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A miniaturized optical biosensor for the detection of Hg^{2+} based on acid phosphatase inhibition

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ABSTRACT

In the present study, a simple low cost and portable optical biosensor has been fabricated for the detection of Hg^{2+} based on inhibition of acid phosphatase activity. Poly-dimethoxysiloxane (PDMS) sensor chip module containing reaction and detection wells was fabricated. Acid phosphatase from the seeds of *Macrotyloma uniflorum* was immobilized at the bottom of reaction well to carry out the enzymatic reaction using *p*-nitrophenyl phosphate as a synthetic substrate. The detection was based on the measurement of transmitted light intensity through the yellow coloured solution of *p*-nitrophenol (λ_{405}) liberated as a result of enzymatic reaction and was measured in terms of volts. The optical system was successfully employed for the detection of Hg^{2+} based on inhibition of enzyme activity. Response of the sensor was found to be linear in the range of 0.01–10 mM. The biosensor was stable up to 20 days of storage at 4 °C without any appreciable loss in activity.

1. Introduction

Heavy metals, even in small concentrations, are one of the most serious environmental pollutants worldwide because of their non bio-degradable nature that can lead to severe health hazards [1]. Numerous uses of metals in metal plating factories, mining industries, tanning, dye and chemical manufacturing industries, etc., are major sources of environmental pollution [2]. Mercury, lead, chromium, cadmium, copper and zinc are amongst the most frequently occurring metal contaminants and mercury is known to be the highest threat for the environment as well as for human health. Mercury occurs in elemental, inorganic and organic form. Elemental Hg, liquid at room temperature volatizes readily and is rapidly distributed in body through vapours, but is poorly absorbed. Exposure to inorganic mercury can result in dermal toxicity whereas, organic mercury, being lipid soluble, gets absorbed via gastrointestinal track, lungs, skin and can cross placenta and into breast milk [3]. Consequently, environmental awareness is growing among consumers and industrialists while legal constraints on emissions, both at national and international levels, are becoming increasingly strict [4].

In this context, reliable, efficient and cost-effective wastewater treatment technologies are needed for monitoring of heavy metal pollutants that adversely affect human health. Various sophisticated analytical techniques like inductively

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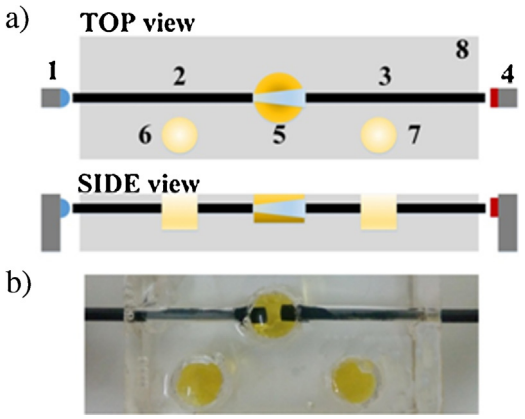

Fig. 1. (a) Schematic presentation of top and side view of PDMS sensor chip (indicated with number 8) used for the Hg^{2+} detection. Numbers 1 and 4 indicates light source and the detector. Number 5 indicates detection well. Optical fibers used to launch light from the source to the detection well and the one to collect transmitted light from the detection well to the detector are indicated with numbers 2 and 3. Acid phosphatase was immobilized in the reaction well as indicated with numbers 6 and 7 on the PDMS chip. (b) Photograph of fabricated PDMS sensor chip.

coupled plasma mass spectrometry, cold vapour atomic absorption spectrometry, UV visible spectrophotometry and X-ray absorption spectroscopy are being routinely used for heavy metal analysis [5–8]. The preciseness in measurements offered by these techniques are shadowed by the disadvantages of high cost, the need for trained personnel and bulkiness. These disadvantages limit their use only in the laboratory. Therefore, there is a demand for portable along with low cost and selective sensors [9–12].

Herein, a low cost, portable biosensor has been developed for the detection of Hg^{2+}, based on acid phosphatase inhibition.

2. Experimental

2.1. Materials

PDMS was procured from Dow Corning Europe SA. Acid phosphatase was purified from the seeds of *Macrotyloma uniflorum*. All other chemicals were purchased from Sisco Research Laboratory India, Hi-Media and Merk India and were used as procured. All solutions were prepared in De-ionized water unless otherwise mentioned.

2.2. Fabrication of biosensor

Acid phosphatase inhibition based biosensor for the detection of mercury was fabricated in our laboratory (Fig. 1) that involved: (a) Fabrication of PDMS sensor chip module containing micro-reactor well to carry out enzymatic reaction and the detection well for placing optical fibers in order to measure the transmitted light intensity through the reaction solution (Fig. 1); (b) Immobilization of acid phosphates in the detection well of the sensor chip

A special type of sensor chip having detection well of 120 μl capacity and reaction well of 200 μl capacity was fabricated by using PDMS (Fig. 1a). Yellow colored solution was added into the wells in order to visualize wells in PDMS chip (Fig. 1b). Plastic optical fibers were placed within the PDMS chip for transmission and collection of transmitted light through the solution in the well and indicated with numbers 2 and 3 respectively (Fig. 1a). Optical fibers were aligned exactly face to face so as to get maximum transmission intensity in the form of voltage. Length of optical fibers used on both sides of the PDMS chip was 4 cm each. Distance between the two optical fibers within the detection well was kept constant to 0.8 mm (optimized) as measured by digital micrometer screw gauge. Light was introduced in to the detection well through one of the optical fibers (indicated by 2 in Fig. 1a) using LED having λ_{max} of 410 nm to match with maximum absorption of p-NPP (solution under test). Second optical fiber (indicated by 3 in Fig. 1a) was used to collect transmitted light through the well and transmit it to the photo-detector (200–1100 nm, SFH 350, Siemens) indicated with number 4 in Fig. 1a. Reaction mixture was placed in the detection well and transmission intensity through the reaction mixture was measured in the form of voltage.

PDMS was chosen as a base material for fabrication of sensor chip firstly because of its highly hydrophobic nature that will facilitate easy cleaning of the detection well in which aqueous based solutions are to be used; secondly its chemical inertness towards chemicals to be used in the proposed experiment. Separate reaction and detection wells were fabricated. The detection well (with embedded optical fibers) can be washed with distilled water to be readily used repeatedly (the present well was used at least 200 times and can be used further experimentation). Since in the reaction well, the enzyme gets inactivated by Hg^{2+}; it cannot be reused immediately. The reaction well after first use needs to be cleaned thoroughly before immobilization of the fresh enzyme. The process of immobilization being time consuming, it is always better to have multiple reaction wells ready for large number of sample analysis. In the present experiments, reaction and detection wells were made on the same PDMS chip module for the compactness and miniaturization.