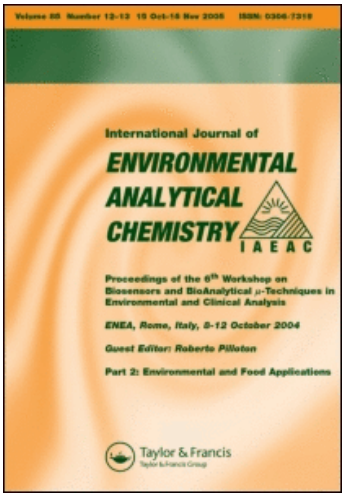

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Surface plasmon resonance-based DNA biosensor for arsenic trioxide detection

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Surface plasmon resonance-based biosensor has been fabricated using self-assembled monolayer (SAM) of β-merceptoethanol (MCE) deposited on gold (Au) substrate. The double stranded *calf thymus* deoxyribonucleic acid (dsCT-DNA) has been immobilised onto MCE/Au electrode using *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide (EDC/NHS) chemistry. This dsCT-DNA/MCE/Au bioelectrode has been characterised using cyclic voltammetry, scanning electron microscopy (SEM) and X-ray photoelectron spectrometry (XPS), respectively. The detection limit of arsenic trioxide has been estimated as $0.01 \times 10^{-6}\,\mathrm{g\,mL^{-1}}$.

Keywords: surface plasmon resonance; β-merceptoethanol; self-assembled monolayer; DNA; arsenic trioxide

1. Introduction

The increased use of heavy metals over the past few decades has resulted in the release of toxicants in our environment. Besides this, industrial waste including arsenic, cadmium, copper, lead and mercury are presently the major source of pollution. Among these, arsenic has been considered by WHO as a serious toxicant present in drinking water. The systemic and chronic exposure to arsenic leads to various serious disorders such as vascular diseases including hypertension, irritation of skin and mucus membrane as well as determatitis, etc. And injection of inorganic arsenic increases the risk of developing bladder liver, kidney and skin, cancer [1]. Arsenic reagents have been used as anticancer agents in traditional Chinese medicine [2].

Arsenic trioxide (As_2O_3), a novel chemotherapeutic agent treating acute promyelocytic leukaemia (APL), as well as other arsenic agents, induces apoptosis of tumour cells with dependence on the cellular redox state. Long-term clinical trial studies have shown that arsenic trioxide is very effective in the treatment of several types of leukaemia including acute promyelocytic leukaemia. Mouron *et al*. [3] have evaluated the degree of damage induced by cadmium and arsenic salts in a human cell line using comet assay. The classical methods for the analysis of metals (arsenic, cadmium, copper, iron, etc.) such as atomic absorption spectroscopic, ion chromatography and mass spectrometric techniques are carried out in laboratories after sampling [4]. These techniques are both expensive

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and time-consuming. In this context, biosensors have been considered to yield cost-effective and faster response for the detection of genotoxicants.

DNA biosensors have recently been proposed for water quality monitoring, food analysis and for clinical diagnosis. The detection of genotoxicants using DNA biosensor has become possible because of its faster response and easy portability [5]. A variety of DNA biosensors based on conducting polymers, self-assembled monolayers (SAMs) and graphite have been developed using physical adsorption, entrapment and covalent binding technique for detection of toxicants [5,6]. Conducting polypyrrole (PPy)-polyvinyl sulfonate (PVS) film-based DNA biosensor has been reported for detection of *o*-chlorophenol and 2-aminoanthracene, 3-chlorophenol, malathion, chlorpyrifos using physical adsorption [5] and electrochemical entrapment [7–11], respectively. Ferancova *et al*. [12] have developed carbon nanotubes-based modified electrode for studies relating to the interaction of tin (II) and arsenic (III) compounds using cyclic voltammetry and obtained response time as about 10 min. Ozsoz *et al*. [2] have reported interaction of As_2O_3 with *calf thymus* double-stranded DNA (dsDNA), *calf thymus* single stranded DNA (ssDNA) and 17-mer short oligonucleotide using differential pulse voltammetry (DPV). Carbon paste electrode (CPE)-based DNA biosensor has been found to have detection limit of 1 ppm [2]. However, these thick multilayer-based biosensors have limited sensitivity and cannot be used under harsh experimental conditions. Further, it has been observed that reaction kinetics of the immobilised biomolecules in a thick polymer film may result in sluggish transport of charge carriers within a polymer matrix [13]. It may be mentioned that physical adsorption technique has several disadvantages such as leaching, long incubation time, non-reproducible results during washing. And, electrochemical entrapment technique for immobilisation of DNA requires large amount of biomolecules resulting in increased cost, etc. It may be noted that covalent method of immobilisation is often useful since the covalently coupled biomolecules on desired substrates including SAMs, are likely to result in improved, reusable and reproducible biosensor response.

Self-assembled monolayers are known to provide suitable matrices for the immobilisation of biomolecules. This has been attributed to high reproducibility, molecular level control, orientation, distribution and direct electron transfer [14]. The carboxyl (–COOH) terminal of SAMs has been used for DNA immobilisation and for hybridisation detection by surface plasmon resonance (SPR) spectroscopy [15]. Zhao *et al*. have reported covalent immobilisation of dsDNA onto different terminal groups of SAMs on gold substrates through layer-by-layer self-assembly [16]. Single stranded oligonucleotides have been immobilised on 6-mercapto-1-hexanol SAM [17] and on SAM of 2-aminoethanolthiol (AET) on gold surface using 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) [18].

Surface plasmon resonance is a simple and direct surface-sensitive analytical technique based on monitoring of change in the refractive index (RI), occurring in close vicinity of a thin metal film surface. This technique can be used to fabricate an SPR biosensor for real time measurement of biomolecular interactions without any labelling [19]. We report results of studies relating to application of the SPR technique to monitor binding of double stranded *calf thymus* deoxyribonucleic acid (dsCT-DNA) with EDC/NHS on hydroxyl terminated (OH) terminal SAM of β-merceptoethanol (MCE) on gold electrode for investigation of interaction of dsCT-DNA with arsenic trioxide. The dsCT-DNA/MCE/Au bioelelctrode characterised by cyclic voltammetry, XPS, SEM, FTIR has been utilised for detection of arsenic trioxide (As_2O_3) using SPR.

2. Experimental

2.1 *Chemical and reagents*

Double *stranded calf* thymus DNA (dsCT DNA) was obtained from Bangalore Genei Pvt. Ltd., India. Arsenic trioxide (AS_2O_3) was procured from Loba Chemie PVT. Ltd., India. Gold-coated BK-7 glass plates (24 mm diameter) were procured from Autolab, Netherlands. MCE, NHS, *N*-(3-dimethy-l-aminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDC), *tris* base, ethylene diamine tetraacetic acid (EDTA), potassium monohydrogen, potassium dihydrogen phosphate were purchased from Sigma-Aldrich, USA. All chemicals were of molecular biology (MB) grade. Deionised water (Milli Q 10 TS) was used for the preparation of reagents and the solutions and glasswares were autoclaved prior to being used.

2.2 *Preparation of self-assembled monolayer on gold substrate*

Prior to the SAM preparation, gold plates were cleaned in 'piranha' solution (7 : 3; concentrated H_2SO_4 : 30% H_2O_2, respectively) followed by thorough rinsing with distilled water, a final rinse with the absolute ethanol. These cleaned gold plates were immersed into 1 mM ethanol solution of MCE compounds for 24 h at room temperature. The monolayers of OH group were obtained on gold substrate. These SAM modified electrode were rinsed with the ethanol to get rid off unbound compound.

2.3 *Activation of DNA and covalent binding on MCE–Au electrode*

The phosphate group of the double stranded *calf thymus* DNA (1.0 mg mL^{-1}) is activated with 100 mM of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) and 200 mM of NHS for the 24 h at room temperature. The dsCT-DNA covalently binds with MCE/Au electrode by forming phosphate ester bond between OH group of MCE/Au electrode and phosphate group of DNA [20,21].

This activated dsCT-DNA is immobilised onto MCE/Au electrode using SPR technique (Figure 1). During experiments, 120 s are given for the base line correction with deionised water followed by addition of 60 μL of dsCT-DNA into the channels and kept for 7200 s at 25°C.

2.4 *Characterisation of SAM of MCE*

Surface plasmon resonance technique has been used for the immobilisation of dsCT-DNA and detection of arsenic trioxide using an Autolab SPR, Eco Chemie (Netherlands) based on the traditional Kretschmann configuration. The linearly *p*-polarised from a laser (670 nm) is directed through prism onto the gold electrode. The intensity of reflected light as a function of time has been measured over a range of 4000 millidegrees. In the experiments, gold-coated glass electrode is coupled with the plane face of the prism via index matching fluid. All experiments are carried out at 25°C. FT-IR and UV–Vis spectroscopy studies have been conducted using Shimadzu, 160A and Perkin Elmer BX, respectively. Cyclic voltammetric studies have been carried out on an Autolab Potentiostat/Galvanostat from the Eco Chemie (Netherlands) using a three-electrodes system. XPS measurements have been carried out on a Perkin–Elmer (Phi) Model 1257 system, working at a base pressure of 5×10^{-10} Torr.

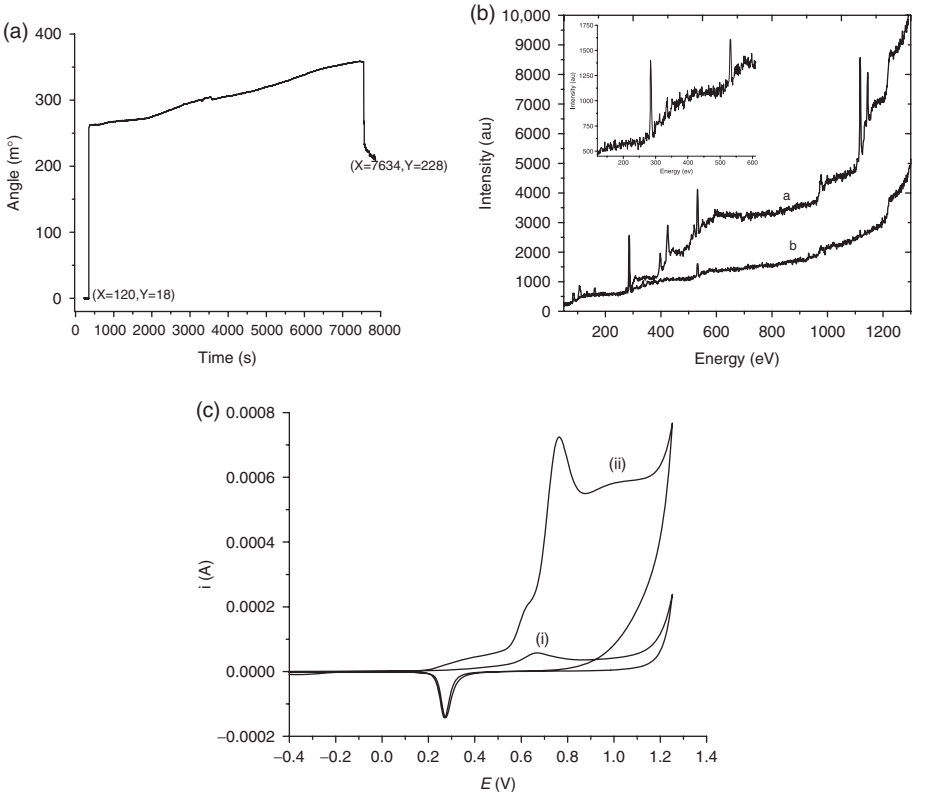


Figure 1. (a) SPR curve for dsCT-DNA binding onto MCE/Au electrode; (b) XPS spectra: (a) MCE/Au electrode, (b) dsCT-DNA/MCE/Au bioelectrode; (c) Cyclic voltammograms of (i) MCE/Au electrode and (ii) dsCT-DNA/MCE/Au bioelectrode in phosphate buffer (50mM, pH 7.0) in the range of −0.5 to 1.3V at 20 mV s^{-1} Scan rate.

3. Results and discussion

3.1 *Covalent binding of dsCT-DNA onto MCE/Au electrode using SPR technique and characterisation*

The covalent binding of dsCT-DNA onto MCE/Au electrode has been done using SPR technique (Figure 1(a)). Prior to immobilisation of the dsCT-DNA onto MCE/Au electrode, the base line is corrected for about 120 s during the first phase of the experiment. After the completion of 120 s the MCE/Au electrode is inserted in the SPR cuvette. For binding, dsCT-DNA solution (60 μL) is run onto MCE/Au electrode for about 7200 s at 25°C during the association phase. After completion of the association phase, dsCT-DNA/MCE/Au electrode is washed with auto-claved deionised water during the dissociation phase (100 s) to remove any unbound dsCT-DNA from the MCE/Au electrode. Figure 1(a) shows the steady increase in binding of dsCT-DNA onto MCE/Au electrode. The amount of dsCT-DNA binding is calculated by the change in the SPR angle obtained before and after the completion of the dissociation phase. The change in SPR is obtained as about 210.0 millidegrees on MCE/Au electrode). Using the *ESPRIT* kinetic evaluation software, it is found that 14.87 ng of dsCT-DNA is bound to the MCE–Au

electrode over a reaction spot (8.5 mm^2). The results of control experiments carried out under similar conditions with dsCT-DNA onto MCE/Au electrode (i.e. without EDC/NHS activation) do not result in any change in the angle (millidegrees) (data not shown) revealing that EDC/NHS activation plays important role in the covalent binding of DNA to the MCE/Au electrode. Results of various SPR experiments repeated at least four times reveal similar amount of DNA bound to the MCE-SAM/Au electrode.

The surface morphologies of MCE/Au and dsCT-DNA/MCE/Au bioelectrodes have been investigated using scanning electron microscopy (Supplementary data). The surfaces of MCE/Au electrode and dsCT-DNA/MCE/Au bioelectrode have been characterised using XPS technique (Figure 1(b)). In the XPS spectra of MCE–Au SAM, the observed peaks at 83, 162, 286 and 531 eV for gold, sulfur, carbon and oxygen reveal the formation of MCE/Au (i). In the case of dsCT-DNA/MCE/Au bioelectrode, XPS peaks at 84, 287, 531, 134 and 408 eV can be correlated to gold, carbon, oxygen, phosphorus and nitrogen, respectively. The additional peaks at 134 and 408 eV obtained for the dsCT-DNA/MCE/Au bioelectrode (ii) indicate the presence of DNA onto MCE/Au electrode. The inset in Figure 1(b) is the XPS spectra of dsCT-DNA/MCE/Au bioelectrode (curve (ii)) in the range, 100–600 e/V.

Cyclic voltammetric studies have been conducted for monitoring the redox potential of MCE/Au electrode and dsCT-DNA/MCE/Au bioelectrode in phosphate buffer (50 mM, pH 7.0) in the range of −0.5 to 1.3 V. Figure 1(c) shows cyclic voltammogramms indicating oxidation current of about 7.4×10^{-5} A for bare gold (curve (i)) and 7.2×10^{-4} A for the dsCT-DNA/MCE/Au bioelectrode (curve (ii)). The increased oxidation current is attributed to the guanine oxidation of DNA indicating immobilisation of DNA onto MCE/Au electrode.

3.2 *SPR and cyclic voltammetric response of dsCT-DNA/MCE/Au bioelectrode*

The response studies of dsCT-DNA/MCE/Au bioelectrode have been observed using both SPR technique as well as cyclic voltammetry as a function of arsenic trioxide concentration.

In the SPR system, firstly the base line of the dsCT-DNA/MCE/Au bioelectrode is monitored with the auto-claved deionised water for about 120 s. After the base line correction, dsCT-DNA/MCE/Au bioelectrode is subject to different concentrations of arsenic trioxide for about 900 s during the association phase. After the completion of the association phase, the dsCT-DNA/MCE/Au bioelectrode is washed with auto-claved deionised water for about 120 s to remove any unbound arsenic trioxide from the dsCT-DNA/MCE/Au bioelectrode (dissociation phase). The control experiments (curve (i) Figure 2(a)) have been conducted using the 0.0×10^{-6} g mL^{-1} (only deionised water) of arsenic trioxide. Figure 2(a) shows variation in SPR angle as the arsenic trioxide concentration increases (0.01, 1.0, 2.5, 5.0, 7.0 and 10.0×10^{-6} g mL^{-1}), indicating interaction of As_2O_3 with the DNA molecules on the dsCT-DNA/MCE/Au bioelectrode. Figure 2(b) exhibits the variation of SPR angle obtained from Figure 2(a) and log of arsenic trioxide concentration. It has been found that change in SPR angle can be expressed as:

Change in SPR Angle = 50.53 + 58.31 (ln concentration of Arsenic Trioxide) Equation (1).
This dsCT-DNA/MCE/Au bioelectrode has the value of sensitivity as 58.31 $m^{\circ}\,cm^{-2}$ with linear regression of variation as 0.9991.

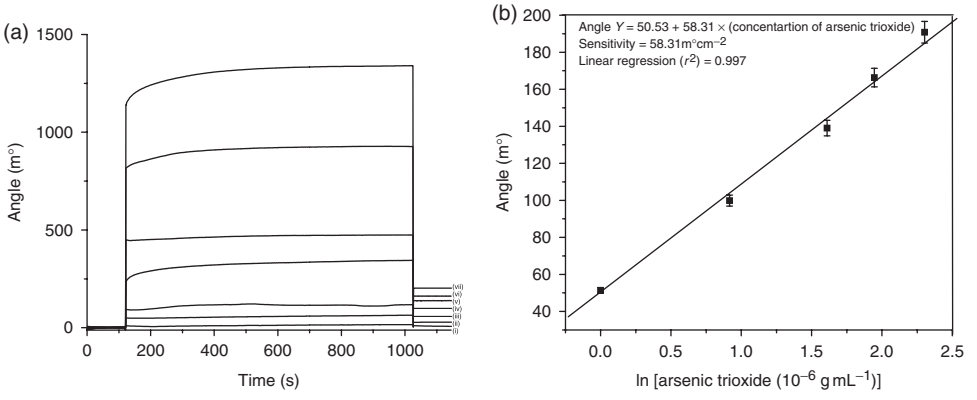


Figure 2. (a) Variation in SPR signal as a function of arsenic trioxide concentration [(i) H_2O (ii) $0.01 \times 10^{-6}\,g\,mL^{-1}$, (iii) $1.0 \times 10^{-6}\,g\,mL^{-1}$, (iv) $2.5 \times 10^{-6}\,g\,mL^{-1}$, (v) $5.0 \times 10^{-6}\,g\,mL^{-1}$, (vi) $7.0 \times 10^{-6}\,g\,mL^{-1}$, (vii) $10.0 \times 10^{-6}\,g\,mL^{-1}$] in freshly prepared dsCT-DNA–MCE–Au bioelectrode (b) variation in the change in the SPR angle obtained from (a) with arsenic trioxide concentration.

Figure 3 shows the current linear plot of the oxidation peak of the dsCT-DNA/MCE/Au bioelectrode obtained using cyclic voltammetry as a function of arsenic trioxide concentration at scan rate of $20\,mV\,s^{-1}$ in phosphate buffer (50 mM, pH 7.0). It is observed that guanine oxidation current decreases with increasing concentration of arsenic trioxide (0.01×10^{-6}, 0.5×10^{-6}, 1.0×10^{-6}, 2.5×10^{-6}, 5.0×10^{-6}, 7.0×10^{-6}, $10.0 \times 10^{-6}\,g\,mL^{-1}$) within 30 s. Beside this, it is found that guanine oxidation peak area decreases linearly with increase in the arsenic trioxide concentration ($0.01–10.0 \times 10^{-6}\,g\,mL^{-1}$), indicating about 95% reduction at $10 \times 10^{-6}\,g\,mL^{-1}$ arsenic trioxide concentration. The response time and detection limit for arsenic trioxide have been obtained as 30 s and $0.01 \times 10^{-6}\,g\,mL^{-1}$, respectively.

Change in current = 47.39 + 19.47 (ln concentration of Arsenic Trioxide) Equation (2).
This dsCT-DNA/MCE/Au bioelectrode has the value of sensitivity as $19.48\,\mu A\,cm^{-2}$ with linear regression of variation as 0.996.

The observed change in SPR angle (Figure 2(b)) and decrease in the DNA oxidation current (Figure 3) indicate the interaction of arsenic trioxide with the dsCT-DNA moieties of dsCT-DNA/MCE/Au bioelectrode, and it has been reported that the small molecules (arsenic trioxide) usually have two possibilities for interaction with dsCT-DNA. Firstly, the electrostatic interaction may occur between As_2O_3 and negatively charged sugar phosphate backbone. Secondly, the interaction may arise between the NH_2 group of nitrogenous bases (guanine) and oxygen of As_2O_3 [2] (Scheme 1).

3.3 *Interaction of arsenic trioxide with dsCT-DNA/MCE/Au bioelectrode*

To delineate the interaction of As_2O_3 with DNA, UV–Visible and FT-IR measurements have been undertaken in the solution phase.

FTIR and UV–Vis spectroscopic studies of blank DNA and on addition of arsenic trioxide conducted in the *tris* buffer (10 mM)-EDTA (1 mM) at (pH 8.0) have been conducted. Figure 4(a) exhibits results of the UV–Visible studies carried out using DNA and DNA + arsenic trioxide. On addition of arsenic trioxide into the dsCT-DNA solution,

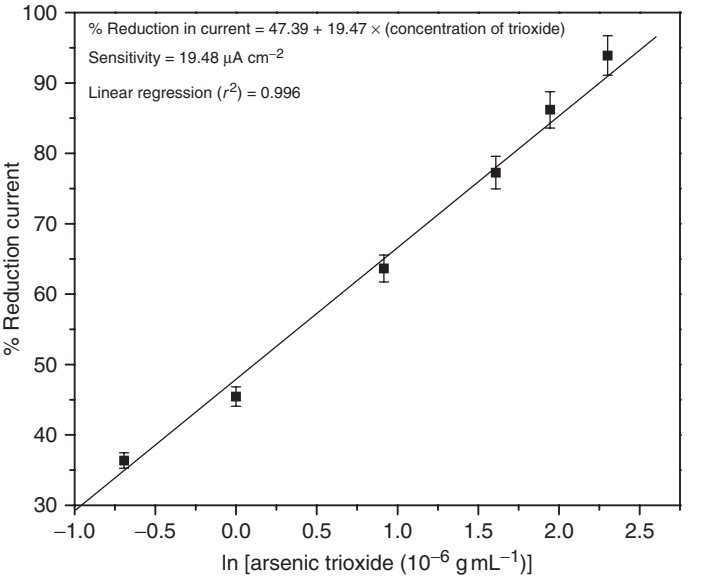
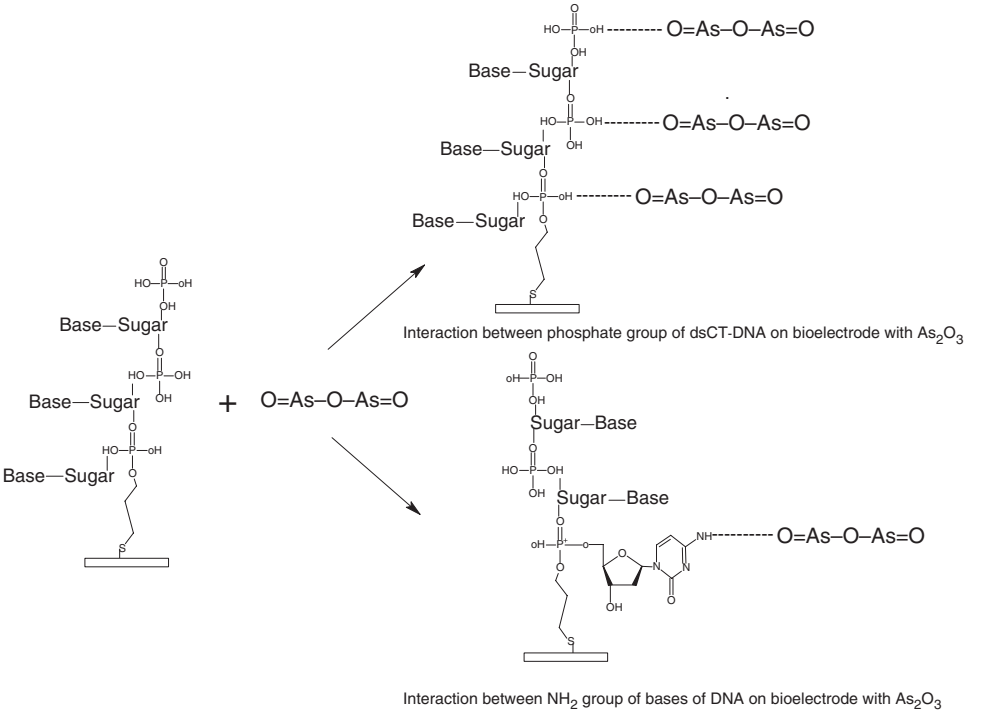


Figure 3. A linear plot of percentage current reduction obtained from cyclic voltammetric as a function of in [arsenic trioxide concentration] ($0.01–10 \times 10^{-6}$ g mL^{-1}).



Scheme 1. Interaction of dsCT-DNA/MCE/Au bioelectrode with As_2O_3.

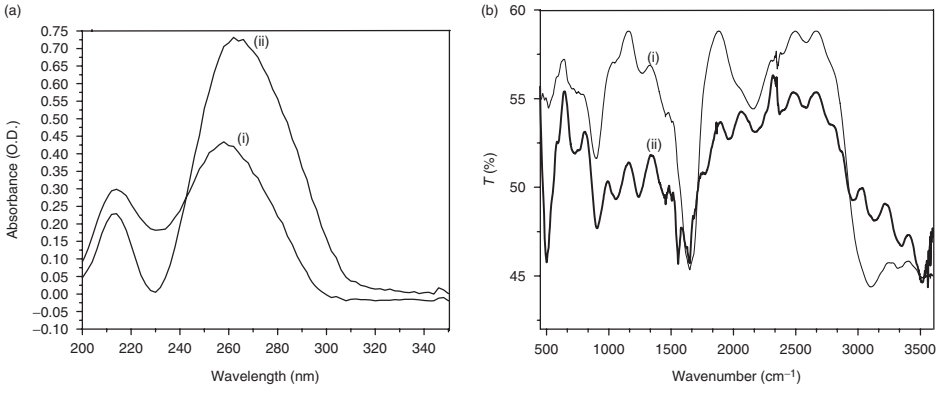


Figure 4. (a) UV–Visible spectra of (i) dsCT–DNA, (ii) dsCT–DNA + arsenic trioxide and in *tris* (10 mM)-EDTA (1 mM) (pH 8.0); (b) FTIR spectra of (i) dsCT-DNA, (ii) dsCT-DNA + arsenic trioxide and in *tris* (10 mM)-EDTA (1 mM) (pH 8.0).

the magnitude of absorption peak increases with shift of the peak towards higher wavelength, resulting in the bathochromic shift. This is attributed to the formation of complex between arsenic trioxide and dsCT-DNA molecule. And there is also a possibility of interaction of phosphate group and the bases of dsCT-DNA molecule with oxygen of As_2O_3 (Scheme 1).

Figure 4(b) exhibits FTIR spectra of blank dsCT-DNA and dsCT-DNA+ arsenic trioxide in *tris* buffer [*tris* (10 mM)–EDTA (1 mM) (pH 8.0)]. The dsCT-DNA peaks are obtained at 480, 600, 900, 1064, 1260, 1460, 1652, 2155, 2585, 3120 and 3333 cm^{-1} (curve (i)). The peaks seen at 1652 and 3108 cm^{-1} are specific for N–H stretching and binding, respectively. In the case of interaction of As_2O_3 with DNA, the magnitudes of DNA peaks at 1652 and 3108 cm^{-1} decrease indicating interaction of As_2O_3 with DNA bases (curve (ii)) and the absorption peak seen at 1546 cm^{-1} is due to hydrogen bonding between NH_2 group of guanine and oxygen of As_2O_3. The 718 cm^{-1} peak pertains to the formation of P–O bond due to interaction of the phosphate group of DNA and oxygen of As_2O_3 (Scheme 1).

It may be remarked that dsCT-DNA/MCE/Au electrode is not presently selective for arsenic trioxide and can be utilised for initial screening of drinking and wastewater.

4. Conclusions

We have covalently immobilised dsCT-DNA onto MCE/Au electrode. This bioelectrode has been characterised using SEM, cyclic voltammetry and XPS, respectively. The dsCT-DNA/MCE/Au bioelectrode has been utilised for detection of 0.01×10^{-6} g mL^{-1} of As_2O_3 concentration. The mechanism of interaction of the As_2O_3 with dsCT-DNA has been proposed. This device can be used for initial screening of genotoxicants including other heavy metal oxides and pesticides etc. Efforts should be made to develop geno-electrodes based on whole cells for selective detection of desired toxicants.

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