Chapter II
Materials & Methods

A brief sketch of the materials and methods used in the investigations is presented in the chapter. The general methods for the fabrication of the modified electrodes are described in this chapter. Details about the general regents and instruments used in the investigations are also given in this chapter.

2.1 Reagents

The reagents and solvents used were of analytical grade and were procured from local vendors. Double distilled water was used throughout the studies. Guanine, adenine, thymine, cytosine, dopamine, uracil, ascorbic acid, uric acid and ortho nitrobenzaldehyde were analytical grade chemicals which were obtained from Sigma Aldrich (USA). Potassium ferricyanide, graphite powder, cobalt chloride, cobalt nitrate, copper sulphate, ferrous ammonium sulphate, tin (II) chloride, potassium chloride, sodium nitrate, sodium fluoride, monosodium dihydrogen phosphate, disodium hydrogen phosphate and sodium hydroxide were purchased from Merck and all chemicals were used as such without any further purification. All other reagents used for this studies were obtained from Merck, India. DNA samples from calf thymus were purchased from Himedia, India. Prior to electrochemical experiments, the solutions were deaerated with high purity nitrogen and maintained under nitrogen atmosphere during experiments.
2.2 Instruments used

2.2.1 Electrochemical studies

![Photograph of Electrochemical Workstation](image)

**Fig. 2.1 Photograph of Electrochemical Workstation**

All the electrochemical measurements were performed using CHI 760C (Fig. 2.1) electrochemical workstation (USA) interfaced with a PC. A conventional three electrode system, consists of working electrode, counter electrode and reference electrode was employed. Working electrode used was aluminium electrode (Al) modified with suitable chemical modifications, counter electrode used was platinum and calomel was used as the reference electrode. All voltammograms were recorded in the polarographic mode. The pH measurements were carried out in an Elico pH meter.

2.2.2 Surface characterization studies

The morphology, elemental composition and phase composition of the modified aluminium electrode coatings have been analyzed by Scanning electron
microscopy (SEM), Energy dispersive X-ray spectroscopy (EDAX), Atomic Force Microscopy (AFM) and X-ray diffraction analysis.

The surface morphology and nanostructure of the coatings were analyzed by (A Hitachi SU-70 and JEOL JSE 5600)/ Field Emission Scanning Electron Microscopy (JSM 840A Scanning Electron Microscope, JEOL-Japan). The SEM also had an energy dispersive X-ray spectrometer (EDS), attachment, using which quantitative analysis of the elemental composition of the coatings was performed. Atomic force microscopy (AFM) was used to analyze the coatings in semi contact mode using NT-MDT model solver Pro M and Veeco, Santa Barbara, USA. The phase composition and the crystallinity of the modified electrode were determined by X-ray diffraction (XRD, Bruker D-8 Advance-Germany) with a CuKα radiation λ=1.5406Å generated at 35 kV and 25 mA. Data were collected over the 2θ range 20-80° with a step size of 0.010° and a count time of 0.2 s.

2.3 Pretreatment of Aluminium electrode

2.3.1 Solvent degreasing

The mechanically polished aluminium specimens were degreased with acetone. The specimens were wiped in a single direction with cotton choked in acetone to remove organic impurities such as oil, grease, dirt etc.

2.3.1 Alkaline cleaning

The degreased aluminium specimens were alkaline cleaned in 5% (w/v) NaOH solution at 30°C for 2 min, to remove the naturally formed non-uniform oxide film on its surface.

2.3.2 Washing/Rinsing

After alkaline cleaning the specimens were thoroughly washed with running tap water and rinsed with deionized water to remove the excess alkaline cleaning solution.
2.3.3 Desmutting

Desmutting was carried out by immersing the specimens in 25% (V/V) HNO₃ at 30°C for 1 min in order to remove the smut (insoluble oxides other than aluminium oxide).

2.3.4 Chemical polishing

Chemical polishing was carried out by immersing the aluminium specimens in the bath containing 75 % phosphoric acid, 20 % sulphuric acid and 5 % nitric acid for 5 minutes at 90°C. Then the specimens were washed in hot water thoroughly and then rinsed in deionised water.

2.4 Fabrication of chemically modified aluminium electrodes

2.4.1 Fabrication of tin nanorods/AAO modified aluminium electrode

Tin nanorods (SnNR) modified aluminium electrode was prepared in two steps: the first step involved electrochemical synthesis of ordered AAO by anodization and in the second step, the SnNR were fabricated on AAO template by electrochemical deposition method.

An aluminium sheet (purity 99.99%, thickness 0.3 mm, area 1 cm²) was used as substrate for the preparation of AAO template. To obtain highly ordered nano pore arrays on AAO, a two-step anodization process was employed. The primary step anodization was carried out by DC power supply at 40 V in 0.3 M H₂C₂O₄ at room temperature for 2 h using Al as anode and graphite as cathode. Then the produced alumina layer was eroded by immersing in a solution of phosphoric acid (6.0 wt.%) and chromic acid (1.8 wt.%) at 60°C. The secondary step anodization was carried out to obtain a regular array of AAO at below 5°C keeping other conditions similar to that used for the primary step anodization. In order to ensure the
electrochemical deposition, the thickness of barrier layer was reduced by decreasing the anodizing voltage systematically with 2.0 V/min intervals after the anodization was finished [1]. After anodization, the pores in AAO were then widened by etching in 5.0 wt.% phosphoric acid for 30 min and then washed with de-ionized water.

Fabrication of the tin nanostructures was carried out by DC electrodeposition in a simple two-electrode electrochemical cell consists of the AAO template as cathode and lead plate as the anode using an electrolyte containing 7.0 g/L SnCl₂ and 25.0 g/L C₆H₁₇N₃O₇. The current density was adjusted to 0.5 mA/cm² and to facilitate the mass transfer, the electrolyte was agitated by a magnetic stirrer.

The schematic diagram of the entire process of formation of the modified electrode was represented in Fig. 2.2.

![Schematic diagram](image)

**Fig. 2.2 Schematic illustration for formation of tin nanorods.**

2.4.2 Fabrication of CoOx nanoflowers modified aluminium electrode

An aluminium sheet (purity 99.99%, thickness 0.3 mm) was used as a substrate for the preparation of CoOx nanoflowers. Before electrodeposition, the aluminium was annealed at 450°C for a half an hour and etched in 5% sodium hydroxide for 2 min to remove the native barrier layer followed by rinsing in distilled water. Prior to electrodeposition, electrode was dipped in phosphoric acid for 5 min to bump off the oxide layer.

The electrodeposition of cobalt oxide was performed in a standard three-electrode glass cell at 20 °C, using the aluminium electrode as working electrode, a
saturated calomel electrode (SCE) as the reference electrode and Pt foil as the counter electrode. The CoOx films were electrodeposited from aqueous solution containing 0.6 M Co(NO$_3$)$_2$ and 0.05 M NaNO$_3$ using a CHI 760c Electrochemical Workstation. The electrodeposition was carried out at a constant potential of -1.0 V vs calomel electrode for various times. After the electrochemical deposition, the electrode was washed with deionized-water, dried at 85 °C and then annealed at 250°C in an air oven for 1 h in order to transform Co(OH)$_2$ into Co$_3$O$_4$.

2.4.3 Fabrication of Fe$_3$O$_4$-Graphite modified aluminium electrode

An aluminium sheet (purity 99.99%, thickness 0.3 mm) was used as substrate for the preparation of Fe$_3$O$_4$-graphite nanoparticles. The electrodeposition of Fe$_3$O$_4$-Gnp on Al electrode was performed in a standard three-electrode glass cell with the aluminium electrode as working electrode, a saturated calomel electrode (SCE) as the reference electrode and Pt foil as a counter electrode. The precursor films were electrodeposited from aqueous solution containing 3.93 g/L ferrous ammonium sulphate and 5.44 g/L sodium acetate using a CHI 760c electrochemical Workstation. The graphite powder (20-100 g/L) with size less than 10 μm was added to the bath.

The cathodic electrodeposition of Fe$_3$O$_4$/graphite composite films on aluminium was carried out at 90 °C for various time durations. The deposition potential was -1.2 V vs calomel electrode. After the electrochemical deposition, the electrode was washed with deionized-water and dried at room temperature.

2.4.4 Fabrication of Cobalt hexacyanoferrate modified aluminium electrode

The CoHCF modified electrode was prepared by electroless method. For this purpose, an aluminium sheet (purity 99.99%, thickness 0.3 mm) was used as
substrate and it was annealed at 450°C for half an hour and degreased with acetone. Then the Al electrode was chemically polished in a mixture of concentrated sulfuric, nitric and phosphoric acids.

After getting the mirror like surface of aluminium, the deposition of cobalt was carried out by electroless method. The cleaned surface of electrode was immersed in the solution containing 1 M CoCl₂ saturated by sodium fluoride. The thickness of cobalt enclosed on the electrode surface depends on the immersion time of the electrode in the plating solution and the concentration of CoCl₂ (in this work the plating time was varied from 1 to 5 min). The aluminium electrode enclosed by metallic cobalt was engrossed in 0.1 M KCl and 10 mM K₃[Fe(CN)₆] as the derivatization reagent, for chemical transformation of metallic cobalt to CoHCF film.

2.4.5 Fabrication of Copper oxide modified aluminium electrode

After getting the mirror like surface of aluminium, the deposition of copper was carried out by electrodeposition method. The cleaned surface of electrode was dipped in the solution containing CuSO₄ (0.5 M) and H₂SO₄ (0.3 M) at room temperature for various times. The electrodeposition of CuO on Al was performed in a standard three-electrode glass cell with the aluminium electrode as working electrode, a saturated calomel electrode (SCE) as the reference electrode and Pt foil as a counter electrode at -0.2 V. The thickness of copper enclosed on the electrode surface depends on the deposition time of the electrode in the plating solution and the concentration of copper sulphate in the solution (plating time was varied from 1 to 5 min).
The aluminium electrode enclosed by metallic copper was annealed in an air for an hour at various temperatures. After annealing the modified electrode, metallic copper was converted into a copper oxide and used for electrocatalytic oxidation of ascorbic acid and dopamine. The total surface concentration of the catalyst in the film per unit surface area of the electrode ($I'_0$) was determined from the area under the anodic cyclic voltammograms of the Copper oxide coated Al electrode.

2.5 Electrochemical measurements

2.5.1 Electrochemical characterization of SnNR/AAO/Al

Cyclic voltammetry, electrochemical impedance spectroscopy and differential pulse voltammetry (DPV) were performed using CHI 760c (CH Instrument Inc., USA) electrochemical workstation. This instrument uses the latest analog and microcomputer design to provide high performance, better precision and greater versatility in electrochemical measurements. This instrument was employed for performing voltammetric studies.

A conventional three-electrode system was used with platinum disk act as a counter electrode, calomel as the reference electrode and modified aluminium as the working electrode. During electrochemical measurements, electrons travel from the Al/alumina-Sn/solution interface and through the alumina to the Sn nanorods that are electrically connected to the aluminium plate. For cyclic voltammetry experiments, the potential was scanned between -1.6 and -0.2 V at a scan rate of 100 mV/sec in 0.25 M phosphate buffer. The electrochemical impedance spectroscopy (EIS) experiment was performed in the presence of 5.0 mM $K_3[Fe(CN)_6]$ as a redox probe prepared in 0.1 M KCl. The impedance was measured in the frequency range from 100 MHz to 100 kHz at open circuit potential of -0.3 V vs. calomel reference electrode with voltage amplitude of 5 mV. DPV experiments were performed in the potential range from -1 to -0.3 V for the selective determination of ONB at the pulse
amplitude of 50 mV and scan rate of 100.0 mV/sec. All experiments were performed at room temperature.

2.5.2 Electrochemical characterization of CoOx/Al

Cyclic voltammetry, electrochemical impedance spectroscopy and differential pulse voltammetry (DPV) were performed using CHI 760c (CH Instrument Inc., USA) electrochemical workstation. A conventional three-electrode system was used with platinum disk as a counter electrode, calomel as the reference electrode and modified aluminum as the working electrode. For cyclic voltammetry experiments, the potential was scanned between 0.5 and 1.1 V at a scan rate of 100 mV/sec in 0.25 M phosphate buffer. The electrochemical impedance spectroscopy (EIS) experiment was performed in the presence of 5.0 mmol/L K₃[Fe(CN)₆] as a redox probe prepared in 0.1 mol/L KCl. The impedance was measured in the frequency range from 100 MHz to 100 kHz vs. calomel reference electrode with a voltage amplitude of 5 mV. DPV experiments were performed in the potential range from 0.5 to 1.2 V for the selective determination of guanine at the pulse amplitude of 50 mV and a scan rate of 100.0 mV/sec. All experiments were performed at room temperature.

2.5.3 Electrochemical characterization of Fe₃O₄-Gnp/Al

The electrodeposition of Fe₃O₄-graphite nanoparticles and electrochemical measurements were performed with a CHI 760c electrochemical workstation (CH Instrument Inc., USA). The accumulation procedures was carried out at the potential of 0.3 V for 240 s. After a quiet time of 2 s, the DPV measurements were performed in 0.25 M PBS (pH 7.0) with different concentrations of adenine or guanine or their mixtures by scanning from 0.4 to 1.2 V. The DPV parameters are as follows: pulse
amplitude 0.1 V, pulse width 0.05s and pulse period 0.2 s. CV was conducted in 0.1 M pH 7.0 PBS containing adenine and guanine with different scan rates. The electrode can be used for the next measurement after a continuous sweep for three cycles at the same potential range in the buffer solution and repeating the above assay procedure.

2.5.4 Electrochemical characterization of CoHCF/Al

The electrochemical analyzer from CHI instruments Model 760 was employed for various electrochemical studies performed. For cyclic voltammetry experiments, the potential was scanned between 1.1 and 0 V at a scan rate of 50 mV/s in 0.1 M phosphate buffer. The electrochemical impedance spectroscopy (EIS) experiments were performed in the presence of 5.0 mM K₃[Fe(CN)]₆ as a redox probe prepared in 0.1 M KCl. The impedance was measured in the frequency range from 100 MHz to 100 kHz at open circuit potential of -0.3 V vs. calomel reference electrode with voltage amplitude of 5 mV and the respective semicircle diameter corresponds to charge-transfer resistance (Rct). All experiments were performed at room temperature. DPV experiments were performed in the potential range from 1.0 to 0.5 V for the selective determination of guanine at the pulse amplitude of 50 mV and scan rate of 100.0 mV/sec.

For all electrochemical experiments cobalt hexacyanoferrate (CoHCF) modified aluminium was used as a working electrode. Saturated calomel electrode was used as reference electrode in this investigation. A platinum disk was used as a counter electrode. Pt electrode was cleaned successively with dilute detergent solution, isopropyl alcohol and sodium hydroxide solution. Finally it was rinsed with distilled water.
2.5.5 Electrochemical characterization of CuO/Al

Cyclic voltammetry, electrochemical impedance spectroscopy and differential pulse voltammetry (DPV) were performed using CHI 760c (CH Instrument Inc., USA) electrochemical workstation. A conventional three-electrode system was used with platinum disk act as a counter electrode, calomel as the reference electrode and CuO modified aluminum as the working electrode. For cyclic voltammetry experiments, the potential was scanned between -0.5 and 1.2 V at a scan rate of 100 mV/sec in 0.25 M phosphate buffer. The electrochemical impedance spectroscopy (EIS) experiment performed in the presence of 5.0 mM \( K_3[Fe(CN)_6] \) as a redox probe prepared in 0.1 M KCl. The impedance was measured in the frequency range from 100 MHz to 100 kHz vs. calomel reference electrode with a voltage amplitude of 5 mV and the respective semicircle diameter corresponds to charge-transfer resistance (Rct). DPV experiments were performed in the potential range from -0.5 to 1.0 V for the determination of ascorbic acid and dopamine at the pulse amplitude of 50 mV and a scan rate of 100.0 mV/sec. All experiments were performed at room temperature.

2.6 Preparation of analytes

A 5 µg mL\(^{-1}\) solution of guanine was prepared daily by dissolving appropriate amount of guanine in 100 ml of alkali media (NaOH 0.1 M). Similarly 5 µg mL\(^{-1}\) solution of adenine was prepared daily by dissolving appropriate amount of adenine in 100 ml of alkali solution.

Stock solution of ascorbic acid (1.0 \( \times \) 10\(^{-3}\) M) and dopamine were prepared daily in deoxygenated distilled water and stored in a dark and cool place.
Stock solution of ONB \((1.0 \times 10^{-3} \text{ M})\) was prepared freshly prior to electrochemical measurements in deoxygenated distilled water and stored in a dark place.

The phosphate buffer (PBS) solution was made up from the appropriate quantities of \(\text{NaH}_2\text{PO}_4\) and \(\text{Na}_2\text{HPO}_4\) adjusted to pH 7 by NaOH. The supporting electrolytes of other pH were prepared by adding suitable quantities of \(\text{NaH}_2\text{PO}_4\) and \(\text{Na}_2\text{HPO}_4\) were taken and checking the pH of the medium using Elico pH meter.

2.6.1 Preparation of ss-DNA samples

Thermally denatured dsDNA was produced according to the previous report [2]. In short native calf thymus dsDNA samples were dissolved in water, and then the solution was heated in a boiling-water bath \((100 \, ^\circ\text{C})\) for about 10 min. Finally, the solution was rapidly cooled in an ice bath. Generally, thermal denaturation involves the rupture of hydrogen bonds, the disturbance of stacking interaction, but not any breakage of a covalent bond. So, thermally denatured dsDNA may act as ss-DNA. The obtained solution was diluted to an appropriate concentration daily using phosphate buffer solution (pH 7.2).
References
