound β-carotene as the biological component. This paper is the first to report the utilization of a biopolymer synthesized from natural palm kernel oil in spectrometry biosensor application.

II. Methodology

A. Materials

Monoester polyol from palm kernel (PKO) was produced as described by Badri et al. [22]. 2,4-diphenylmethane diisocyanate (MDI) was purchased from Cosmo Polyurethane Sdn. Bhd., Klang, Malaysia, tetrahydrofuran (THF) was supplied by Merck Sdn. Bhd., Shah Alam, Malaysia. Polyethylene glycol (PEG) was supplied by Fluka Chemie Sdn. Bhd., Kuala Lumpur, Malaysia, while Cu in the form of $CuSO_4.5H_2O$, Pb in the form of $Pb(NO_3)_2$, Zn in the form of $ZnSO_4.7H_2O$, and Al in the form of $Al(NO_3)_3$ were provided by Systerm, Shah Alam, Malaysia.

B. Preparation of Biopolymer

A volume of 12 mL of THF was added into 10 g of PKO and stirred until PKO dissolved completely. MDI was then added to form urethane prepolymer [23]. 2.1 g of PEG was added followed by agitation at 200 rpm for one hour at room temperature. After that, 350 µL of the mixture was casted onto the clear surface of polystyrene cuvette (4.5 mL, 10 mm light

path) (Chemopharm Sdn. Bhd., Petaling Jaya, Malaysia) and left in room temperature for 72 hours to allow the complete evaporation of the solvent. The dried biopolymer was light-yellow in colour, translucent, and homogenous.

C. The OD for Detection and the Response from Polyol

In order to determine the OD for the detection, 2 mL of distilled water and Cu solutions (0.1 ppm and 1.0 ppm) were added respectively into cuvettes with dried biopolymer. OD readings at λ = 300 nm – 600 nm were measured at time zero minute and 20 minutes after the exposure using spectrophotometer Biochrom Libra12 (Chemopharm Sdn. Bhd., Petaling Jaya, Malaysia). OD at λ = 450 nm was selected to be used for the detection of heavy metals. All the tests in this experiment were conducted in triplicate (n = 3) unless stated otherwise.

The response of polyol was determined by adding distilled water and Cu solutions (1 ppm and 10 ppm) to all the chemicals required for the biopolymer synthesis- polyol, MDI, THF, and PEG. The response from each of the chemical to heavy metal was determined by the change of OD at λ = 450 nm at time zero minute and 20 minutes after the exposure.

D. Determination of Optimum Exposure Time

Four Cu solutions with concentrations of 0.01 mg/L, 0.10 mg/L, 1.00 mg/L, and 10.00 mg/L were prepared. 2 mL of the respective Cu solutions were transferred into cuvettes with dried biopolymer. Distilled water without Cu was added to cuvette with dried biopolymer as negative control. The change of OD was measured at λ = 450 nm with 15 minutes intervals for 60 minutes in room temperature and lighting condition.

E. Heavy Metals and Al Tests

The tests on Cu, Pb, Zn, Al (0.01 ppm, 0.10 ppm, 1.00 ppm, and 10.00 ppm) were conducted with OD at λ = 450 nm under room condition and exposure time set at 15 minutes.

III. Results and Discussion

A. Preparation of Biopolymer

There are several approaches to produce polyurethane, e.g. prepolymerization, single step polymerization, and quasiprepolymer technique [23]. Single step polymerization can be carried out by mixing diisocyanate and catalyst with polyol, producing a thin layer of polymer [24]. Quasiprepolymer technique utilizes the reaction between polyol and diisocyanate, producing urethane prepolymer with high content of free isocyanate. The method involves creating partial reaction between diisocyanate with polyol [25]. Prepolymerization on the other hand involves two-steps of reaction. Firstly, the formation of urethane prepolymer takes place by mixing polyol and diisocyanate. Then, the extension of the urethane prepolymer occurs by adding chain extender which leads to the formation of polyurethane. In this work, the prepolymerization approach was used to produce the biopolymer from PKO. The presence of β-carotene gave yellowish colour to PKO. The equation of formation of polyurethane via prepolymerization is shown in Figure 1.

The prepolymerization method produced the biopolymer in low temperature with literally no shrinkage. These characteristics of the biopolymer show advantages in preserving the biomolecules as well as creating a homogenous layer of film on the surface of cuvette. In addition to that, the biopolymer showed strong adsorption to the surface of the cuvette, which facilitated the immobilization process. The production of biopolymer is shown in Fig. 2.

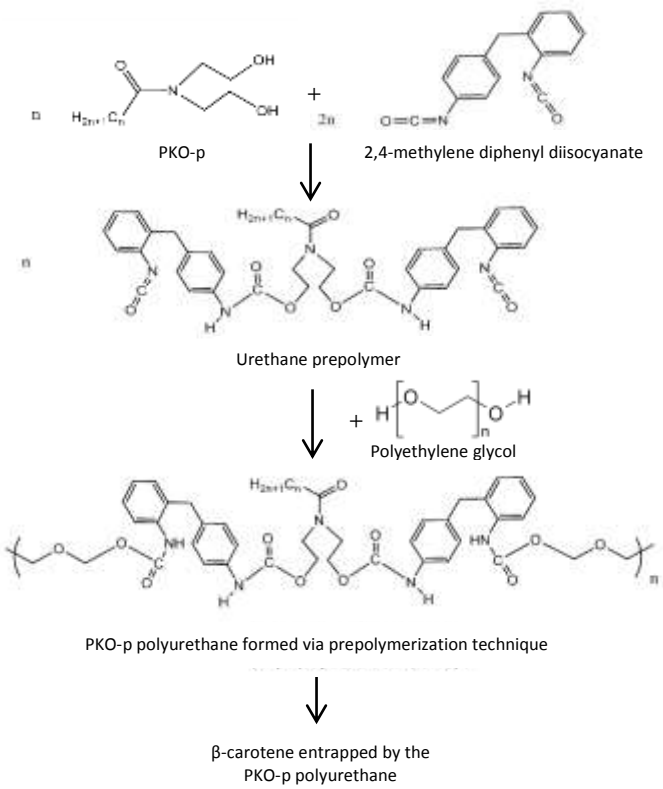

Fig. 1. The formation of PKO-p polyurethane and the entrapment of β-carotene.

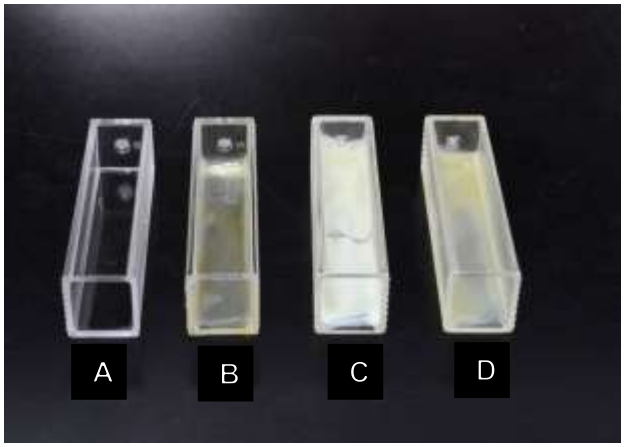

Fig. 2. The empty cuvette (A), cuvette with dried biopolymer before the exposure (B), the wet biopolymer after exposure to the solution with Cu (C), and the dried biopolymer after the exposure (D).

B. The OD for the Detection and the Response from Polyol

β-carotene is not soluble in water and forms an emulsion in water [26]. The emulsification caused the change of biopolymer from yellowish translucent substance to murky white non-translucent substance. The presence of PEG stabilized the emulsion formed [27]. The process of micro emulsification had made the spectrophotometry detection possible [28].

The spectrometry tests showed that the OD increased with the reduction of wavelength (Fig. 3). The shorter wavelength caused a higher scattering effect which reduced the amount of light reaching the sensor. Stable signals were produced by wavelengths 280 nm – 600 nm. However, the test with Cu solutions revealed a wavelength of ≤ 300 nm produced signals that exceeded the maximum detection limit of the spectrophotometer (≥ 3 Abs). Through statistical analysis, the detection wavelength at λ = 450 nm was selected. The signal produced at λ = 450 nm showed lower deviation and better dosage-dependent response to Cu.

The tests on PEG, polyol, THF, and MDI with distilled water and Cu solutions (1.0 ppm and 10.0 ppm) showed that PEG didn't cause significant increase in OD at λ = 450 nm with all the analytes. Furthermore, THF precipitated after the contact with analytes, while high hydrophobicity of MDI prevented the use of spectrophotometry tests. The test on polyol however showed significant increase in OD (Average increase of 6.79% in 1 ppm and 18.43% in 10 ppm of Cu solutions), thus the reaction of β-carotene containing polyol to Cu was confirmed.

C. Determination of Optimum Exposure Time

By taking the OD readings in 15mminute intervals for a duration of 60 minutes, the response curves of the biopolymer to 0.01 ppm, 0.10 ppm, 1.00 ppm, and 10.00 ppm Cu were obtained (Fig. 4). The OD increase at 15 minutes of exposure corresponded to the increase of the concentration of Cu, thus the exposure time of the biopolymer was set at 15 minutes.

The biopolymer responded to all Cu solutions by the emulsification of β-carotene, which had been detected using spectrophotometer at λ = 450 nm. In negative control, the OD increased slowly to 13.24% in 60 minutes of exposure to distilled water. With the presence of Cu, the rate of OD increase was higher than in the negative control. The results suggested that the presence of Cu led to the oxidization of β-carotene [29-31] and accelerated the emulsification. However, the real reaction of Cu in the increasing of OD of the biopolymer is yet to be studied.

D. Heavy metals and Al Detection

The biopolymer was tested with different concentrations of Cu, Pb, Zn, and Al. The OD produced by all heavy metals was higher compared to the OD of the control (Fig. 5). The statistical analysis showed the average standard deviations of the responses for Cu, Pb, Zn, and Al were ± 0.21%, ± 1.24%, ± 2.84%, and 0.26% respectively. Low standard deviation indicates high reproducibility, which is an advantage for biosensor application.

In order to estimate linear detection ranges for all tested metals, linear trend-lines were plotted on the scatter graphs. The results for the estimated linear detection range are shown in Table 1.

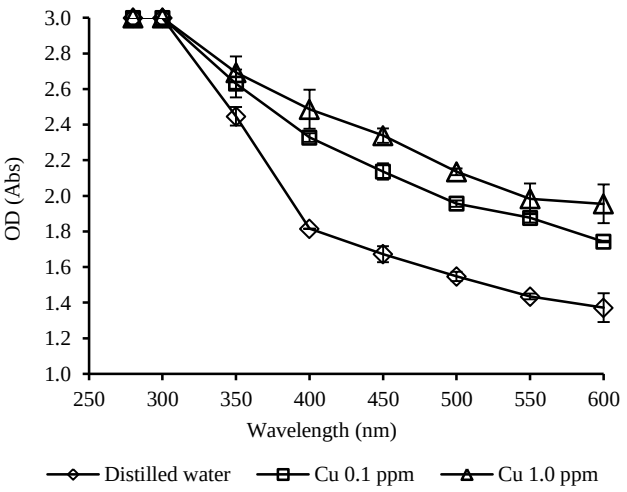

Fig. 3. The OD of the biopolymer with different wavelengths (50 nm interval)

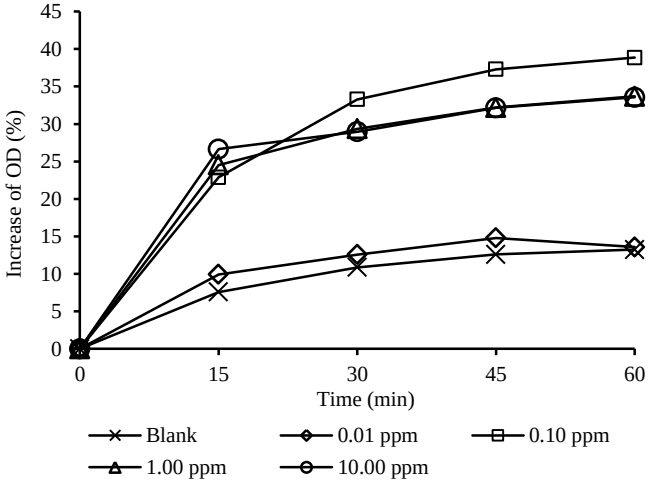

Fig. 4. The increase of OD for 60 minutes of exposure of biopolymer to different concentrations Cu.

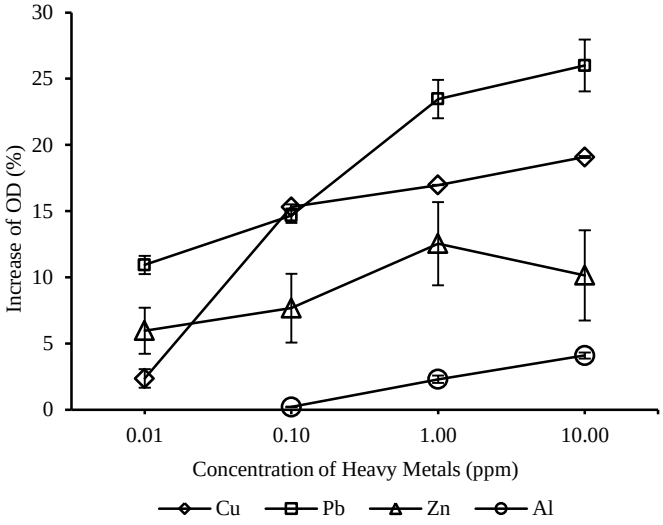

Fig. 5. The increase of OD after 15 minutes of exposure of biopolymer to different concentrations of Cu, Pb, Zn, and Al, compared to the negative control

Tab. 1. The estimated linear detection range for heavy metals and Al

Heavy Metal	Linear Equation	Linear Range (ppm)	r^2
Cu	y = 1.885x + 13.35	0.10 – 10.00	0.9953
Pb	y = 5.402x + 5.26	0.01 – 10.00	0.9554
Zn	y = 3.285x + 2.1567	0.01 – 1.00	0.9280
Al	y = 1.945x - 3.6417	0.10 – 10.00	0.9982

The biopolymer with β-carotene has a high potential to be used in biosensor application. The biopolymer was synthesized with the biological reporter (β-carotene) contained in one of the chemical ingredients (polyol), thus extra step to immobilize the biological reporter as described in many biosensors was no longer required [11, 32-34]. The strong adsorption of the biopolymer to the surface of cuvette ensures that the biopolymer remains intact on the supporting matric for a long period of time. The tests showed the biopolymer had high homogeneity of β-carotene with very low standard deviations compared to a few other biosensors [17, 32, 35].

Liu et at. [36] reported the construction of organic-inorganic hybrid-based biosensor with unique immobilization of organic detection element on silica surface. However, the sensor required complicated fabrication which involved very high temperature in certain steps. The biosensor as described in this work could be fabricated mostly under room temperature. The responses towards heavy metals and Al (considered as light metal) showed the biosensor designed in this work can be potentially used to detect wide range of metals. The working condition under room temperature with pH near to 7 could be an advantage for this biosensor to be used for environmental sample assessment.

This biosensor could be used for qualitative detection for Cu, Pb, and Zn at concentrations of 0.01 mg/L to 10.00 mg/L, and for the detection of Al at concentrations of 0.1 mg/L to 10.0 mg/L. High r^2 values in the estimated linear detection range indicates the possibility of diversification of the biopolymer applications in quantitative detection.

IV. Conclusion

A novel biopolymer-based biosensor has been developed using naturally occurring β-carotene from palm kernel oil as reporter group. The tests showed that the biosensor was sensitive to Cu, Pb, Zn, and Al. The biosensor showed good potential in terms of reproducibility and rapid response to heavy metals and Al. In addition, the presence of β-carotene naturally in palm kernel oil eased the immobilization of biological reporter. The operation of the biosensor with simple one-step detection using simple spectrophotometry equipment was another advantage in the construction of a practical biosensor.

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