Zno Nanoparticle Based Isfet Biosensor for the Detection of Mercury (Ii) Ions

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ABSTRACT
Alkaline phosphatase (AP) based ion sensitive field-effect transistor (ISFET) biosensor was investigated by the incorporation of ZnO nanoparticles for the detection of heavy metals. Enzyme was immobilized on SiO_2 surface of electrode by cross-linking a mixture of AP and bovine serum albumin in saturated glutaraldehyde vapour. The proposed biosensor was optimized for characteristics like substrate concentration and cell density. Enzyme dephosphorylates substrate 2-Phospho-L-ascorbic acid trisodium salt (PAA) to generate current equivalent to ascorbic acid (AA) whereas the presence of mercury would hinder the formation of AA, which indirectly corresponds to the concentration of metal ion present. The electrode responds linearly in concentration range of 10^{-12} M to 10^{-3}M for mercury and showed rare interference of silver, alkali metals, alkaline earth metals and transition metals. The results obtained by the ZnO NPs modified ISFET transducers suggested that incorporation of NPs strongly influenced the biosensor performances as they increase sensitivity and also improves the detection limit.

Keywords: ISFET biosensor, Alkaline phosphatase, ZnO nanoparticles, Hg(II) determination

1. INTRODUCTION

Mercury (II) is a perilous metal that has pulled in much consideration in ecological and toxicological domain. Even at low concentration, it can cause numerous disorders of central nervous system, fatal minamata disease and kidney failure [1-3]. Current main approaches for mercury determination are atomic emission spectrometry [4] or ultraviolet spectrophotometry [5] or HPLC etc, which provide satisfactory results but are not cost effective and are time consuming. An alternative to these methods is biosensor technology, which has perceived potential advantages over existing techniques. One of the potential strengths of biosensor is that it is a selective detection method, which gives bioavailable content. It is also cost effective, reduces analysis time and no skilled trainer is required.

Among a variety of biosensors, ion-selective field-effect transistor (ISFET) integrated with biomaterial are receiving great attention in the field of detection of pesticides. ISFET biosensors has semiconductor structures that offer numerous advantages such as rapid response, small size, low output impedance, highly reliable, on-chip integration of biosensor arrays and portable microanalysis system [6].

In the present work, inhibition based microbial ISFET biosensor was fabricated. Chlorella sp. with surface bound active alkaline phosphatase enzyme was immobilized on the Ta_2O_3 electrode surface using bovine serum albumin and glutaraldehyde as crosslinker for the detection of Hg^{2+} ions. The major problem with the proposed system was immobilization that decreases permeability for H^+ ions (key analyte) in the detection of pesticide. To overcome this hurdle, ZnO nanoparticles (NPs) was incorporated in the immobilized matix. ZnO NPs are best suited as they are semiconductor in origin, thus possess a high specific surface area, high stability, high conductivity and being non-toxic, an excellent candidate for bio sensing properties.

Thus, in the proposed biosensor, alkaline phosphatase dephosphorylates 2-phospho-L-ascorbic acid to ascorbic acid, hence producing H^+ ions producing equivalent current to its concentration. Presence of Hg^{2+} would deactivate the enzyme; hinder the production of H^+ ions that would indirectly relate to pesticide concentration.

2. EXPERIMENTAL

2.1. Algal culture

Chlorella sp. was identified, grown and sub-cultured in BG-11 [7] media after every 20 days. AP is a surface bound enzyme, so, to induce its maximum activity, it was starved in phosphate free BG-11 media for 15 days, before immobilizing it on the surface of electrode.

2.2. Chemicals

2-Phospho-L-ascorbic acid trisodium, bovine albumin serum (BSA), $MgCl_2$, mercury nitrate, zinc acetate, Silver nitrate, magnesium sulfate, sodium nitrate and gluteraldehyde were of analytical grade and purchased from Sigma-Aldrich, India and used without further purification. Hydrochloric acid, potassium hydroxide, ammonia solution (25%, GR grade) were obtained from Loba Chemie, India. Stock solutions of mercury and other metal ions were prepared in the concentration range 10^{-12} M to 10^{-1} M in water.

2.3. Instruments

All experiments were performed on ISFET pH sensor model no. 3330, Agilent U8001A single output DC power supply (0-30 V, 3A) and Agilent U1233A True RMS Multimeter. Powder X-ray diffraction (XRD) patterns for ZnO nanoparticles were obtained using a

XPERT-PRO diffractometer (Cu Kα, λ = 0.15406 nm, 45 kV, 40 mA, step size = 0.013°). The size and shape of ZnO was analyzed by TEM (JEOL JSM-6510LV). Sample for TEM was prepared by dropping sample powder onto standard carbon coated copper grid. Fourier transform infrared (FTIR) studies were examined using Agilent Resolutions Pro (Cary 660) by preparing KBr pellets in volume ratio of 1:100 (sample:KBr).

2.4. Synthesis of ZnO

ZnO nanoparticles were prepared from $Zn(acac)_2$ salt precursor and NaOH. 100 mL solution of 0.2 M metal salt precursor was put on stirring for 15 minutes at 60^0C. Sodium hydroxide (0.5 M) solution was heated upto 60^0C. Hot sodium hydroxide solution (100 mL) was added to $Zn(NO_3)_2$ solution with continuous stirring within one minute. The reaction mixture was allowed to stir at 60^0C for 1 hour. The reactor was then allowed to cool at room temperature without stirring. White colored precipitates of ZnO began to form in mother liquid. The solution was left for overnight and washed several times with water to remove impurities. Prior to each washing, 15 minutes of ultra-sonication was done for proper washing. After washing, the precipitates were dried in air oven at 80 ^{0}C [8].

2.5. Biosensor preparation

15µL of 3 mg per mL of ZnO NPs were placed on Ta_2O_3 electrode and air-dried at room temperature. Then, 10 µL of 1mL solution of the starved algae (6 million cells per mL) with 7.5% (w/v) BSA was deposited on the surface of ZnO/Ta_2O_3 electrode by drop method [9]. The electrode was then placed in glutaraldehyde for crosslinking of amines provided by BSA for about 30 minutes, followed by drying for 40 min at room temperature. After drying, the immobilized electrode was washed with distilled water before further experiments.

2.6. ISFET study

The pH sensitivity of Ta_2O_5 ISFET (V_{gs}) was biased at 0.70V to obtain drain-source current corresponding to H^+ ions formed during the enzymatic reaction. The study was performed by dipping Ta_2O_5 ISFET immobilized electrode in solution containing $MgCl_2$ (10^{-3} M) acting as an enzyme activator and 2-Phospho-L-ascorbic acid (PAA) trisodium salt as a substrate. Enzyme alkaline phosphatase acts upon the substrate PAA (20 mM) converting it into ascorbic acid (AA) thus producing H^+ ions which produces current equivalent to its concentration. The role of ZnO NPs in proposed biosensor can be explained on the basis of their highly conductive nature and most importantly being nano-porous in nature. These properties help to enhance the active surface area for strong adsorption for effective immobilization of biomolecule (Chlorella sp. algae) as well as the permeability of the immobilized membrane.

For Hg^{2+} measurement, immobilized electrode was first incubated into solution containing different concentration of mercury (10^{-11} M to 10^{-3} M) for 10 mins and then dipped in the reaction solution containing 20 mM of substrate PAA to study the response. The current generated in experimental setup was compared with the control set (i.e., in the absence of Hg^{2+} ions), thus, the percentage inhibition was calculated by following formula:

$I\% = (I_o - I)/ I_o \times 100$

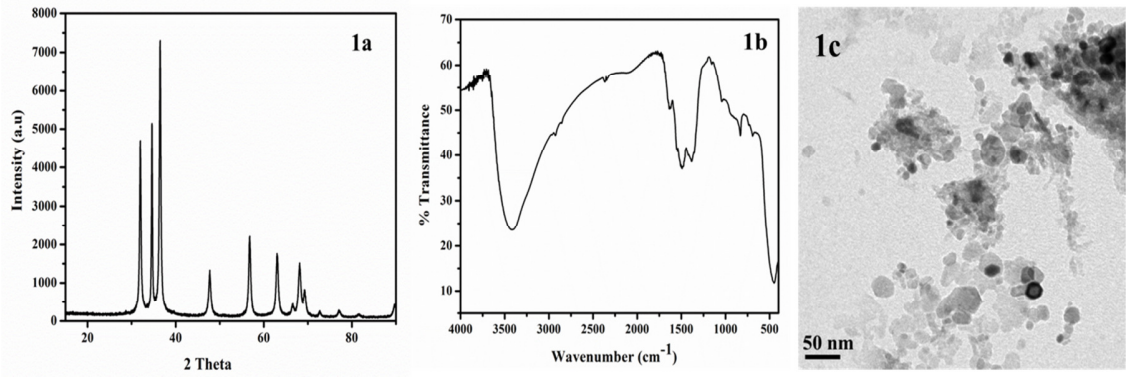
Where, I_o represents the peak current of control, I is peak current after pesticide inhibition. All the experiments were done under nitrogen atmosphere and at room temperature.

3. RESULT AND DISCUSSION

3.1. Structural and morphological analysis of ZnO nanoparticles

The synthesized ZnO nanoparticles were characterized using XRD, TEM, and FT-IR techniques, which are explained below:

XRD pattern of the powder material was studied with diffraction angle 15° - 80° as shown in Fig 1a. The diffraction peaks for 2θ = 31.71°, 34.40°, 36.42°, 47.58°, 56.84°, 63.12°, 66.52°, 68.12°, 69.18°, 72.58°,



and 77.08° were observed, which are matching with the

ZnO hexagonal phase of JCPDF No. 36-1451. The crystallite size of ZnO, determined using Scherrer formula is found to be ~ 20 nm.

FT-IR spectra of ZnO NPs are shown in Fig. 1b. The absorption peaks at 470 cm^{-1} corresponding to Zn–O vibrations, suggest the presence of ZnO NPs. Absorption peak observed at 3433 cm^{-1} is due to O–H stretching vibrations. In addition, the small intensity peaks at 1636, 1482 and 1429 cm^{-1} were assigned to COO^- ions, which were difficult to remove completely even after intense washing.

The morphology and the grain size of ZnO NPs were elucidated from TEM image as shown in Fig. 1c. It is apparent that nano ZnO particles appeared as dark black spheres against light background. The grain size is estimated to be ~ 20 nm.

Fig.1. (a) XRD pattern (b) FT-IR spectrum (c) TEM of ZnO nanoparticles.

3.2. Heavy metal determination

Detection principle: Alkaline phosphatase (AP) is a metalloenzyme containing Mg^{2+} and Zn^{2+} ions and a number of amino acids like histidine, aspartate, glutamate, serine and cysteine [10]. Hg^{2+} ions inhibit enzyme activity by irreversibly binding to the cysteine residue of enzyme. The –SH group present in cysteine would acts as soft base and Hg^{2+} as soft acid, forming stable metal enzyme complex. Moreover, mercury also has higher tendency to form back bonding with nitrogen of amino acids present in enzyme. Thus, Hg^{2+} ions have greater degree of inhibition as compared to silver, alkali metals, alkaline earth metals and transition metals.

4. CONCLUSION

An Algal/ZnO/Ta_2O_5- ISFET biosensor is developed for the determination of Hg^{2+} ions based on inhibition of alkaline phosphatase enzyme present on the surface of algae Chlorella sp. with sensitivity in the concentration range of 10^{-12} - 10^{-3} M. Hg^{2+} interacts with cysteine residue, which causes enzyme inhibition and form the basis of its determination. It was found that the proposed biosensor can detect mercury ions

Interfering species	Change in current percentage	
	10^{-4}M	10^{-5}M
Zn (II)	+1.26	+1.23
Fe (II)	+0.85	+0.87
Mg (II)	-2.09	-2.18
Ag (I)	-2.01	+2.11
Na (I)	+1.66	+1.59

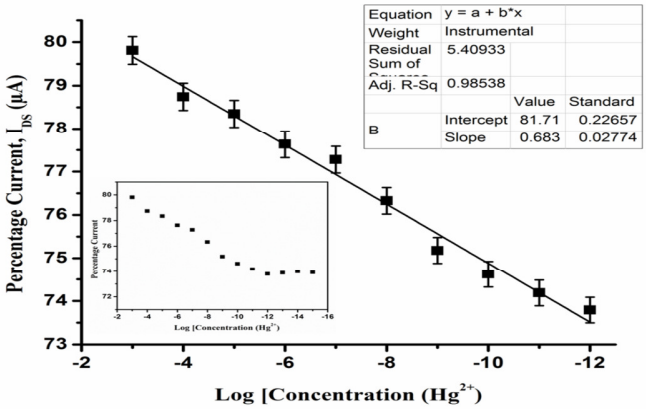
With optimized conditions for enzyme activity, the algal immobilized Ta_2O_5 ISFET electrode was used for.

mercury determination. The electrode responded linearly in the concentration range of 10^{-12} - 10^{-2} M as shown in Fig 2. Silver ions are most potent interferent in determination of Hg^{2+}, so inference study was done by taking equal amounts of silver of different concentrations (1×10^{-4} M and 1×10^{-5} M) were added to 1×10^{-4} M of Hg^{2+}solution in the reaction setup and the change in current magnitude was measured. Other metal ions such as Zn (II), Fe (II), Mg (II), and Na (I) were also studies and are reported in Table 1. It can be seen that no noticeable change was noticed in any of the cases. Thus, proposed biosensor is selective to Hg^{2+} and showed rare interference of silver, alkali metals, alkaline earth metals and transition metals. Lower detection limit (LOD) for Hg^{2+} was determined as 1.2×10^{-12} M, which was calculated from following equation [18]: DL= 3 S/ b, where, S represents standard deviation of blank solution and b is slope of the calibration curve.

Interference study on the working of algal immobilized Ta_2O_5 ISFET electrode in the presence of interference species

.2. Curve of the percentage current (I_{DS}) vs concentration of mercury ions

even in the presence of silver, alkali metals, alkaline earth metals and transition metals.



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