Fabrication of a Sensor using Seed Lectin in Voltammetric Detection of Heavy Metals

Elmer-Rico E. Mojica[*], **Florinia E. Merca** and **Rene L. Micor**

[*]Institute of Chemistry
University of the Philippines Los Baños, College, Laguna

This study made use of a seed lectin as biological component of a metal sensor. Red kidney bean (*Phaseolus vulgaris*) seeds that served as source of lectin, were extracted with Tris buffered saline (TBS, 0.01 M in 0.15 M NaCl pH 7.5) solution, followed by ammonium sulfate precipitation (0-90%) and gel chromatography using Sephadex G-150. Hemagglutination assay was used to determine the presence of lectin in the collected gel chromatography fractions. Positive fractions were pooled together and freeze-dried. The freeze dried sample was mixed with carbon powder and Nujol oil to form a paste, which was used to fabricate a sensor to detect different heavy metals in aqueous solutions by electrochemical methods like cyclic voltammetry (CV) and differential pulse anodic stripping voltammetry (DPASV). Among the 6 heavy metals tested, only lead ions showed current signal. Eight percent (8%) lectin modifier composition and 0.1 M HCl as supporting electrolyte gave the optimum and highest signal. Electrodes that were pre-soaked with human erythrocyte solution gave a higher signal for lead ions.

INTRODUCTION

Lectins are a group of proteins/glycoproteins that exhibit distinct sugar specificity and show interaction with specific mono- or oligosaccharides in free or conjugated form (Sharon & Lis 2003; Etzler 1986). These have elicited considerable interest due to a variety of interesting biological properties exhibited such as blood-group specific hemagglutination, mitogenicity, and the ability to distinguish between normal and malignant cells. In some plants, lectins may function as defense proteins whereas in others, they may be responsible for the symbiosis between plant roots and nodulating Rhizobia. With their carbohydrate-binding specificity, they have been widely used in cell separation techniques, in isolation and purification procedures in biochemistry, cell biology, pharmacology, immunology and other related areas (Sharon & Lis 2003).

Lectins are widespread in nature and have been isolated from plants, microorganisms, vertebrates and invertebrates (Lis & Sharon 1986). Although lectins are present in various parts of plants such as seeds, fruits, roots, leaves and bark in most plants, they are relatively far more abundant in the seeds. Legume seeds have been found to be rich sources of lectins and therefore many seed lectins have been isolated, purified, and characterized from species that belong to the Leguminoseae (Lajolo and Genovese 2002; Brinda et al. 2004; Kim et al. 2004)

Studies have shown that lectins can bind to certain metals. The lectin Concanavalin A, derived from jack beans (*Canavalia ensiformis*), was found to bind with Mn^{2+} and Ca^{2+} in addition to sugars (Magnuson et al. 1983). Moreover, being a protein, lectin can contain amino acids that can serve as binding materials with metals. This property of lectins makes them desirable as a biological component of a metal sensor or a chemically modified electrode (CME). A chemically modified electrode is an electrode modified by incorporating chemicals or reagents with chemical and biological modifying moieties to increase its sensitivity and specificity to certain substances.

*Corresponding author: emojica2@buffalo.edu

These types of sensors have been recently introduced for wide application in electrochemistry particularly in the analysis of trace metals (Arrigan 1994). These sensors are more advantageous than standard methods like atomic absorption spectroscopy (AAS) because of their fast analytical response, ease of fabrication, low cost, and suitability for miniaturization (Wang 2000).

But there is limited literature on the use of lectins as biological component for electrochemical electrodes. One study made use of a protein mixture containing lectin from antipolo (*Artocarpus blancoi*) as biological component of a metal sensor that detects mercury and lead ions. Mojica and Merca (2005) utilized lectin from sea cucumber as modifier of CPE, and found the fabricated electrode to be specific only to mercury. This study dealt on the fabrication of a metal sensor containing lectin isolated and purified from red kidney bean (*Phaseolus vulgaris*) seeds. The fabricated sensor was characterized electrochemically by cyclic voltammetry (CV). For application in the detection of heavy metals, differential pulse anodic stripping voltammetry (DPASV) was used.

MATERIALS AND METHODS

Preparation of Crude Extracts
Seeds of red kidney bean (*Phaseolus vulgaris*) were obtained from the public market in Los Baños, Laguna, Philippines. The seeds were soaked in distilled water and then grounded using a blender. The ground sample was then extracted with n-hexane using 1:5 w/v ratio for 3 h at 4°C cold temperature. After defatting with n-hexane, the ground sample was air dried and then extracted with 0.01 M Tris buffered saline (TBS, 0.15 M NaCl pH 7.5) solution at 1:5 w/v ratio. The resulting mixture was homogenized for 60 min, stirred for 24 h at 4°C and filtered using cheesecloth. The filtrate was centrifuged at 10,000 rpm for 20 min. The clarified crude extract (supernatant) was assayed for hemagglutination using human erythrocyte (blood type O).

The crude extract was precipitated at 90% saturation with ammonium sulfate. The resulting precipitate was collected through centrifugation at 10,000 rpm at 10°C for 10 min. The collected precipitate was dissolved in minimum amount of buffer and dialyzed against a dilute solution of TBS using a Sigma dialysis tubing with a molecular weight cut-off of 12,000 at 10°C. Polyethylene glycol was added to concentrate the dialyzed sample.

The concentrated sample was loaded to a Sephadex G-150 column and eluted with 0.01 M TBS pH 7.5

Eluted fractions of 3.0 mL were collected and assayed for hemagglutinating activity. Fractions with hemagglutinating activity were pooled and freeze dried. The homogeneity of the pooled fractions showing agglutinating activity was determined by performing native polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions in a discontinuous polyacrylamide slab gel system following the method of Leammmli (1970). The gel was stained with 0.1% Coomasie Blue R-250 in (5:4:1) water:methanol:acetic acid mixture for 8 h at room temperature followed by destaining the gel for 1 h in destaining solution I (10% acetic acid and 50% methanol) and in destaining solution II (10% acetic acid and 5% methanol), until the band is clearly defined.

Preparation of the Lectin Modified Carbon Paste Electrode
A 100 mg mixture of carbon powder and lyophilized lectin was hand mixed thoroughly with 20 μL mineral oil binder (Nujol) to form a carbon paste. The amount of lectin used was varied from 0-14% by mass composition of the prepared mixture. The metal sensor was fabricated by filling the cavity of a cut straw tip connected to a Cu wire with the carbon paste at the tip. The surface of the electrode was smoothened using a weighing paper.

Voltammetric Analysis
All voltammetric measurements were carried out in a 3-electrode cell (platinum as auxiliary electrode, Ag/AgCl as reference electrode, and the fabricated sensor as the working electrode) connected to Metrohm 693VA processor. The processor is interfaced to a personal computer via a RS232 connection, which converts the generated data into an ASCII format. The data obtained was processed using Microcal Origin version 6. Cyclic voltammetric measurement was performed with the fabricated sensor to evaluate the potential window and reversibility of any redox reaction. Cyclic voltammetry (CV) is the most widely used technique for acquiring qualitative information about electrochemical reactions (Wang 2000).

Differential pulse anodic stripping voltammetry (DPASV) were made on the fabricated sensor before and after preconcentration in different metal solutions (cobalt, cadmium, lead, mercury, copper, zinc and nickel at 100 μg/L) which were prepared by diluting each metal standard solution (1000 μg/L) obtained from J.T. Baker with deionized distilled water. The parameters used for the DPASV on the different heavy metals are the same as the one used by Mojica et al. (2005).

Pre-concentration was done on an open circuit wherein the sensor was dipped in the analyte solution in a cell with constant stirring for 5 min. The voltammetric readings before and after pre-concentration in different analytes were compared. Any peak that was generated after pre-concentration of the electrode is due to the analyte present in the solution.

The amount of purified lectin in the metal sensor and the different supporting electrolytes (HCl, KNO_3 and NaOH) were also tested to obtain the parameters that will give the optimum signal. In addition, the effect of erythrocyte on the binding of metal was also done by pre-soaking the electrodes in an erythrocyte solution before preconcentration with metal.

RESULTS AND DISCUSSION

Isolation of Lectin

A 0-90% saturation with ammonium sulfate was done on the crude extract to remove all undesired proteins, and at the same time to keep the isolated lectin in stable form by preventing possible denaturation. Gel filtration of the crude extract that passed through a Sephadex G-200 column yielded some fractions, which gave positive results when assayed for agglutination. The pooled and freeze-dried positive fractions showed a single band in the PAGE profile indicating the presence of a purified lectin (Figure 1). Although the molecular weight of the isolated lectin was not obtained, the observed PAGE profile is enough to say that the collected lectin is homogenous.

Figure 1. Native polyarylamide gel electrophoresis (PAGE) profile of the pooled positive fractions. A single band was observed in a gel containing 8% acrylamide confirming the purity of the lectin.

Electrochemistry Application

The isolated lectin was then utilized as the biological component of a metal sensor or as a modifier. The electrochemistry of the seed lectin modified metal sensor was examined by cyclic voltammetry (CV) prior to metal pre-concentration. CV is usually used as a pre-conditioning mechanism to remove any extraneous matter incorporated onto the electrode surface. CV showed that the seed lectin modified metal sensor is electroinactive at a potential range of approximately -1000 mV to +1000 mV (Figure 2). It also showed that the modifier had undergone inert redox reaction at minimal extent as exhibited by the broadening of the cyclic voltammogram. Therefore, it can be inferred that any signal observed during voltammetric analysis of lead at potential range of -1000 mV to +1000 mV can be solely attributed to the analyte itself.
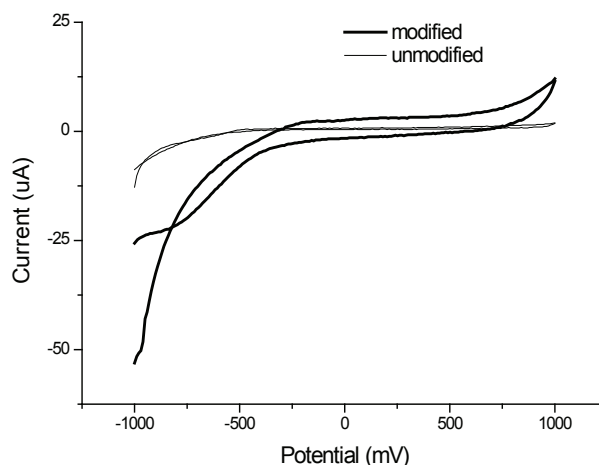

Figure 2. Cyclic voltammogram of metal sensor containing red kidney bean seed lectin at a sweep rate of 50 mV/s at 0.1 M HCl supporting electrolyte.

To determine which metal will bind with the modifier, differential pulse anodic stripping voltammetry (DPASV) was performed. Result of the DPASV with different metal ions using the seed lectin modified metal sensor is shown in Figure 3. No distinct peak was observed in the DPASV of the metal sensor before pre-concentration in different metal solutions. However, a distinct peak was observed at around -500 mV upon pre-concentration with 5 sample solutions containing lead ions (Pb^{2+}). No peak signals or current responses were observed upon pre-concentration of the modified metal sensor in the other sample solutions that contained the other 6 metal ions.

The ability of the seed lectin modified metal sensor to detect lead can be possibly due to the affinity of the lead ions by ion-exchange mechanism or complexation with the different functional groups of the amino acids present in the red kidney bean lectin.
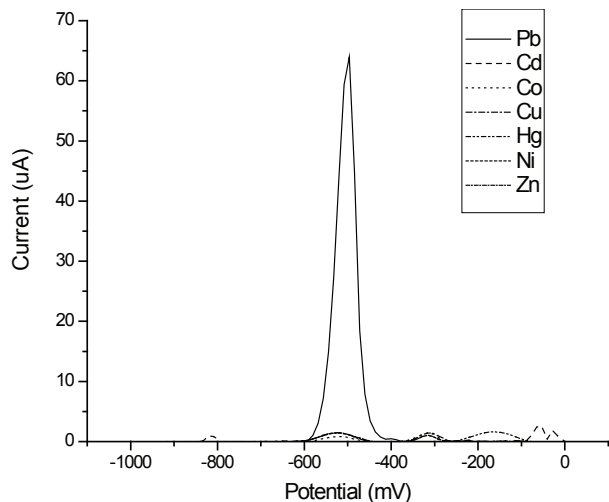
Figure 3. DPASV of the metal sensor at the different metal ions (100 mg L-1) after 5 minutes pre-concentration.

For optimization purposes, the effects of the different amounts of the seed lectin in the metal sensor were determined by varying the compositions from 0 to 14%. The generated current signal was observed to increase from 2% to 8% seed lectin composition (Figure 4). The increase could be due to an increase in the binding sites where the lead ions could attach. However, starting at 10% seed lectin composition, the current signal obtained began to decrease. That decrease could be due to the decreasing amount of the carbon powder, which could affect the current of the metal sensor in terms of conductivity.
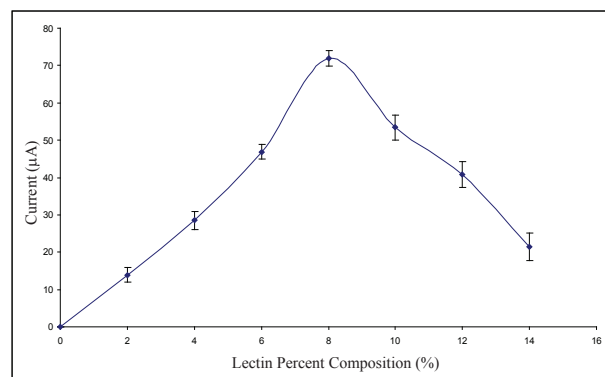

Figure 4. Effect of different concentration of red kidney bean seed lectin in the metal sensor generated signal. (Conditions: 100 mg L 1 Pb ions solution; 0.1 M HCl supporting electrolyte and 5 min pre-concentration)

Another parameter that was optimized was the supporting electrolyte, which is responsible in maintaining good electrical contact between the electrodes in the solution. An acid, base, and a salt solution with 0.1

M concentration were used. Among the 3 supporting electrolyte, hydrochloric acid (0.1 M HCl) gave the sharpest and highest peak at about -500 mV (Figure 5). Hydrochloric acid gave the optimum current response because it is an acid, allowing it to displace lead ions from the binding positions due to its 'protons' ability (Ramos et al. 1993). Since it aids in the stripping of the lead ions on the electrode surface, it enhances the peak current response of the metal sensor.
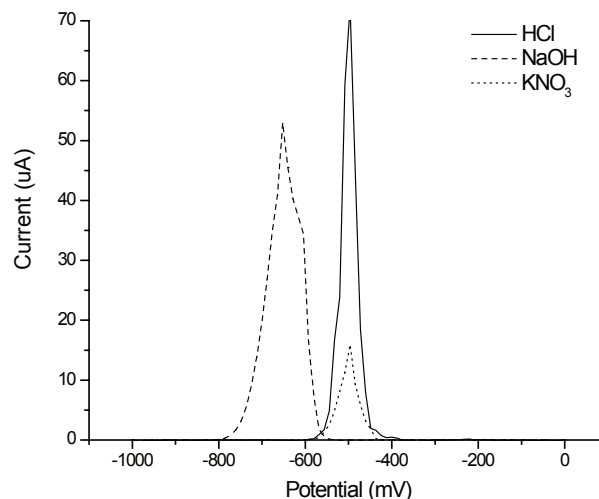

Figure 5. Differential pulse anodic stripping voltammogram of metal sensor containing red kidney bean seed lectin with different supporting electrolyte solutions. (Conditions: 100 mg/L Pb ions solution and 5 min pre-concentration)

Effect of Erythrocyte Solution
The effect of the erythrocyte solution on the seed lectin modified metal sensor was done by soaking the fabricated sensor in a 1% erythrocyte solution (human blood type O), before being used for pre-concentration of lead ions. Result showed that there is an increase in signal (almost twice) for metal sensor that was pre-soaked in erythrocyte solution (Figure 6). These could be due to interaction of the lectin and erythrocyte. The erythrocyte might have altered the lectin in the metal sensor since there is agglutination, and studies have shown that lectin undergoes several morphological changes when it binds with erythrocyte (Anderson and Lovrien 1981; Anderson et al 2002). This agglutination could lead to some conformational changes in the lectin, which could lead to the exposure of more binding sites to lead ions.

Figure of Merits
The relationship between the concentration of lead (II) and peak current was found to be linear in the range of 1 to 5 mg/L (r^2= 0.9743), and in the range of 1 to 10 mg/L
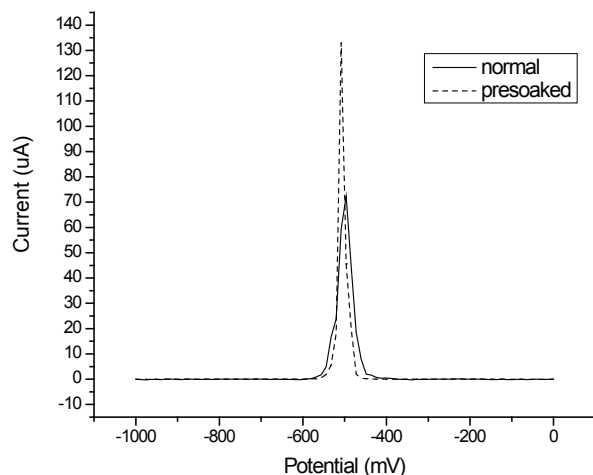
Figure 6. Differential pulse anodic stripping voltammogram of metal sensor presoaked in erythrocyte solution. (Conditions: 100 mg/L Pb ions solution; 0.1 M HCl supporting electrolyte and 5 min preconcentration)

(r^2=0.9905) for sensor pre-soaked in erythrocyte solution. The detection limit (signal to noise [S/N] ratio of 3) was found to be 0.2191 for the normal sensor, while for the pre-soaked sensor the value was found to be 0.1072. Deviations from linear relationship were observed at the concentration higher than the calibration range. This could be due to the possible saturation of the binding sites.

Application
The prepared sensor was used to determine the lead content in laboratory waste sample containing various heavy metals and organic compounds. DPASV using the optimized paramaters showed an average lead content of 4.58 ± 0.78 and 6.59 + 0.34 mg/L (pre-soaked sensor) for the laboratory waste sample. This is lower than the value obtained using the AAS method (14.10 ± 0.03). The discrepancy could be due to the presence of the other components in the sample. The organic compounds may have denatured the lectin in the sensor.

To further test the electrode, a deionized water sample was used and spiked with known concentration of lead ions. No lead was detected in the water sample and spiking it with 5.0 mg/L lead ions gave a mean value of 4.87 ± 0.15 or a percent recovery of 97.4% for the normal sensor, and a mean value of 4.98 ± 0.10 or a percent recovery of 99.6% for the pre-soaked sensor. These were compared with flameless AAS which gave a reading of 5.01 ± 0.05 on the spiked samples or a 100.2% recovery.

SUMMARY AND CONCLUSION

Lectin from red kidney bean (*Phaseoulus vulgaris*) seeds were isolated by extraction with Tris buffered saline (TBS, 0.01 M in 0.15 NaCl pH 7.5) solution, ammonium sulfate precipitation (0-90%), and gel chromatography using Sephadex G-200. The presence of lectin in the collected fractions was detected by hemagglutination assay. Positive fractions were pooled together and freeze-dried. The freeze-dried fractions were then mixed with carbon powder and Nujol oil to form a paste, which was then used to fabricate a sensor that successfully detected lead ions among the 7 heavy metals tested. An 8% lectin content of the sensor and the use of 0.1 M HCl as supporting electrolyte gave the optimum signal. In addition, soaking the sensor in human erythrocyte solution improved the obtained signal.

LITERATURE CITED

ANDERSON RA and LOVRIEN RE. 1981. Erythrocyte membrane sidedness in lectin control of Ca2^{+}-A23187 mediated discocyte/echinocyte conversion. Nature 292:158-161.

ANDERSON RA, PAQUETTE S and LOVRIEN R. 2002. Lectin-erythrocyte interaction with external transmembrane glycophorin saccharides controlling membrane internal cytoskeleta. J Agric Food Chem 50:6599 – 6604.

ARRIGAN DWM. 1994. Voltammetric determination of trace metals and organics after accumulation at modified electrodes. Analyst 119:1953-1966.

BRINDA K, MITRA N, SUROLIA A and VISHVESHWARA S. 2004. Determinants of quaternary association in legume lectins. Prot Sci 13: 1735-1749.

ETZLER ME. 1986. Distribution and function of plant lectins. In: The Lectin. Properties, Functions and Applications in Biology and Medicine. Liener IE, Sharon N, Goldstien IJ (eds.) Orlando, FL: Academic Press. p. 371–435.

KIM BS, OH KT, CHO DH, KIM YJ, KOO WM, KONG KH and KIM HH. 2004. A sialic acid-binding lectin from the legume *Maackia fauriei*: comparison with lectins from *M. amurensis*. Plant Sci 167: 1315-1321.

LAJOLO FM and GENOVESE MI. 2002. Nutritional significance of lectins and enzyme inhibitors from legumes. J Agric Food Chem 50: 6592-6598.

LIS H and SHARON N. 1986. Applications of lectins and biological properties of lectins. In: The Lectins. Properties, Functions and Applications in Biology and Medicine Liener IE, Sharon N, Goldstein IJ (eds.) USA: Academic Press, Inc. p. 265-370.

MAGNUSON JA, ALTER GM, APPEL DM, CHRISTIE DJ, MUNSKE GR and PANDOLFINO ER. 1983. Metal ion binding to concanavalin A. J Biosci 5: 9-17.

MOJICA ERE and MERCA FE. 2005. Anodic stripping voltammetric determination of mercury (II) using lectin-modified carbon paste electrode. J Appli Sci 5:1461-1465.

MOJICA ERE, TOCINO AB, MICOR JRL and DEOCARIS CC. 2005. A feather-trode sensor for detecting lead ions. Philipp J Sci 134: 51-56.

RAMOS JA, BERMEJO E, ZAPARDIEL A, PEREZ JA and HERNANDEZ L. 1993. Direct determination of lead by accumulation at a moss-modified carbon paste electrode. Anal Chim Acta 273:219-227.

SHARON N and LIS H. 2003. Lectins, 2nd ed. Kluwer Academic Publishers. p. 5-14.

WANG J. 2000. Analytical Electrochemistry, 2nd ed. USA: VCH Publishers. p. 94-100.