# PHENOSAFRANINE FUNCTIONALIZED MULTIWALLED CARBON NANOTUBES MODIFIED ELECTRODE FOR THE DETERMINATION OF Hg(II)

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## ABSTRACT

The preparation of chemically modified electrode has attracted a lot of attention during the past three decades and number of methods has been proposed for modifying the electrode surface. The utility of CMEs in the analysis is growing day by day because of the selectivity and sensitivity associated with these electrodes. The recent trend is to use nano materials which are having amazing electrical condution to achieve better sensitivity. The modified electrodes have attracted considerable interest in electrochemistry. This involves coating of substrates of biological, environmental and pharmaceutical importance. In this investigation, characterization of Phenosafranine functionalized MWCNTs modified graphite electrode and its electrocatalytic behavior for some analytes have been experimented.

**Keywords:** Multi walled Carbon Nanotubes, Chemically Modified Electrodes, Cyclic Voltammetry, Differential Pulse Voltammetry, Phenosafranine, chemically modified electrode, Hg(II).

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## INTRODUCTION

### 1.1 Carbon Nanotubes

The search for new advanced materials is an important area of contemporary research in numerous disciplines of science and the development of many new technologies. Great attention has been paid in recent years to nano- structured materials of different chemical composition, produced as nanoparticles, nanowires or nanotubes. Similarly, there has already also been great interest in their preparation, properties and application in the literature. In various fields of chemical analysis, there have been an increasing number of applications of Carbon Nanotubes (CNTs).

CNTs, also known as buck tubes, are allotropes of carbon with cylindrical nanostructure which were discovered by

Iijima in 1991[1]. CNTs are molecular scale tubes of graphite carbon with outstanding properties. They are among the stiffest fibers known. CNTs constitute a novel class of nanomaterials with remarkable applications in diverse domains. These are fullerene related, tube like structures which may be considered as a rolled up sheet of graphene. Their name is derived from their size, since the diameter of a nanotube is in the order of a few nanometers (approximately 1/50,000th of the width of a human hair).

The nature of bonding of a Nanotube is described by applied quantum chemistry, specially, orbital hybridization. Each carbon atom is covalently bonded to three of its neighbors and the 4th electron is free to move over the whole structure, that is, it is delocalized ($sp^2$ hybridization). These nanotubes naturally align themselves into “ropes” held together by Vander waal forces.

In analogy to graphite modification of carbon, the unit cells of CNTs are planar hexagons. These have a variety of helical structures [2], depending on the graphene sheet rolling up phenomenon. The CNTs form bundles, which are entangled together in the solid state giving rise to a highly complex network. Depending upon the arrangement of the hexagon rings along the tubular surface, CNTs can be either metallic or semi conducting.

There are two main types of CNT with high structural perfection:
1) Single-wall Carbon nanotubes (SWNTS) [3, 4]
2) Multi-wall Carbon nanotubes (MWCNTs) [1]


SWCNT MWCNT

**Fig- 1.1. Structures of MWCNT and MWCNT**

SWNTs consist of a single graphite sheet rolled seamlessly, with a diameter of 1-2 nm. SWNTs usually are observed arranged in a regular pattern of bundles that consist of 10 to 100 of nanotubes in parallel and in contact with each other.

MWCNTs are composed of concentric and closed graphene tubules, each with a rolled-up graphene sheet, formed with a range of diameters typically from 2 to 25 nm. These are first CNTs to be discovered.

Both SWNT and MWNTs may generally be synthesized using methods which include arc-discharge [5, 6], Laser ablation [7, 8], chemical vapor deposition (CVD) [9] and the gas phase catalytic process (Hip co) method [10]. But unfortunately, no method has yet been introduced for the synthesis of pure CNTs; the methods proposed yet lead to impure products such as C-coated metal catalysts, carbon coated metal and carbon nanoparticles/amorphous carbon as well as structural defects like dangling bonds. These impurities, however, may be removed using a variety of purification techniques.

### 1.1.1 Properties of CNT:

Some properties of CNT are sated below:

- ***Electrical***: Their structural parameters indicate how much the nanotube is twisted. CNTs can be highly conductive and hence can be metallic. Their conductivity has been shown to be a function of their chirality, the degree of twist as well as their diameter [11].

- ***Mechanical:*** The small diameter of CNTs also has an important effect on the mechanical properties compared with traditional micron size graphitic fibers [12]. The most striking effect is the opportunity to associate high flexibility and high strength with high stiffness. These properties of CNTs open the way for a new generation of high performance composites [13]. The nanotube as a whole is very flexible because of the great length [14].

- ***Chemical:*** High specific surface & sigma-pi rehybridization facilitate molecular adsorption, doping & charge transfer on nano tubes which in turn modulates electronic properties

- ***Thermal & Thermoelectric***: Nanotubes display very high thermal conductivity. Therefore it is expected that CNT reinforcements in polymeric materials may also significantly improve the thermal & thermo mechanical properties of these composites [11].

### 1.1.2 Applications of CNT:

The CNTs or Buckytubes are an example of true nanotechnology: only a nanometer in diameter, but molecules that can be manipulated chemically and physically. These open incredible applications in materials, electronics, chemical processing, and energy management.

Carbon nanotubes have extraordinary electrical conductivity, heat conductivity and mechanical properties. They are probably the best electron field emitter. These are polymers of pure carbon and can be reacted by using the tremendously rich chemistry of carbon. This provides opportunities to modify the structure and to optimize solubility and dispersion. These extraordinary characteristics give CNTs potential in numerous applications.

### (i) Field Emission:

CNTs are the best known field emitters of any material. This is understandable, given their high electrical conductivity, and the unbeatable sharpness of their tip (the sharper the tip, the more concentrated will be an electric field, leading to field emission; this is the same reason lightening rods are sharp). The sharpness of the tip also means that they emit at especially low voltage, an important fact for building electrical devices that utilize this feature. CNTs can carry an astonishingly high current density, possibly as high as 1013 A/cm2. An immediate application of this behaviour receiving considerable interest is in fieldemission flat-panel displays. Instead of a single electron gun, as in a traditional cathode ray tube display, here there is a separate electron gun (or many) for each pixel in the display. The high current density, low turn-on and operating voltage, and steady, long-lived behavior make CNTs attract field emitters to enable this application. Other applications utilising the field-emission characteristics of CNTs include: general cold-cathode lighting sources, lightning arrestors, and electron microscope sources.

### (ii) Conductive plastics:

Over the last half century plastics have been used as a replacement for metal. For structural applications, plastics have made tremendous headway, but not in electrical conductivity as these are good electrical insulators. This deficiency is overcome by loading plastics up with conductive fillers, such as carbon black and graphite fibres. The loading required to provide the necessary conductivity is typically high, however, resulting in heavy parts, and more importantly, plastic parts whose structural properties are highly degraded.

It is well established that the higher aspect ratio of filler, the lower loading required to achieve a given level of conductivity. In this sense CNTs are ideal, since they have the highest aspect ratio of any carbon fibre. In addition, their natural tendency to form ropes provides inherently very long conductive pathways even at ultra-low loadings.

Applications that exploit this behaviour of CNTs include EMI/RFI shielding composites and coatings for enclosures, gaskets, and other uses; electrostatic dissipation (ESD), and antistatic materials and (even transparent!) coatings; and radar-absorbing materials.

### (iii) Molecular Electronics

The idea of building electronic circuits out of the essential building blocks of materials - molecules - has seen a revival the past five years, and is a key component of nanotechnology. In any electronic circuit, but particularly as dimensions shrink to the nanoscale, the interconnections between switches and other active devices become increasingly important.

Their geometry, electrical conductivity, and ability to be precisely derived, make CNTs the ideal candidates for the connections in molecular electronics. In addition, they have been demonstrated as switches themselves.

### (iv) Structural Composites

The world-record properties of CNTs are not limited to electrical and thermal conductivities, but also include mechanical properties, such as stiffness, toughness, and strength. These properties lead to a wealth of applications exploiting them, including advanced composites requiring high values in one or more of these properties.

### (v) Catalyst Supports

CNTs have an intrinsically high surface area; in fact, every atom is not just on a surface - each atom is on two surfaces, the inside and outside, combined with the ability to attach essentially any chemical species to their sidewalls provides an opportunity for unique catalyst supports. Their electrical conductivity may also be exploited in the search for new catalysts and catalytic behaviour.

### (vi) Biomedical Applications

The exploration of CNTs in biomedical applications is just underway, but has significant potential. Cells have been shown to grow on CNTs, so they appear to have no toxic effect. The cells also do not adhere to the CNTs, potentially giving rise to applications such as coatings for prosthetics and anti-fouling coatings for ships.

The ability to chemically modify the sidewalls of CNTs also leads to biomedical applications such as vascular stents, and neuron growth and regeneration.

### (vii) Other Applications

Carbon nanotube fibre is so strong that they are the only fibre that could be spun into a space elevator (a sky bridge connecting a counter weight in geosynchronous orbit to a position on the ground) without snapping. Recently, CNTs have been proposed as a building material for armor so strong that bullets bounce right off it.

There is a wealth of other potential applications for CNTs, such as solar collection; nano-porous filters; catalyst supports; and coatings of all sorts. There are almost certainly many unanticipated applications for this remarkable material that will come to light in the years ahead and which may prove to be the most important and valuable of all.

### 1.1.3 Application in Electrochemistry:

The field of research that has been growing extensively in CNTs is electrochemistry**.** CNTs are electrochemically inert materials similar to other carbon-based materials used in electrochemistry, i.e. glassy carbon, graphite, and diamond. They possess distinct electrochemical properties because of their unique electronic structure. Generally, these are treated as excellent electrode materials.

The carbon atoms of CNTs at the sidewall and the end of the tubes are not same and their behavior can be compared with the basal plane and edge plane of highly oriented pyrolytic graphite (HOPG), respectively [15]. Compton *et al*. observed similar electron transfer rate constants for CNTs-modified and the edge plane HOPG electrodes. So, CNTs act as an efficient electron transfer promoter (Fig1.2) [16-18]. It has been found that rate of electron transfer was determined to be faster in the case of edge plane defect sites and are responsible for much of the chemical and electrochemical activity of CNTs [19]. The electroactive sites on MWCNTs are located at the tube ends.

The length of the aligned CNTs also has a significant effect on the electron transfer rate. The electron transfer rate constant varied inversely with the mean length of the CNTs. An experiment on the electron transfer rate constant of the surface attached ferrocene methylamine on CNTs reveal that when the nanotube dispersed randomly, the rate constant was found to be 40 times slower than that obtained at vertically aligned nano tubes modified electrode. The electron transfer rate at the CNTs modified electrode could be further increased by dialyzing the CNTs after purification and shortening treatment [20].

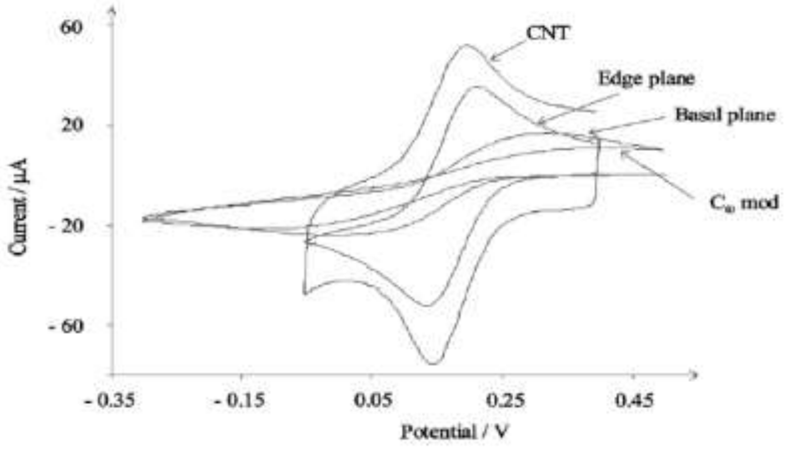


**Fig 1.2. Cyclic voltammograms for the reduction of 1 mM ferrocyanide at different electrodes at a scan rate of 100 mV$s^{-1}$ [10].**

The electrocatalytic activity and the electroanalytical performance at CNTs modified electrode are strongly dependent on the mode of production of the CNTs, either by CVD or the ARC discharge process [21]. CNTs produced by CVD appear to be more electrochemically reactive in their voltammetric study than these produced using the ARC methodology. The differences in the electrochemical reactivity are attributed to the smaller fraction of exposed edge plane at ARC-CNTS and higher density of edge plane defects at CVD-CNTs.

For electrochemical applications, CNTs are typically activated in strong acids, which opens the CNTs ends and forms oxygenated species, making the ends hydrophilic and increasing the aqueous solubility of CNTs. The electrochemical behavior of CNTs varies considerably with the methods used for purification and preparation, including oxidation treatment. For analytical application CNTs are most often used to modify other electrode materials or as part of a composite electrode.

### 1.1.4 Modification on CNT as a material:

- Recently CNTs have been proposed as a building material for armor. It is so strong that bullets bounce right off it.
- Used as sensors
- Nanotube films show promise for use in displays for computers, cell phones, ATMs etc.
- CNTs have been implemented in nano electromechanical systems, including mechanical memory elements(NRAM) and nanoscale electric motors(nanomotors)
- Bulk CNTs have been used as composite fibers in polymers to improve the mechanical, thermal and electrical properties of the bulk product.
- Used as paper batteries:

  A paper battery is a battery engineered to use a paper thin sheet of cellulose infused with ligned CNTs [22]. The nanotube act as electrodes, allowing the storage devices to conduct electricity.
- Used in solar cells:

  Solar cells use CNT complex formed by mixing fullerene and CNT to form snake like structures.
- Used as Infra red Devices:

  SWNTs are good absorber and emitters of light in the IR.

### 1.1.5 Chemical Functionalization at CNTs surface:

#### (i) CNT Oxidation

The emerging area of Nanotube chemistry was the development of an oxidation process for SWCNTs or MWCNTs involving extensive ultrasonic treatment in a mixture of concentrated nitric and sulfuric acid [23]. Such drastic conditions lead to the opening of the tube caps as well as the formation of holes in the sidewalls, followed by an oxidative etching along the walls with the concomitant release of carbon dioxide. The final products are nanotube fragments, whose ends and sidewalls are decorated with a high density of various oxygen containing groups (mainly carboxyl groups). Under less vigorous condition, such as refluxing in nitric acid, the shortening of the tubes can be minimized. The Chemical modification is then limited mostly to the opening of the tube caps and the formation of functional groups at defect sites along the sidewalls. Nanotubes functionalized in this manner basically retain their pristine electronic and mechanical properties [24].

#### (ii) Electrochemical Modification of Nanotubes

The Electrochamical properties of CNTs mainly steems from the superior electrocatalytic properties and their high surface-to-volume ratio, as compared to other carbon materials that are widely used as electrode materials [25]. Electrochemistry has been developed into an elegant tool for the functionalization of CNTs in a selective and controlled manner. To this end, a constant potential (potentiostatic) or a constant current (galvanostatic) is applied to a CNTs electrode immersed in a solution that contains a suitable reagent, where by a highly reactive (radical) species is generated through the electron electron transfer between the CNTs and the reagent. Many organic radical species have a tendency to react with the starting reagent or to self-polymerise, resulting in polymer coating on the tubes.

#### (iii) Covalent immobilization of CNTs

The oxidation introduced carboxyl groups represents useful sites for furher modifications, as they enable the covalent coupling of molecules through the creation of amide and ester bonds. By this method the nanotubes can be provided with a wide range of functional moieties, for which purpose bifunctional molecules (e.g. diamines) are often utilized as linkers. SWCNTs or MWCNTs with a good solubility in organic solvents can be obtained by covalent [26] or ionic [27] attachment of long-chain aliphatic amines onto the carbonyl groups.

Through Electrochemical coupling of aromatic diazonium salts, phenyl residues have been covalently grafted on to samples of CNTs [28]. In one of the first experiments performed in this direction, phenyl rings bearing various substituents were covalently linked to the surface of CNTs.

### (iv) Photochemical Functionalization of Nanotubes

The chemical functionalization routes based upon thermally activated chemistry or electrochemistry, photochemical approaches have been employed to a much lesser extent up to now. Photoirradiation has been used to generate reactive species such as nitrenes in the course of sidewall addition reactions [29]. In these cases however, the photoactivation exclusively employs the azido compound as the nitrene precursor. photochemical modification of CNTs has been reported to date, which is sidewall osmylation [30]. Initially, it was observed that CNTs exposed to osmiumtetroxide ($OsO_4$) under UV-light irradiation show a pronounced increase in electrical resistance.

## 1.2 Chemically Modified Electrode

Chemically Modified Electrodes (CMEs) comprise a relatively modern approach to electrode systems [31, 32] that finds utility in

1. A wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity to electrode surface chemistry, electrostatic phenomena at electrode surfaces, and electron and ionic transport phenomena in polymers.
2. The design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection, and electro-organic syntheses.

Compared with other electrode concepts in electrochemistry, the distinguishing feature of a CME is that a generally quite thin film (from a molecular monolayer to perhaps a few micrometers-thick multilayer) of a selected chemical is bonded to or coated on the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rotational, chemically designed manner. The range of electrode surface properties includes, but is more diverse than, that of ion-selective electrodes (ISEs) which also involve, in their highest forms, rotational design of the phase-boundary, partition and transport properties of membrances on or between electrodes.

CMEs can operate both amperometrically (and voltammetrically) and potentiometrically, they are generally used amperometrically, a faradaic (charge transfer) reaction being the basis of experimental measurement or study, whereas ISEs are generally used in potentiometric formats where a phase-boundary potential (interfacial potential difference) is the measured quantity [33].Gas-sensing electrodes (e.g., for $CO_2$, $NH_3$, $NO_X$) are also potentiometrically based [34] although the oxygen electrode, which functions amperometrically, is an exception [35]. Chemically sensitive field effect transistors (CHEMFETs) are basically non-faradaic electrode systems in whichelectric field variations in the semiconductor gate region control the magnitude of the source-drain current [36]. Enzyme-based electrodes detect the product(s) of a reaction between an immobilized enzyme layer and a reaction substrate in many ways, including both amperometric and potentiometric means. The distinction between CMEs and amperometric enzyme-modified electrodes is thus very narrow, the latter being based on a natural biological catalyst, but also with a rational (bio) molecular electrode design goal in mind.

### 1.2.1 Necessity to Modify an Electrode

- To obtain fundamental information about the mechanism of electron transfer ar an electrode surface.
- To impart the electrode surface some chemical specificity not available at the unmodified electrode.
- To mediate a reaction which is kinetically unfavorable at an unmodified surface.
- To prevent undesirable reactions from competing kinetically with desired electrode process [37].
- Using nano materials which are having high surface to volume ratio, electrode can be made more sensitive to the analyte.
- To preconcentrate a reactant, so that even low concentrate can be measured can be measured analytically.

### 1.2.2 Electrodes for Modification

The electrodes used for modification must exhibit certain necessary parameters in order to yield satisfactory attachment of modifier. The performance criteria for an electrode include electron transfer kinetics, background current, reproducibility, adsorption properties and pragmatic concerns such as case of use and availability.

A range of electrode materials have found applications for electroanalysis. Among those the most popular have been Mercury [38], Gold [39], Platinum [40], Nickel [41], and Carbon electrodes [42]. Now days carbon electrodes are mainly used for modification [43].

### 1.2.3 Superiority of the Modified Electrode over Bare electrode

- The electron transfer is mediated by the redox group present as thin layer on the surface, which is ultimately required to achieve the goal.
- Some electrode reaction may occur at high over potentials due to poor kinetics. These can be reduced to a lower potential by modified electrodes.
- Modified electrodes prevent undesirable reactions from competing kinetically with the desired electrode process.
- The modified electrodes enhance the selectivity and sensitivity.

### 1.2.4 Methods for Modifying the Electrode Surface

A chemically modified electrode consists of two parts, namely the electrode and a layer of chemical substrate. Chemically modified electrodes are prepared by incorporating a redox component onto the bare electrode surface. The principle routes of modification of the electrode surface with redox components can be classified as follows:

(i) Chemisorption
(ii) Covalent Modification
(iii) Polymer coating
(iv) Electrochemical coating
(v) Mechanical immobilization
(vi) Carbon Composite electrodes
(vii) Sol-gel method
(viii) Screen Printing

The divisions between these categories however are not distinct and individual strategies for Modifying electrode surfaces often exhibit which fall into more than one of the above categories.

**(i) Chemisorption**

The absorption of the species of interst into the electrode surface is called chemisorptions. It is one of the oldest and simplest techniques for anchoring redox centers to electrode surface. The first deliberate modification of an electrode surface by exploiting the concept concept of chemisoption was done by Lane and Hubbar [44, 45], who chemisorbed a variety of olefin-bearing species on to Pt electrode. Although these early studies were on Pt surface, the majority of adsorption studies have been performed on pyrolytic graphite and glassy carbon electrode [46].

**(ii) Covalent Modification**

In this method the molecules are covalently bonded to silicon or germanium surface to provide a coating over the surface. Murray’s group [47, 48] first articulated a general study for the covalent attachment of redox centers on to the electrodes surface. The approach was based on the use of silane reagents that has been employed by chromotographs in the derivatization of silica surface for the preparation of bonded phases. The work of Watkins et al [49] was the published one on covalent immobilization of an optically active molecule on a carbon electrode. Since then there has been extensive work on broadening and applying covalent synthetic scheme for attachment to electrode surface. The covalent schemes include procedure for attaching both monomolecular layer and multimolecular layer onto the surface.

**(iii) Polymer Coating**

In 1978, Miller’s and Bard’s group independently showed that CMEs could be prepared by Coating electrode surface with polymer films [50, 51]. There was many reason in using as electrode Modifying agents. The quantity of polymer deposited can be reliably and reproducibly varied. Polymer films can be made completely insoluble in contacting solutions thus loss of modifier from the electrode is avoided. Many organic polymers have tremendous chemical stability and hence the desired electroactive chemical functionality can be synthesized. Finally, other types of functional groups can be added to the polymer to increase film conductivity.

### (iv) Electrochemical Coating

In this method the electrolysis of the compound is carried out with the suitable substrate using the electrode to be modified. Platinum, graphite and glassy carbon electrodes have been used for modification. A large number of electrodes were constructed based on the electrochemical deposition mostly using inorganic materials such as ferrocyanides, phthalocyanines, ferrocenes, metal oxides and other compounds [52].

### (v) Mechanical immobilization

This is a simple method used for the study of insoluble materials. This method involves the transfer of solids to the electrode surface by mechanical immobilization and the electrochemical properties of the materials are studies by stripping voltammetry. This technique is called abrasive stripping voltammetry and a number of insoluble materials have been studied by this technique [53].

### (vi) Carbon wax composite electrodes

Composite graphite electrode are prepared by mixing graphite powder, the sensor material and wax. The mixture is heated in a water is heated in a water bath to melt the paraffin. This liquid paste is again carefully mixed before it is cast into appropriate polyethylene tubes. After its solidification, the tubing is removed and the electrode rods are covered with a layer of pure paraffin for insulation. This layer is removed at the upper for contact and the circular surface of the other part is also removed to provide a sensor surface. Composite electrodes are used to study a number of insoluble solid materials [54].

### (vii) Sol-gel method

During the last decade, a new method of host matrices fabrication by sol-gel process for the encapsulation of a wide range of chemical and biological molecules in an active and stable form in the three-dimensional porous network. The sol-gel process shows a great promise for the encapsulation of chemical agents in connection with the preparation of chemical sensors. The use of sol-gel chemistry to produce sensing material is of considerable interest now days. This is due to a number of advantages, including low temperature encapsulation of biorecognition elements, tenability of physical characteristics, optical transparency, mechanical rigidity and lower chemical reactivity [55-57].

### (viii) Screen printing

Inexpensive, reproducible and sensitive disposable electrochemical sensors have been produced by the application screen printing technology. The sensors are used for the determination of trace level of important compounds in biological fluids. The large the number of organometallic compounds coated over carbon electrodes by these method and their application have been described [58-61].

## 1.3 Redox Mediator:

Voltammetry sensors are used to exploit the potential, between a reference and working electrode, to cause the oxidation or reduction of an electroactive species. The modification facilitates the charge transfer between the electrode and the analyte in solution at much lower potentials. The electrode catalyst used for this purpose include organometallic mediators such as ferrocenes, phthalocyanides, ruthenium oxides, metalloporphyrines and several organic mediators including dopamine and quinine.

### 1.3.1 Organometallic mediators:

### Ferrocene

The ferrocene groups of mediators have been successfully applied to the quantification of several compounds and have been particularly important in the development of sensors for the determination of glucose in a variety of sample matrices reported on amperometric enzyme electrodes for the determination of circulating levels of Glucose in blood for patients suffering from diabetes mellitus. Substituted ferrocenes have been developed to improve the long term stability of electrode [62].

### Phthalocyanines

Each of the transition metal complexes of the phthalocyanine macrocycle is electroactive and several complexes have been investigated for numerous types of applications, eg. Electratalysis, photoconductivity, etc., [63]. However, Cobalt phthalocyanine (CoPC) has shown in the most promise for the electrocatalytic determination of biologically important compounds. One of earliest reports regarding applications was described by Zagal et al., who employed chemically modified pyrolytic graphite electrode for the oxidation of cysteine. The modified electrodes were prepared by adsorption of Cobalt phthalocyanine tetrasulphonate (CoTSPC) on to the polished graphite surface from an aqueous solution containing $10^{-5}$mol $dm^{-3}$ concentration of the mediator.

### Hexacyanoferrate

Potassium hexacycnoferrate has been successfully employed as a mediator following adsorption onto graphite foil and more commonly in aqueous solution or when electrostatically immobilized with poly-4-vinylpyridine (PVP). The PVP was incorporated into the carbon pasting in order to co- immobilize the hexacyanoferrate ions; the resulting electrodes displayed a rapid current response to ethanol but suffered from poor stability which was associated with a gradual loss of the mediator into solution.

### 1.3.2 Organic mediators

Several organic dyes such as Phenazines, Phenothiazines, Phenoxazines and their corresponding derivatives have been widely used for electrode modification. These types of dyes have been either polymerized, adsorbed or covalently attached to the electrode surface. As these dyes gives negative formal potentials it was often used for the determination of several substrates in negative redox potentials. Substrates such as, NADH, NADPH, $H_2O_2$, NO2- etc., were determined using these dye modified electrodes. Even some metal ions having negative reduction potentials have been determined using these organic dyes. Also it has an application in biosensing, i.e. it was easily immobilized with some coenzyme such as HRP, Glucose Oxidase, dehydrogenase etc., and this enzyme modified electrode was used for the determination of corresponding biomolecules.

### 1.3.3 Nano Particles

### Gold Nano Paticles

During the past few decades there has been singnificant interest in understanding the size dependent physical and chemical properties of gold nanoparticles due to their immense importance in a variety of application such as catalysis, optoelectronics, novel template in biomineralization, in medicine(diagnostics and therapeutics) etc. Another important property of gold is form strong linkages with thiol group, which help us to immobilize different types of ligands with thiol group to electrode.

The reaction between gold and thiol is an oxidative addition of S-H group to the gold surface, followed by the reductive elimination of hydrogen.

$$\text{R-S-H} + \text{Au}^0_n \rightarrow \text{R-S}^-\text{Au}^+.\text{Au}^0_n + 1/2\text{H}_2$$

The combination of hydrogen atoms at the metal surface to yield molecule hydrogen is thought to be an important exothermic step in the overall chemisorptions energetic. The bonding of the thiolate to gold surface is gold surface is very strong where the hemolytic bond strength is approximately 40 kcal $mol^{-1}$.

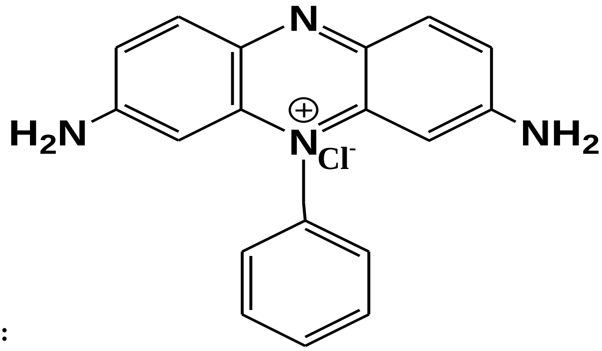
### 1.4 Present modification:

The present study involves the modification of the surface of graphite electrode by Phenosafranine (PS) immobilized MWCNTs.

### 1.4.1. Phenosafranine:

Safranines are the azonium compounds of symmetrical 2,8-dimethyl-3,7-diamino-phenazine. They are obtained by the joint oxidation of one molecule of a para-diamine with two molecules of a primary amine; by the condensation of para-aminoazo compounds with primary amines, and by the action of para-nitrosodialkylanilines with secondary bases such as diphenylmetaphenylenediamine. They are crystalline solids showing a characteristic green metallic luster; they are readily soluble in water and dye blue or violet. They are strong bases and form stable monacid salts. Their alcoholic solution shows a yellow-red fluorescence. Phenosafranine is not very stable in the free state; its chloride forms green plates. It can be readily diazotized, and the diazonium salt when boiled with alcohol yields aposafranine or benzene induline, $C_{18}H_{12}N_3$. F. Kehrmann showed that aposafranine could be diazotized in the presence of cold concentrated sulfuric acid, and the diazonium salt on boiling with alcohol yielded phenylphenazonium salts. Aposafranone, $C_{18}H_{12}N_2O$, is formed by heating aposafranine with concentrated hydrochloric acid. These three compounds are perhaps to be represented as ortho- or as para-quinones. The "safranine" of commerce is a ortho-tolusafranine. The first aniline dye-stuff to be prepared on a manufacturing scale was mauveine, which was obtained by Sir William Henry Perkin by heating crude aniline with potassium bichromate and sulfuric acid. Mauveine was converted to parasafranine (1,8-dimethyl Safranine) by Perkin in 1878 by oxidative/reductive loss of the 7-N-para-tolyl group.

The structure of Phenosafranine is as shown in the figure.



### 1.4.2. Analyte Determination:

The Phenosafranine functionalized CNTs is used to determine some metal ions like Hg (II) ion .

**Mercury (II)**

Contamination of the environment with heavy metal ions especially by mercury ions has drawn considerable concern. As mercury can accumulate in the vital organs and tissues to bind with sulfur-containing proteins and enzymes, some important cell functions are inactivated which lead to a wide variety of diseases [64]. Therefore, it is critical to develop simple and practical assays to detect $Hg^{2+}$. To date, a number of $Hg^{2+}$ detection methods have been developed [65-68].

## AIM AND SCOPE

Modification of various electrode surfaces is an important research area particularly in electrochemistry, material science, molecular electronics. Chemically modified electrodes (CMEs) have been continued to be a major concern during the last two decades due to their wide potential use in analytical and technical applications. The utility of CMEs in the analysis is growing day by day because of the selectivity and sensitivity associated with these electrodes. The recent trend is to use nano materials which are having amazing electrical conduction to achieve the selectivity and sensitivity. In particular, the development of the electrochemical sensors based on carbon nanotubes (CNTs) was achieved a great interest and since from their discovery, CNTs have attracted the attention of many scientist around the world. This extraordinary interest stem from their outstanding structural, mechanical and electronic properties. For sensing applications, CNTs have many advantages such as small size with large surface area, excellent electron transfer promoting ability when used as electrodes modifier in electrochemical reactions and easy protein immobilization with retention of its activity for potential sensors. Here, we summarize PS-MWNT modified electrode and its electro catalytic behavior for some substrates.

The aim of the present investigation includes:

- Functionalization of MWCNTs with PS using EDC and NHS.
- Fabrication of PS-MWNTs modified graphite electrode.
- To study the application of the modified electrode for the determination of Mercury (II) ions.

The present investigation has been carried out in the following manner:

- Carboxylation of MWCNTs
- Coupling of Phenosafranine with MWCNTs through carbodiimide coupling method
- Characterization of MWCNTs, MWCNTs-COOH and PS-MWCNTs by UV-Deflective Reflectance UV-visible spectroscopy, FTIR spectroscopy, Confocal Raman Microscopy and EDAX
- Morphology of MWCNTs, MWCNTs-COOH and PS-MWCNTs was studied by SEM
- Fabrication of PS-MWCNTs modified electrode
- Optimization of parameters influencing the electrode response
- Characterization of PS-MWCNTs graphite electrode by Cyclic Voltammetry
- Study of the factors influencing the electrochemical characteristics of the modified electrode
- Studies on analytical utility of the PS-MWCNTs modified graphite electrode for the determination of Mercury (II) ion

- Amperometric determination of the above said ions under dynamic conditions.

## EXPERIMENTAL SECTION

This chapter provides a brief account of the materials used, solutions prepared, equipments used and experiments carried out during the course of the present investigation.

### 3.1. Materials/Chemicals:

1. MWCNTs
2. Phenosafranine
3. EDC
4. NHS
5. Conc. HCl
6. Conc. $HNO_3$
7. Ammonium Nitrate
8. Mercury (II) Chloride

### 3.2. Preparation of the Solutions

### 3.2.1 Ammonium Nitrate

About 0.8004g of ammonium nitrate substance is weighed and transferred into 100ml standard flask with doubly distilled water to get 0.1M solution.

### 3.2.2 Mercury (II) Chloride

About 0.0679g of mercury (II) chloride is weighed and transferred into 25ml standard flask with doubly distilled water to get 0.01M solution.

About 0.6788g of ammonium nitrate substance is weighed and transferred into 25ml standard flask with doubly distilled water to get 0.1M solution.

### 3.3 Functionalization of MWCNTs with Phenosafranine

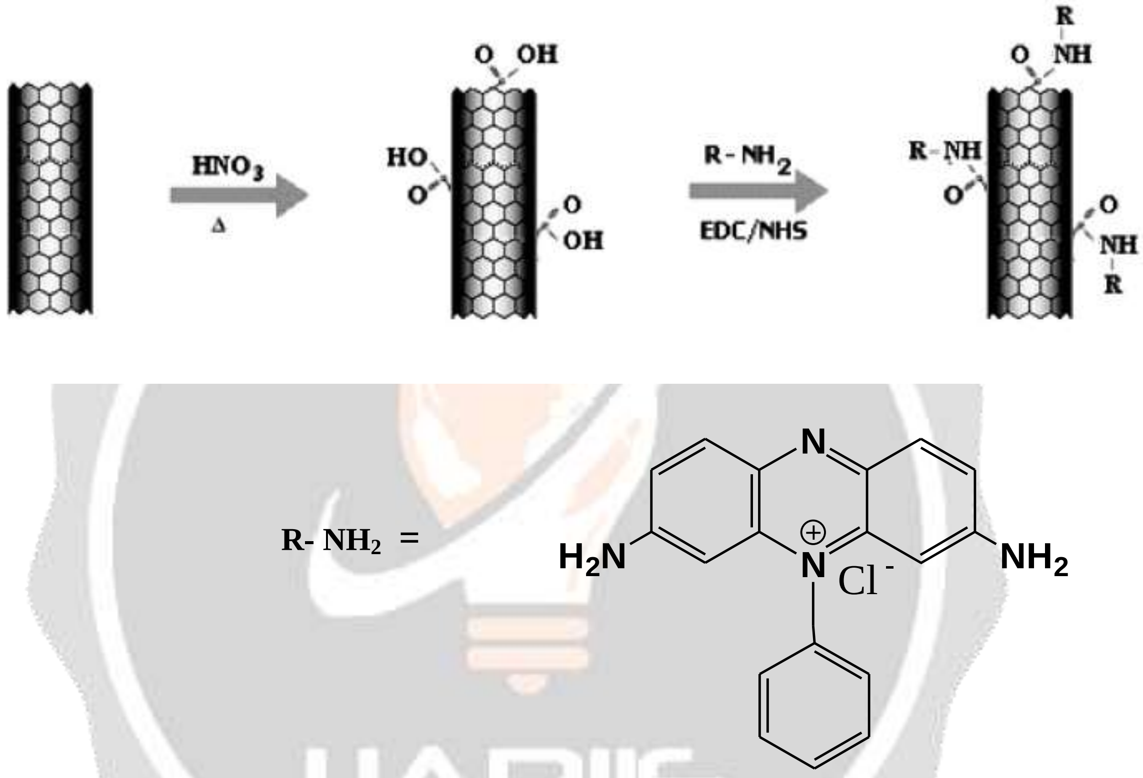
The functionalization of MWCNTs with PS includes the following steps

### Step 1: Carboxylation of MWCNTs

About 500mg of MWCNTs was refluxed in Conc. HCl for 30 mins in order to eliminate metal catalyst and then the CNTs was centrifuged. The residue was kept in an oven at $225^0$ C for 18 hours and at $350^0$ C for 2 hours. Then it was dispersed in 5.6 M $HNO_3$ solution and refluxed for 12 hours. The resulting suspension was centrifuged and the precipitate was washed with water to obtain carboxylic group functionalized MWCNTs.

**Step 2: Amidation**

The redox mediator PS was attached to the carboxylic acid groups of the MWCNTs using water coupling agents EDC and NHS by forming amide linkages between the amino group and –COOH group of MWCNTs [74]. This was done by immersing the activated MWCNTs (100mg) in a freshly prepared 10ml aqueous solution of EDC (10mg/ml). With stirring, 300mg of NHS was added to the solution. The pH of the solution was adjusted to 7 and 100 mg of PS was added to this solution. The reaction was allowed to occur at room temperature for 2h and then the CNTs were washed thoroughly with water. The reaction mechanism was shown in scheme **3.1**.



**Phenosafranine**

**Scheme. 3.1**. Reaction Mechanism for the functionalization of MWCNTs with Phenosafranine using EDC and NHS.

### 3.4 Fabrication of the modified electrode

Paraffin impregnated graphite electrode was used for electrode preparation

The PIGEs were prepared by immersing graphite rods into molten paraffin wax under vaccum until air bubbles ceased to evolve from rods. After re-establishing to atmospheric pressure, the rods were removed before the paraffin solidified. One end of the electrode was carefully polished on a smooth surface to get a mirror finished surface of the electrode. The PS-MWCNTs modified electrode was prepared by rubbing the polished surface onto a fine powder of PS functionalized MWCNTs placed on a smooth glass plate. The resulting electrode was rinsed with doubly distilled water and used for further experiments.

### 3.5. Characterization Techniques:

### 3.5.1 Cyclic Voltammetry

Cyclic voltammetry [75] is perhaps most versatile electroanalytical technique for the study electroactive species. Its versatility combined with ease of measurement has resulted in extensive use of CV in the fields of electrochemistry, inorganic chemistry, organic chemistry and biochemistry. The effectiveness of CV results from its capability for rapidly observing the redox behavior over a wide potential range. The resulting voltammogram is analogous to a conventional spectrum in that it conveys information as a function of energy scan. The overall view of the CV experiments excitation signals and response for a reversible redox process is shown in Fig 1.3.

CV consists of scanning linearly the potential of a stationary working electrode, using a triangular potential waveform. Depending on the information sought, single of multiple cycles can be used. During the potential sweep, the potentiostat measures the current resulting from the applied potential. The resulting plot of current versus potential is termed as cyclic voltammogram. The CV is characterized by several important parameters. The peak current for a reversible electron transfer is given by,

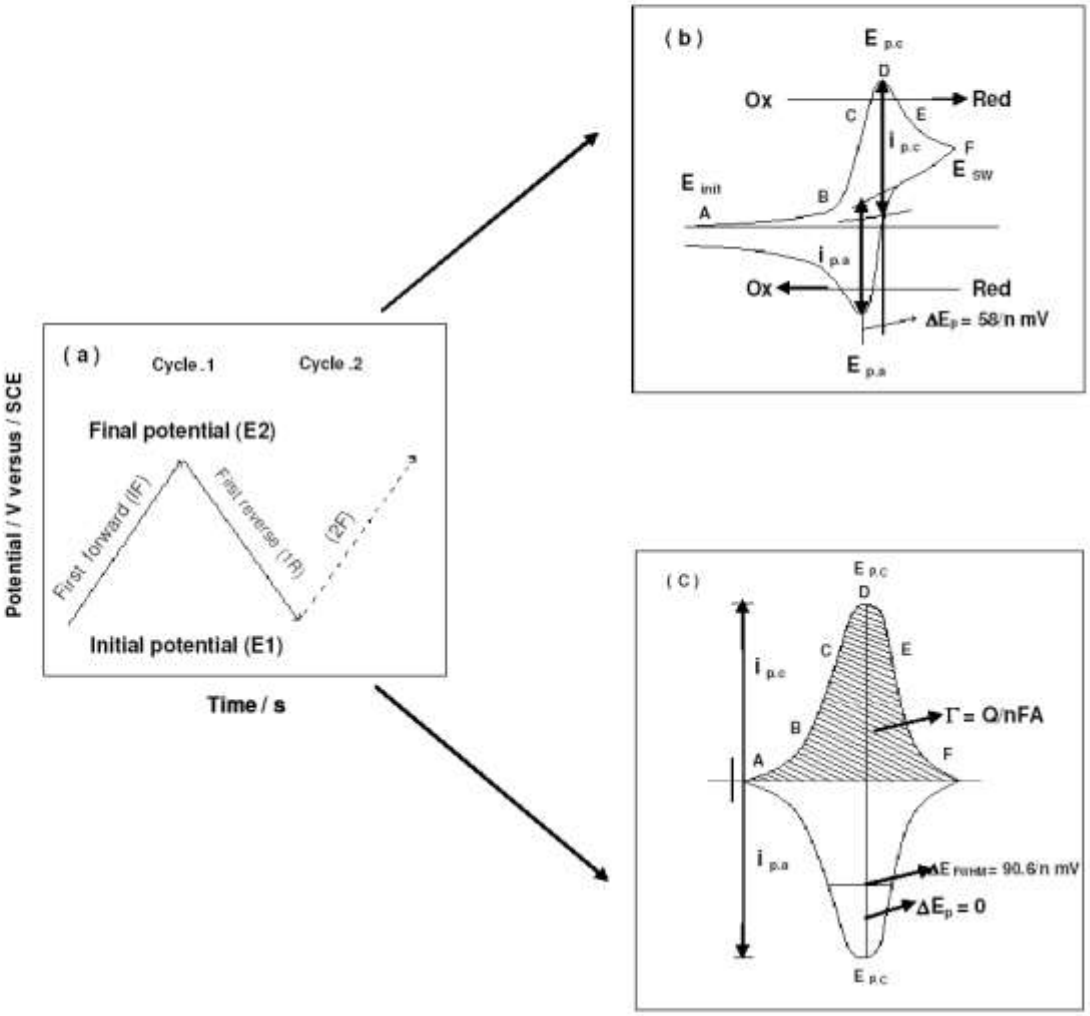
$$\mathbf{Ip = 0.4463\ n\ FA\ [nE/RT]\ Cox\ D^{1/2}}\ \boldsymbol{v}^{1/2} \qquad (1)$$

At 25$^{o}$C, this is equal to **Randles- Sevick** equation.

$$\mathbf{Ip = (2.69\ X\ 10^{5})\ n^{3/2}\ A\ C\ D^{1/2}}\ \boldsymbol{v}^{1/2} \qquad (2)$$

Where,

*v* - Scan Rate
C -Concentration
n -Number of electrons
A -Area of electrode
D -Diffusion Co-efficient



## RESULTS AND DISCUSSION

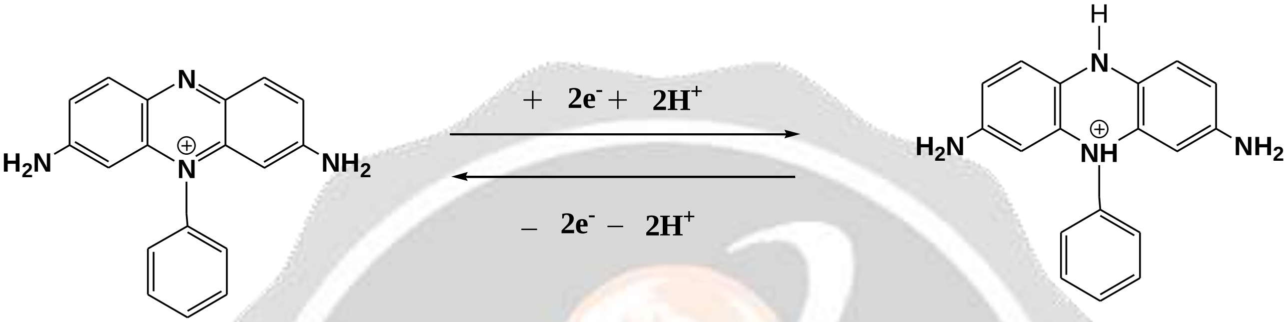
### 4.1. Covalent immobilization of Phenosafranine on MWCNTs:

To immobilize PS with MWCNTs, EDC and NHS have been used. EDC and NHS used for this reaction are classified as zero-length cross-linking reagents because during cross-linking, atoms are eliminated from reactants, thus shortening the distance between the two linked moieties [76]. In this reaction, EDC activates the terminal –COOH groups of the MWCNTs, forming a highly reactive O-acylisourea active intermediate. A subsequent nucleophilic attack by the primary nitrogen of the amino compound brings about the formation of the amide linkage with the release of the soluble substituted urea. This intermediate may react with an amine group of PS yielding a conjugate of the two molecules joined by a stable amide bond. However the intermediate is also susceptible to hydrolysis making it unstable and short lived in aqueous solution. The NHS, which stabilizes as well as facilitates the reaction, reacts with carboxyl-containing compounds to give aminoacyl esters. The stable active esters, thus formed, hydrolyze slowly compared with their rates of reaction with amino groups. Thus, NHS increases the coupling efficiencies of carbodiimides for conjugating carboxylated compounds with primary amine of the mediator.

### 4.2. Characterization of PS-MWCNTs

### 4.2.1. Electrochemical Characterization:

Cyclic Voltammetry was the effective and convenient method for probing the feature of the modified electrode surface. Fig.4.6. shows the cyclic voltammograms of the (a) modified electrode and (b) the bare electrode in the potential range of 0.4 to -1.0 V in 0.1 $NH_4NO_3$ solution. No redox peaks was obtained for bare PIGE. In contrast, a well defined redox peak was obtained for PS-MWCNT modified electrode with a formal potential [$E^0$] of -0.5457 V (cathodic peak potential $I_{pc}$ = - 0.6185 V and anodic peak potential $I_{pa}$ = -0.4729 V ). The oxidation and reduction reaction of PS at the modified electrode is shown in the Scheme. 4.1.



Scheme. 4.1. Redox reaction of PS at the modified electrode surface

Effect of Scan Rate

In order to confirm the current response is associated with PS molecules, the cyclic voltammograms of PS-MWCNTs modified electrode was recorded at different scan rates in the range of 50 to 150mV$s^{-1}$ (Fig 4.7). It was found that the peak current of the modified electrode were directly proportional the scan rate indicating that the electrode reaction is surface confined process.

The cyclic voltammograms of modified electrode at different electrolytes like $Ba(NO_3)_2$, $NH_4NO_3$, $NaNO_3$, $K_2SO_4$, $KNO_3$, KCl and $CH_3COONa$ were studied. The modified electrodes showed different electrochemical responses on varying the background electrolyte. It was found that the electrode give redox peaks in all background electrolytes but it shows a well defined peak in $NH_4NO_3$. Hence $NH_4NO_3$ has been chosen as background electrolyte.

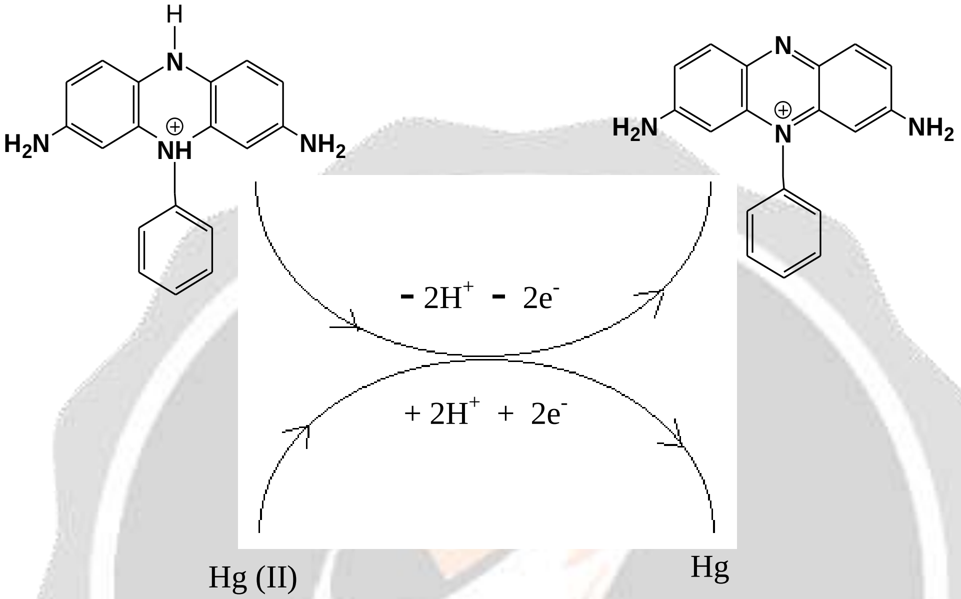
To study the effect of pH, the electrochemical properties of the modified electrode in the pH range 4.5–9 in 0.1 M $NH_4NO_3$ solution were investigated by recording the cyclic voltammograms. The results indicated that the formal potential of the PS-MWNTs redox couple was not changed, but reversibility and peak currents were decreased with increasing pH values (pH > 7). However, the stable and reproducible cyclic voltammograms were observed at neutral pH and hence pH 7.0 was optimized for the subsequent study.

### 4.3. Analytical applicability of the modified electrode.

### 4.3.1. Determination of Hg(II)

To address the issue of analytical applicability, CVs were recorded for the catalytic reduction of Hg(II) with the PS-MWNT modified PIGE. Fig 4.8. shows the CVs obtained with PS-MWNT modified PIGE and bare PIGE in a 0.1 M $NH_4NO_3$ solution without and with Hg (II) ion were recorded. As shown in the figure, in the presence of Hg (II) a dramatic change in the CV with a significant increase in the cathodic current was observed for PS- MWNT modified PIGE. Curve (a) shows the CV of the bare electrode and curve (b) that for the bare electrode in the presence of Hg(II). Almost no reduction current was observed with a bare PIGE in a 0.1M $NH_4NO_3$ solution containing Hg(II) ion solution. The CVs illustrate that the modified electrode can efficiently reduce Hg(II) with a marked decrease in overpotential. The peak current response to Hg(II) as nearly ten times more sensitive when compared with the bare PIGE. This can be mainly attributed to the easy electron transfer of PS and the large surface area and subtle electronic properties of MWNTs. The reduction of Hg(II) ion at the PS-MWCNTs electrode was shown in scheme.4.2.

Also, the catalytic current increased linearly with the Hg(II) concentration in the range from 1.67 to $11.7 \times 10^{-4}$ M with a correlation coefficient of 0.9985 (Fig 4.9 A). The electrocatalytic activity of the modified electrode for Hg(II) determination indicates that the modified electrode based on PS immobilized MWNT has good electrocatalytic properties



Scheme.4.2. Reaction mechanism for the reduction of Hg(II)

As Differential pulse voltammetry (DPV) is very sensitive and selective method for the determination of substrate, it can also be used to determine Hg(II) ion. Fig.4.9. B shows the DPVs of the modified electrode in presence of Hg(II) ion in the concentration range of 1.67 to $11.7 \times 10^{-4}$ M. It was found that the there is a linear increase in the reduction current of the modified electrode. The plot of catalytic current vs concentration is shown in the inset of fig 4.9.B.

After understanding the mechanism for the reduction of Hg(II) we turned our attention to optimizing the analytical response of the PS-MWNT sensor. This was initiated by studying the hydrodynamic voltammetric (HDV) response as a means of determining the optimum potential for the amperometric determination of Hg(II). From the hydrodynamic voltammograms (Fig. 4.10) the optimum potential for the present system was fixed to be -0.7 V. Also HDVs for bare electrode with and without Hg(II) ions were recorded. It was found that similar to CVs bare electrode doesn't reduces Hg(II) that much as that of the modified electrode. This demonstrate that the modified electrode exerts a good electrocatalytic reduction for Hg(II) even under dynamic condition.

### 4.3.2 Amperometric determination of Hg (II) using PS-MWCNTs modified electrode

The dynamic response of the modified electrode under optimal conditions with successive injections of 0.25ml of 0.1 M Hg(II) ion to the $NH_4NO_3$ solution under stirred condition was studied. The results are shown in Figure 4.11. The electrode showed rapid response to the concentration changes under the experimental conditions studied. A good linear relationship was observed between the peak current and the Hg (II) concentration over the range of $0.417 \times 10^{-5}$ M to $5.83 \times 10^{-3}$ M with the correlation coefficient of 0.990.

## SUMMARY AND CONCLUSION

- Phenosafranine was immobilized at the surface of MWCNTs through amide bond using EDC and NHS.
- PS-MWCNTs was loaded on the PIGE using mechanical immobilization and the modified electrode was characterized by Cyclic Voltammetry.
- The CV studies showed that the PS-MWCNTs electrode exhibited redox peaks at formal potential of -0.5457V (cathodic peak potential $I_{pc}$ = - 0.6185V and anodic peak potential $I_{pa}$ = -0.4729V ).

- Effect of scan rate studies showed that peak current of the modified electrode is linearly proportional to the scan rate, suggesting that the redox reaction is surface controlled.

- The electrochemical response of the modified electrode showed a higher catalytic current for Hg(II) at a reduced over potential when compared to that of an unmodified electrode.

- In order to study the suitability of the modified electrode for the determination of Hg(II) in flow system, hydrodynamic voltammetry and chronoamperometric studies have been carried out. The electrocatalytic activity of the modified electrode permits the convenient detection of analytes at lower potential with higher sensitivity. Thus, the applicability of the modified electrode as an amperometric sensor has been demonstrated.

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