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Development and characterization of an amperometric biosensor using Metallothionein for identification and quantification of lead (Pb²⁺) ions

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Lead accumulation in the body can cause severe damage to human health, particularly affecting the nervous system in young children. Hence, determining the amount of lead in drinking water is useful to prevent accumulation in body, which leads to lead poisoning. It is noteworthy that a large fraction of the population that is affected by lead contamination in drinking water lives in the developing world. Hence, this research focuses on the development of an amperometric biosensor to quantify Pb²⁺ ions. The protein metallothionein (MT) was used as the Pb²⁺ recognition molecule in the biosensor. MT was isolated from *Staphylococcus warneri* TWSL_6 strain and physically adsorbed onto paper discs. The loading volume of MT was optimized to be 6 μ l from 7.8 μ g/ μ l MT solution. The MT-loaded paper discs were placed on the electrode system which consists of Ag/AgCl reference electrode, and two graphite paste electrodes as working and counter electrode. The metal ion-MT binding will cause the space-charge in the aqueous solution to move closer to the sensor because of changes in the protein conformation. This system was tested with 1 μ M to 1000 μ M concentrations of Pb²⁺ in 10 mM KCl as the supporting electrolyte. The cyclic voltammograms of the samples were obtained by applying potential from -1 V to +1 V and the current produced at -0.43 V was used for the calibration plots. Thus, the amperometric transducer detects this change directly. There was a clear increase in current for the analysis of Pb²⁺ when carried out with MT-loaded discs. The calibration plots were studied under three sub-series. The same electrode set-ups were used for each sub-series throughout the experiment. The Pb²⁺ concentrations ranging from 650 μ M to 1000 μ M gave a linear trend ($R^2=0.97$) with a slope of 5×10^{-9} A/ μ M for MT-loaded paper discs. The slope and the linearity (R^2) were determined to be 2×10^{-8} A/ μ M and 0.98, respectively for Pb²⁺ concentration series in 10 mM KCl medium ranging from 50 μ M to 500 μ M with MT-loaded paper discs. The sensitivity of the device is 2×10^{-7} A/ μ M with an R^2 of 0.85 in the Pb²⁺ concentration series in 10 mM KCl medium ranging from 1 μ M to 50 μ M. Hence, the LOD value of the developed biosensor is 5.24 μ M and the LOQ value is 17.45 μ M. It can be concluded that metallothionein can be used successfully for the Pb²⁺ ion detection in amperometric biosensors.

Keywords: Amperometric paper-based biosensor, metallothionein, lead (Pb)

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