DETECTION OF TRACE HEAVY METAL IONS BY ANODIC STRIPPING VOLTAMMETRY USING $_{\mathrm{L}}$-CYSTEINE FUNCTIONALIZED CARBON NANOTUBES PASTE ELECTRODE

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
Ag / AgCl	Silver / Silver Chloride
ASV	Anodic Stripping Voltammetry
Bi	Bismuth
Cd	Cadmium
$-CH_2SH$	Thiol Group
CNTPE	Carbon Nanotubes Paste Electrode
CNTs	Carbon Nanotubes
Co	Cobalt
$-COO^-$	Carboxylate Anion Group
-COOH	Carboxyl Group
CPE	Carbon Paste Electrode
Cu	Copper
CV	Cyclic Voltammetry
Cys-CNTPE	$_{L}$-Cysteine Functionalized Carbon Nanotubes Paste Electrode
Cys-MWCNTs	$_{L}$-Cysteine Functionalized Multi-Walled Carbon Nanotubes
DPASV	Differential Pulse Anodic Stripping Voltammetry
ECH	Epichlorohydrin
EDC	N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide
FTIR	Fourier Transform Infrared Spectroscopy
GA	Glutaraldehyde
GCE	Glassy Carbon Electrode
H_2O_2	Hydrogen Peroxide

H_2SO_4	Sulphuric Acid
HCl	Hydrochloric Acid
Hg	Mercury
HNO_3	Nitric Acid
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
KBr	Potassium Bromide
KCl	Potassium Chloride
KNO_3	Potassium Nitrate
LSASV	Linear Sweep Anodic Stripping Voltammetry
MS	Mass Spectrometry
MWCNTs	Multi-Walled Carbon Nanotubes
N	Nitrogen
$NaNO_3$	Sodium Nitrate
$-NH_2$	Amine Group
$-NH_3^+$	Ammonium Group
NHS	N-hydroxysuccinimide
Ni	Nickel
O	Oxygen
-OH	Hydroxyl Group
PANI	Polyaniline
Pb	Lead
PDMcT	Poly(2,5-dimercapto-1,3,4-thiadiazole)
PVDF	Polyvinylidene Fluoride
QCM	Quartz Crystal Microbalance

RSD	Relative Standard Deviations
S	Sulphur
SEM	Scanning Electron Microscopy
Sn	Tin
SPE	Screen-Printed Electrode
SWASV	Square Wave Anodic Stripping Voltammetry
SWCNTs	Single-Walled Carbon Nanotubes
TEM	Transmission Electron Microscopy
XPS	X-Ray Photoelectron Spectroscopy
Zn	Zinc

LIST OF SYMBOLS

a_1	Unit vector of chirality
a_2	Unit vector of chirality
C_{Cd}	Concentration of Cd^{2+}
C_h	Chiral vector
C_{Pb}	Concentration of Pb^{2+}
E	Potential
I_p	Peak current
m	Integer of the chiral vector
n	Integer of the chiral vector
R^2	Correlation coefficient
θ	Chiral angle

PENGESANAN ION LOGAM BERAT SURIH DENGAN VOLTAMETRI PELUCUTAN ANOD MENGGUNAKAN ELEKTROD ADUNAN TIUB-NANO KARBON BERKEBERANGKAPAN $_{\text{L}}$-SISTEINA

ABSTRAK

Pembangunan dan penggunaan biosensor elektrod adunan tiub-nano karbon berkeberangkapan $_{\text{L}}$-sisteina (Cys-CNTPE) untuk pengesanan ion plumbum (Pb^{2+}) dan ion kadmium (Cd^{2+}) dalam sistem akueus telah diperkenalkan. $_{\text{L}}$-sisteina dirangkapkan secara kovalen ke atas tiub-nano karbon dinding berlapis (MWCNTs) dengan menggunakan N-(3-dimetilaminopropil)-N'-etilkarbodiimida-N-hidroksilsuksinimida (EDC-NHS) gandingan karbodiimida sebagai elemen pengecaman dengan keupayaan pengkelatan terhadap Pb^{2+} dan Cd^{2+} yang tinggi. Keberangkapan kovalen $_{\text{L}}$-sisteina di atas MWCNTs telah disahkan dengan keputusan pencirian daripada Spektroskopi Inframerah Transformasi Fourier (FTIR). Cys-CNTPE disediakan dengan mencampurkan tiub-nano karbon dinding berlapis berkeberangkapan $_{\text{L}}$-sisteina (Cys-MWCNTs) dengan minyak mineral dan kemudian dipadatkan ke dalam rongga elektrod. Pengesanan Pb^{2+} dan Cd^{2+} oleh Cys-CNTPE dinilai dengan menggunakan Voltametri Pelucutan Anod Nadi Pembezaan (DPASV). Pengumpulan Pb^{2+} dan Cd^{2+} pada Cys-CNTPE diteruskan dengan pengacauan malar untuk satu tempoh masa tentu sebelum dianalisa. Arus puncak dan kedudukan puncak pelucutan masing-masing berkaitan dengan kepekatan dan jenis ion logam berat. Cys-CNTPE menunjukkan peningkatan dari segi kepekaan dan kememilihan dalam analisis voltametri untuk Pb^{2+} dan Cd^{2+}, disebabkan gabungan sinergi ciri pengecaman $_{\text{L}}$-sisteina dengan ciri elektrik MWCNTs. Pengoptimuman parameter eksperimen dilakukan untuk mendapatkan kepekaan Cys-CNTPE yang tertinggi dalam pengesanan Pb^{2+} dan Cd^{2+}, di mana kesan masa pengumpulan, upaya penurunan, masa penurunan, pH larutan penimbal asetat dan komposisi adunan elektrod telah dikaji. Keadaan optimum yang diperolehi adalah: (i) 10 minit masa pengumpulan, (ii) -1.0 V upaya penurunan, (iii) 5 minit masa penurunan, (iv) 0.1 M larutan penimbal asetat pH 5.0 dan (v) 60:40 (nisbah berat-berat) komposisi adunan elektrod. Dalam keadaan optimum, Cys-CNTPE menunjukkan julat linear pengesanan dari 1 hingga 15 μM dengan had pengesanan 0.1 μM dan 0.2 μM masing-masing untuk Pb^{2+} dan Cd^{2+}. Di samping itu, Cys-CNTPE juga menunjukkan kestabilan jangka panjang untuk 2 bulan, kepersisan dan kebolehulangan semula yang tinggi dengan relatif sisihan piawai kurang daripada 2% untuk lapan ukuran berulang. Potensi Cys-CNTPE untuk pengesanan serentak Pb^{2+} dan Cd^{2+} telah berjaya ditunjukkan. Tambahan pula, Cys-CNTPE menunjukkan kejituan yang tinggi dalam aplikasi sampel sebenar dengan ralat relatif maksimum kurang daripada 4% berbanding dengan Spektrometri Penyerapan Atom (AAS).

DETECTION OF TRACE HEAVY METAL IONS BY ANODIC STRIPPING VOLTAMMETRY USING $_{\text{L}}$-CYSTEINE FUNCTIONALIZED CARBON NANOTUBES PASTE ELECTRODE

ABSTRACT

The development and application of the $_{\text{L}}$-cysteine functionalized carbon nanotubes paste electrode (Cys-CNTPE) biosensor for the detection of lead ion (Pb^{2+}) and cadmium ion (Cd^{2+}) in the aqueous system are described. $_{\text{L}}$-cysteine was covalently attached to multi-walled carbon nanotubes (MWCNTs) via N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide-N-hydroxysuccinimide (EDC-NHS) carbodiimide coupling as a recognition element for its high metal-chelating ability towards Pb^{2+} and Cd^{2+}. The covalent attachment of $_{\text{L}}$-cysteine on MWCNTs was verified with the results from Fourier Transform Infrared Spectroscopy (FTIR) characterization. The Cys-CNTPE was prepared by dispersing the $_{\text{L}}$-cysteine functionalized multi-walled carbon nanotubes (Cys-MWCNTs) into mineral oil and then firmly packed into the well of an electrode. Determination of Pb^{2+} and Cd^{2+} at the Cys-CNTPE were evaluated by Differential Pulse Anodic Stripping Voltammetry (DPASV). Prior to the analysis, accumulation of Pb^{2+} and Cd^{2+} at the Cys-CNTPE proceeded with constant stirring for a specific duration of time. The resulting stripping peak currents and peak positions were correlated to the concentrations and types of heavy metal ions, respectively. From the voltammetry analysis of Pb^{2+} and Cd^{2+}, the Cys-CNTPE showed significantly enhanced sensitivity and selectivity, attributable to the synergistic combination of the recognition properties of $_{\text{L}}$-cysteine and the excellent electrical properties of MWCNTs. Optimization of the

experimental parameters was performed in order to obtain the best sensitivities for the determination of Pb^{2+} and Cd^{2+} at the Cys-CNTPE, in which the effect of accumulation time, reduction potential, reduction time, pH of acetate buffer solution and electrode paste composition were investigated. The optimum experimental conditions were found to be: (i) accumulation time of 10 minutes, (ii) reduction potential of -1.0 V, (iii) reduction time of 5 minutes, (iv) 0.1 M acetate buffer solution pH 5.0 and (v) electrode paste composition of 60:40 (w/w). Under the optimum conditions, the Cys-CNTPE exhibited a detection linear range from 1 to 15 μM with excellent linearity and detection limits of 0.1 μM and 0.2 μM for Pb^{2+} and Cd^{2+}, respectively. In addition, the Cys-CNTPE showed a good long-term stability for 2 months, as well as excellent precision and reproducibility with relative standard deviation less than 2% for eight repetitive measurements. The potential of the Cys-CNTPE for simultaneous detection of Pb^{2+} and Cd^{2+} was successfully demonstrated. Furthermore, the Cys-CNTPE exhibited high accuracy in real sample application with maximum relative error less than 4%, as compared to Atomic Absorption Spectrometry (AAS).

CHAPTER ONE

INTRODUCTION

1.0 Chapter Overview

This chapter presents an overview of the origins and interaction of heavy metals with the ecosystem, heavy metals pollution in Malaysia, the conventional analytical techniques and the biosensor for heavy metals detection. This is followed by a brief discussion of the problem statement, research objectives and the scope of study in this research work.

1.1 Heavy Metals – Origins and Its Interaction with Ecosystem

Heavy Metal is a quasi-scientific term to describe element that exhibit metallic properties, which mainly include the transition metals and some metalloids. However, the inconsistent use of the term “heavy metals” has lead to claiming the term to be meaningless and misleading in the 2002 IUPAC technical report (Duffus, 2002). Many different definitions have been proposed – in terms of density, atomic number or atomic weight and some on chemical properties or toxicity. United States Environmental Protection Agency has defined heavy metals as metallic elements with high atomic weights; can damage living things at low concentrations and tend to accumulate in food chain. To avoid general confusion, “heavy metals” is defined in this work as metallic chemical elements with a density above 5 g/cm^3, which include the transition metals from vanadium (V) to the metalloids arsenic (As), from zirconium (Zr) to antimony (Sb), from lanthanium (La) to polonium (Po), the lanthanides and the actinides (Nies, 1999).

Heavy metals are natural components of the Earth’s crust. They cannot be degraded and are persistent in the environment. Sources of heavy metals can be both of natural or anthropogenic origin: in rocks and soils, surface and groundwater, as well as in the atmosphere (Bradl, 2005). The main anthropogenic sources of heavy metals stemming from human activities are from agricultural activities and industrial activities (Siegel, 2002, Adriano, 2001). In agricultural activities for food production, heavy input of fertilizers and pesticides containing heavy metals, as well as soil amendments with sewage sludge and animal manures have introduced heavy metals into soils and subsequently contaminate the surface water and groundwater (Khairiah et al., 2006, Jordão et al., 2002). The main industrial activities that introduce heavy metals into the environment are metallurgical activities, coal and petroleum combustion, effluent streams and waste disposal (Alkarkhi et al., 2009, Nimmo et al., 1998). Metallurgical activities such as mining, smelting, metal finishing and electroplating are the primary sources of heavy metals pollution to the environment.

These human activities affect the natural geological and biological redistribution of heavy metals through various path of pollution. The effect of heavy metals on the environment is very complex as undesirable transfers occur continually among different environmental sections. The atmosphere is an effective medium, which can transfer the heavy metals and redeposit them in locations far away from their application sites (Latif et al., 2009, Tahir et al., 2009). Emission sources of heavy metals into the atmosphere are from mining activities (mineral dusts from ore extraction process), industrial activities (transportation, coal combustion and fugitive particulate emissions), as well as agricultural activities (fertilizers and pesticides aerosols). Eventually, heavy metals that are released into the air may end up in soils or water. On the other hand, heavy metals applied directly to the soil may be washed off by rain into nearby bodies of surface water and groundwater. These heavy metals are potentially accumulated in soils, sediments and water, uptake by land and marine organisms including crops and vegetable, which consequently transferred to human being through food chain and drinking water supply.

Despite the potential toxicity of heavy metals, some heavy metals are known to be of beneficial requirement by living organisms, such as calcium, copper, iron, manganese, molybdenum, vanadium, strontium and zinc (Irwandi and Farida, 2009). The three heavy metals commonly cited as being of the greatest public health concern are cadmium, lead and mercury that have no known beneficial need on organisms. Moreover, heavy metals are dangerous because they tend to bioaccumulate in living organisms and bioconcentrate in the food chain. Bioaccumulation means compounds accumulate in living organisms anytime they are taken up and stored faster than they are metabolized or excreted, resulting in an increase in the concentration of the compounds in a biological organism. Therefore, excessive levels or exposure to high concentration of heavy metals are hazardous to living organisms. Human are often exposed to heavy metals poisoning, mainly through the ingestion of heavy metals-contaminated food and drinking water, as well as the inhalation of ambient air with high concentrations of heavy metals (Tahir et al., 2009, Ismail et al., 2005). Allergic reactions, neurotoxicity, nephrotoxicity, cancer and cardiovascular disease are examples of the adverse health effects associated with heavy metals poisoning on human, whereby the treatment for most heavy metals poisoning is chelation therapy (Soghoian and Sinert, 2011).

1.2 Heavy Metals Pollution in Malaysia

Malaysia is classified as a newly industrialized country with high-growth industrial economies. The rapid development and economical growth through land development, urbanization and industrialization have resulted in increasing production and usage of toxic chemicals such as trace elements. Subsequently, issues of river pollution, contamination of agricultural soils and many others arise from heavy metals pollution. River pollution, especially Sungai Selangor, Sungai Klang and Sungai Langat were once reported to be heavily contaminated with industrial and domestic wastes, heavy metals and sewage (Consumers Association of Penang, 2007, John, 2006). These rivers supply water to the nearby residence, but heavy metals contamination has put the drinking water source in jeopardy to the public health. Landfills without proper leachate collection and treatment system pose a great risk of contamination to river water or any natural water bodies (Yusof et al., 2009). Leachate and runoff water from landfills are potentially hazardous due to the presence of substances, such as organic contents, inorganic macrocomponents, heavy metals and xenobiotic organic compounds. In Malaysia, a survey conducted in 2007 revealed that only 10 out of 179 landfill sites were equipped with a leachate treatment system (National Solid Waste Management Department, 2007). Uncontrolled landfills without appropriate bottom liners and leachate collection systems can contaminate the surface and groundwater. Heavy metals contamination of agricultural soils is due to the unsustainable farming practices of applying heavy doses of fertilizers and pesticides, which will potentially contaminate the crops and lead to heavy metals poisoning if consumed by human (Zarcinas et al., 2004).

The Straits of Malacca is subjected to a vast variety of pollutants due to its strategic location as a major international shipping lane and the concentrated agriculture, industry and urbanization activities on the west coast of Peninsular Malaysia (Yap et al., 2002). **Table 1.1** shows the sources of heavy metals input in the west coast of Peninsular Malaysia, which include manufacturing industries, agriculture and animal husbandry, agro-based industries and urbanization activities. These human activities jeopardize the ecosystem health and lead to the undesirable environmental changes in the west coast of Peninsular Malaysia and the Straits of Malacca. According to the Malaysia Environmental Quality Report 2009, point sources of water pollution mainly comprise from manufacturing industries (47.15%), sewage treatment plants (46.74%), animal farms (3.72%) and agro-based industries (2.39%), with Johor state having the highest number of water pollution sources followed with Selangor and Penang (Department of Environment, 2009). The main categories of scheduled waste generated in the country are dross/slag/clinker/ash, gypsum, E-waste, oil and hydrocarbon, clinical/pharmaceutical and heavy metals sludge. Of all industries, chemical, water treatment plant/power station, electronic/electrical and metal/engineering industries are the leading industries that contribute to the increasing amount of scheduled waste generated annually.

In order to address the issue of heavy metals pollution, Malaysia government has established and enforced the Environmental Quality Sewage and Industrial Effluents Regulations, as well as the National Water Quality Standards. The environmental monitoring of the surface water and groundwater quality status is vital to ensure that these water sources would comply with the regulations to safeguard the public health for the purpose of environment preservation.

Table 1.1: Sources of heavy metals input in the west coast of Peninsular Malaysia as published in the Malaysia Environmental Quality Report 2009 (Yap et al., 2002).

Sources	**Type of Industry/Product**
Manufacturing Industries	Chemical products Papers and printings Batteries Electroplating Textile and leathers Fertilizers/pesticides/insecticides Plastic-based products Rubber-based products Wood based products Electric and electronic industries Cosmetics Fungicides Fluorescent lights Dental amalgams Art supplies Mining and siltation Cement and cement products Iron, steel and tin workshops Welding fumes Medical equipment Smelting plants Metal fabrications Oil refineries Quarries Beverages and food
Agriculture and Animal Husbandry	Piggeries
Agro-based Industries	Palm oil mills Paddy mills Rubber mills
Urbanization/Population	Dusts (Highway/Industrial) Domestic discharges Landfills (Groundwater)

1.3 Analytical Techniques for Heavy Metals Detection

Conventional analytical techniques for the determination of heavy metals are Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). AAS is one of the most popular techniques for trace analysis of metals and metalloids, owing to its high sensitivity and selectivity (Gilmutdinov, 1999). Two stages are involved in AAS, where the first stage is the atomization of sample analyte with either flame atomizers (Hill, 2005), electrothermal atomizers (Fodor and Ipolyi, 2005), or vapour generation atomizers (Sturgeon, 2005). The second stage is the absorption of electromagnetic radiation at a wavelength specific to the analyte atoms. The absorption signal measured in atomic absorbance is correlated to the concentration of free analyte atoms for quantitative analysis. ICP-MS is a combination of two techniques, which are the inductively coupled plasma (ICP) and mass spectrometry (MS). The operating principle of ICP-MS is the same as in AAS, where the atomization of sample analyte is carried out in ICP and the absorption of radiation by analyte atoms in MS (Beauchemin, 1999). Both AAS and ICP-MS offer high precision, satisfactory sensitivity and selectivity for heavy metals determination. However, they suffer from the disadvantages of high maintenance cost, require skilled personnel and they are mostly laboratory bound.

Voltammetry is an electrochemical technique that imposes a potential scanning and measures the current flowing through the working electrode, which is immersed in the analyte solution containing electroactive compounds (Protti, 2001). It is a versatile technique for studying electrochemical reactions and quantitative analysis of reducible or oxidizable chemicals, notably trace amount of heavy metals (Janegitz et al., 2011, Trojanowicz, 2006). Anodic Stripping Voltammetry (ASV) is one of the most sensitive methods for the determination of trace heavy metals, owing to its inherent sensitivity and selectivity from the combination of a preconcentration step with a voltammetric stripping step. Examples of some commonly used ASV techniques are Linear Sweep Anodic Stripping Voltammetry (LSASV), Differential Pulse Anodic Stripping Voltammetry (DPASV) and Square Wave Anodic Stripping Voltammetry (SWASV). In preconcentration step, a negative potential is applied to the working electrode to accumulate the heavy metal cations on the electrode surface. The heavy metal cations are reduced as heavy metal atoms in the reduction step. Successively, an anodic scanning of potential (scanning towards positive potential) is imposed upon the working electrode, where the heavy metal atoms are stripped back into the analyte solution. The resulting voltammogram is a plot of current against potential with a peak shaped graphic, where the peak height and peak position are correlated to the concentrations and types of heavy metals in the analyte solution. ASV has the advantages of high sensitivity and selectivity in heavy metals detection, in addition to low cost and ease of use.

The main difference between ASV and AAS or ICP-MS is their operating principle. AAS and ICP-MS are optical techniques that can only analyze metal elements, as well as sulphur and phosphorus, whereas ASV is an electrochemical technique, which capable of analyzing reducible or oxidizable organic and inorganic compounds. Furthermore, ASV allows the analysis of metals at different oxidation number, but AAS and ICP-MS can only analyze total metals. The operating and maintenance costs of the conventional analytical techniques are significantly higher as compared to voltammetric technique. The spare parts and accessories for AAS and ICP-MS are very expensive, in addition to the requirement of significant analytical resources for sample analysis. The comparison summary of the conventional analytical techniques and voltammetry is presented in **Table 1.2**. Therefore, voltammetry demonstrates great potential in sensing applications for qualitative and quantitative analysis of trace heavy metals due to its advantages as compared to the conventional analytical techniques.

Table 1.2: Comparison summary of the conventional analytical techniques and voltammetry (Protti, 2001).

	AAS / ICP-MS	**ASV**
Operating Principle	Optical	Electrochemical
Analysis	Metals (Sulphur and Phosphorus)	Reducible or oxidizable organic and inorganic compounds
	Total metals	Metals at different oxidation numbers
Operating and Maintenance Costs	Higher costs ~ Lamps, spare parts and accessories are expensive ~ Require significant analytical resources (fuel and inert gas)	Lower costs ~ Electrodes and accessories for voltammetry are cheaper

1.4 Electrochemical Sensor and Biosensor

Sensor is described as an analytical device that measures a variable quantity and converts it into a processable signal (Ahammad et al., 2009, Grieshaber et al., 2008). The main components of a sensor are the sensing interface, the transducer interface and the signal processor. The sensing interface will perform the recognition event towards the specific target analyte and produce a signal. The transducer interface functions as both transducer element and signal amplifier, where the signal is converted into an electronic signal and amplified prior sending to signal processor. The signal processor will convert the electronic signal to a physical parameter, which describes the process being investigated. The analytical performance in terms of sensitivity, selectivity and stability are the most important aspect of investigation for a sensor. High sensitivity implies that a small change in the measured quantity results in a significant change in the sensor's output signal, whereas high selectivity implies that the sensor only respond to the signal of the measured quantity and reject other interferences. A sensor with high stability is capable of furnishing consistent measurements and signals after a long-term usage.

As discussed in the previous section, ASV demonstrates its advantages as a potential sensor platform for heavy metals detection due to its inherent sensitivity and selectivity. Despite that, the commonly used mercury working electrode in ASV is a major drawback due to the reason that mercury is a toxic pollutant and proper recovery is vital after each analysis (Serrano et al., 2003, Choi et al., 2001). The development of chemically modified working electrode in ASV has been the active research area to seek alternatives for the mercury working electrode. Fundamentally, chemically modified electrodes involve the attachment of specific chemical moiety as a recognition element in the sensing interface to impart selective recognition towards target analyte present in the analyte solution phase (Zen et al., 2003). There are many chemically modified electrodes reported for heavy metals detection, which can be categorized in terms of preparation method: thin film-coated electrode (Pan et al., 2009, Xu et al., 2008, Wu et al., 2003), carbon paste electrode (Guo et al., 2011, Ganjali et al., 2010, Janegitz et al., 2009) and screen-printed electrode (Hwang et al., 2008, Laschi et al., 2006, Honeychurch and Hart, 2003). Nanomaterials/nanoparticles and ionophores are examples of the modifiers that have been investigated as the recognition element in chemically modified electrode for heavy metals detection.

In stripping analysis to detect heavy metals, recent research activities involve the development of biosensor by exploiting amino acids and peptides as recognition elements for their high metal-chelating ability (Gooding et al., 2001). The terminal functional groups of biomaterials offer high binding affinity towards some heavy metals, which can be employed as a metal ion chelating agent to induce high sensitivity and selectivity. Recently, carbon nanotubes (CNTs) have been the material of research interest for electrode modification as the transducer element, owing to their high electrical conductivity, high surface area, excellent mechanical strength and good chemical stability (Ajayan, 1999). The excellent electrocatalytic properties of CNTs improve the electron transfer kinetics between the electroactive species and electrode, which ultimately enhance the analytical performance of the sensor (Rivas et al., 2007, Rubianes and Rivas, 2003). Therefore, functionalization of CNTs with biomaterials has gained much research attention to combine the specific recognition properties of biomaterials with the unique electrical properties of CNTs. The biosensor employing ASV with biomaterials functionalized CNTs working electrode will serve as an excellent sensor platform for heavy metals detection with higher sensitivity and selectivity, excellent precision and stability, low detection limits and good reproducibility.

1.5 Problem Statement

With the rapid pace of development and economical growth in nations across the world, heavy metals contamination is becoming a serious issue that receive attention from people of all levels in the society. Heavy metals are considered as one of the major toxic pollutants due to its long persistency in the environment as they cannot be degraded. Overwhelming utilization, improper disposal and redistribution of chemicals consisting heavy metals have contaminated the soils and water, which in turn, uptake in crop or vegetation and animals, jeopardizing our drinking water and food supply. Ingestion of heavy metals-contaminated food and drinking water, as well as the inhalation of ambient air with high concentrations of heavy metals are the common exposure route to the risk of heavy metals poisoning, which will lead to myriad adverse health effects on human (Tahir et al., 2009, Ismail et al., 2005). Of all heavy metals, cadmium, lead, and mercury are known as being of the greatest public health concerns since they have no known beneficial need on living organisms (Irwandi and Farida, 2009). Therefore, the detection and monitoring of these environmental pollutants in natural water bodies are of great importance to safeguard the public health, as well as to preserve our ecosystem and environment.

The conventional analytical techniques for heavy metals detection are AAS and ICP-MS. Although these techniques are precise with high sensitivity and selectivity, they suffer from the disadvantages of high maintenance cost, require skilled personnel, and they are mostly laboratory bound (Protti, 2001). ASV appears to be a promising alternative technique for heavy metals detection, owing to its inherent sensitivity and selectivity. However, there are drawbacks with the commonly used working electrodes in ASV, such as the poor sensitivities of glassy carbon electrode to detect trace levels of heavy metals (He et al., 2011a, Wu et al., 2003), in addition to the toxicity of mercury working electrode (Serrano et al., 2003, Choi et al., 2001). Although previous research activities have revealed the advantages of CNTs electrode with rapid voltammetric response and stable electrochemical behaviour, the bare CNTs electrode still lack satisfactory sensitivity and selectivity performances for heavy metals detection (He et al., 2011a, Hwang et al., 2008). In this study, a biosensor was developed by attaching $_{\mathrm{L}}$-cysteine to CNTs electrode as a recognition element for its high metal-chelating ability, and therefore to induce high sensitivity and selectivity in detecting trace levels of heavy metals. The hybrid sensing composite combines the recognition properties of $_{\mathrm{L}}$-cysteine and the excellent electrocatalytic properties of CNTs with a synergistic effect, which ultimately improve the analytical performance of the biosensor. The biosensor will serve as an excellent sensor platform to detect heavy metals with high sensitivity and selectivity, excellent precision and reproducibility, low detection limits and good long-term stability, as well as the potential for real time analysis.

1.6 Objectives

The main objective of this research is to develop a L-cysteine functionalized carbon nanotubes paste electrode (Cys-CNTPE) for the biosensor to detect lead ion (Pb^{2+}) and cadmium ion (Cd^{2+}) in the aqueous system. The research objectives to be achieved are listed below:

- To perform functionalization of multi-walled carbon nanotubes (MWCNTs) with L-cysteine as a recognition element for the heavy metals and to investigate the effectiveness of the Cys-CNTPE biosensor to detect heavy metals.
- To characterize the Cys-CNTPE biosensor and to obtain the optimum condition for detecting heavy metals with DPASV.
- To investigate the real sample application of the Cys-CNTPE biosensor and to perform comparison with the conventional analytical technique, AAS.

1.7 Scope of Study

The aim of this study is to develop Cys-CNTPE for the biosensor to detect Pb^{2+} and Cd^{2+} in the aqueous system via voltammetry technique.

Initially, raw MWCNTs were subjected to oxidation treatment with acid solution and oxidizing agent. L-cysteine was then covalently attached to the oxidized MWCNTs in the functionalization step. The cysteine functionalized MWCNTs, oxidized MWCNTs and raw MWCNTs were characterized with Fourier Transform Infrared Spectroscopy (FTIR). The Cys-CNTPE was prepared by mixing the cysteine functionalized MWCNTs with mineral oil, which was then packed into the well of a carbon paste electrode.

Subsequently, the Cys-CNTPE biosensor was evaluated for the determination of Pb^{2+} and Cd^{2+} by using DPASV. A comprehensive study on the effect of experimental parameters towards the determination of Pb^{2+} and Cd^{2+} with Cys-CNTPE biosensor was carried out. The experimental conditions were optimized with respect to the accumulation time, reduction potential, reduction time, pH of acetate buffer solution and electrode paste composition. Calibration plots for Pb^{2+} and Cd^{2+} detection were obtained under the optimum conditions. Reproducibility and stability tests were carried out to investigate the life time of Cys-CNTPE biosensor. In addition, the potential for simultaneous detection of Pb^{2+} and Cd^{2+} was demonstrated.

Finally, the Cys-CNTPE biosensor was subjected to real sample application and the results were compared to AAS.

1.8 Organization of the Thesis

This thesis is divided into five chapters. Chapter one gives an overview of the origins and interaction of heavy metals with the ecosystem, as well as the issue of heavy metals pollution in Malaysia. The advantages of biosensor for heavy metals detection compared to the conventional analytical techniques are discussed. The problem statement, research objectives and the scope of study are presented in the next section, followed by the organization of the thesis.

Chapter two presents the review of literature related to this research work. Fundamental information on CNTs, such as its general characteristics, synthesis methods, purification and functionalization techniques, as well as its various unique properties is presented. This is followed by the report of research works on the development of CNTs based sensor for heavy metals detection. In addition, research literatures related to the development of $_L$-cysteine for the biosensor to detect heavy metals are reviewed.

Chapter three provides an in-depth description on the materials and methodology performed in this study. Information of materials and chemicals used in this study are presented, followed with detailed explanation on the stage-by-stage experimental procedures. The analytical equipments and characterization methods are described in the last section.

Chapter four presents the results and discussion of this research work. The first section examines the results from FTIR characterization test. The voltammetry evaluation of the biosensor with Cys-CNTPE and bare CNTPE are reported in the next section. Subsequently, the optimization study of Cys-CNTPE biosensor is reported, where the effect of accumulation time, reduction potential, reduction time, pH of acetate buffer solution and electrode paste composition towards the determination of Pb^{2+} and Cd^{2+} are discussed. The calibration plots and detection limits for Pb^{2+} and Cd^{2+} detection, as well as the reproducibility and stability of Cys-CNTPE biosensor are reported. The potential for simultaneous detection of Pb^{2+} and Cd^{2+} at the Cys-CNTPE biosensor is investigated. The last section examines the performance of Cys-CNTPE biosensor in real sample application.

Chapter five presents the summary of the findings obtained in this research. Conclusion of the research is made and several recommendations for future studies are suggested.

CHAPTER TWO

LITERATURE REVIEW

2.0 Chapter Overview

This chapter presents the fundamental information on CNTs, such as its general characteristics, synthesis methods, functionalization techniques, as well as the unique properties of CNTs. Subsequently, recent research works on the development of CNTs based sensor for heavy metals detection are reviewed in the next section. In addition, research literatures related to the development of $_{\mathrm{L}}$-cysteine for the biosensor to detect heavy metals are reviewed.

2.1 Carbon Nanotubes (CNTs)

Nanotechnology focuses on the study, design and creation of materials, devices and systems at nanoscale, typically smaller than 100nm (Jianrong et al., 2004). It is based on the fact that at a specific composition, some matters in nanoscale may have new properties that are not displayed by the bulk matters. CNTs were discovered in 1991 by the Japanese electron microscopist Sumio Iijima who was studying the material deposited on the cathode during the arc-evaporation synthesis of fullerenes (Iijima, 1991). With the unique structural, mechanical, electrical and chemical properties of CNTs, they are one of the most commonly used building blocks in nanotechnology and therefore, receiving intense investigation towards the development of their application.

2.1.1 General Characteristics and Functionalization of CNTs

CNTs represent one of the best examples of novel nanostructures derived by bottom-up chemical synthesis approaches. CNTs can be essentially thought of as a rolled-up tubular shell of graphene sheet, which are built from sp^2 carbon units in hexagonal networks (Merkoçi, 2006). CNTs are classified as either single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs). SWCNT is formed by rolling a sheet of graphene into a cylinder and capped by hemispherical ends, which is a result of pentagon inclusion in the hexagonal carbon network of the nanotube wall during the growth process. The ways to roll graphene into tubes are mathematically defined by chiral vector (C_h) and chiral angle (θ) as illustrated in **Figure 2.1**. The chiral vector connects two crystallographically equivalent sites (A and A') on a graphene sheet. It is defined as $C_h = na_1 + ma_2$, where n and m are the integers of the chiral vector considering the unit vectors a_1 and a_2. The chiral angle is taken with respect to the zigzag axis (n,0).

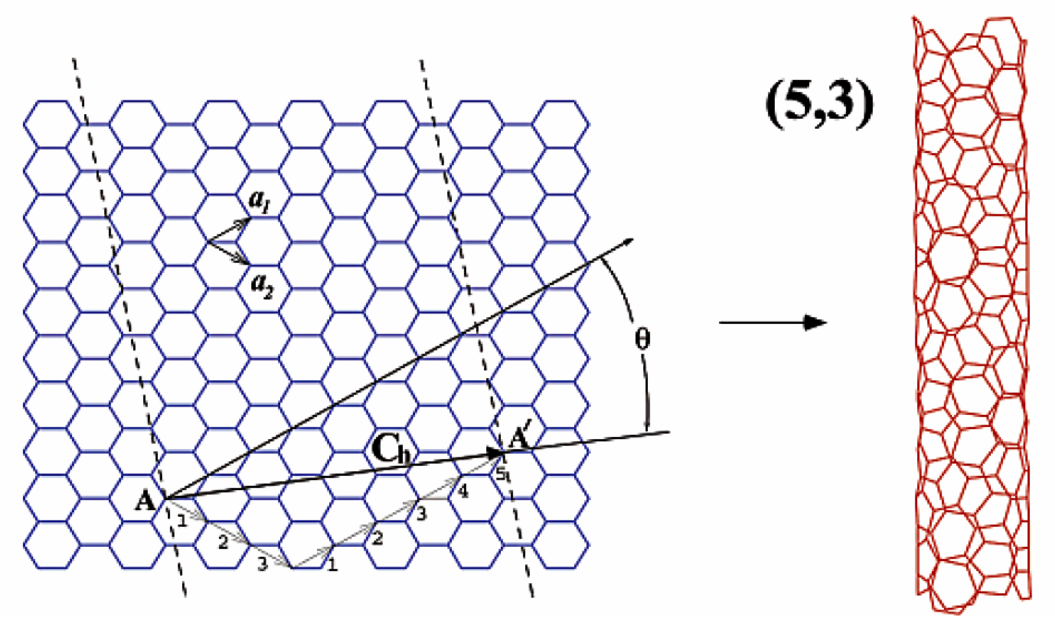

Figure 2.1: Schematic diagram of the graphene sheet and formation of SWCNT by rolling the graphene sheet along the lattice vector AA'. In the diagram: n = 5, m = 3, thus the chirality is (5,3) or $C_h = 5a_1 + 3a_2$ (Charlier, 2002).

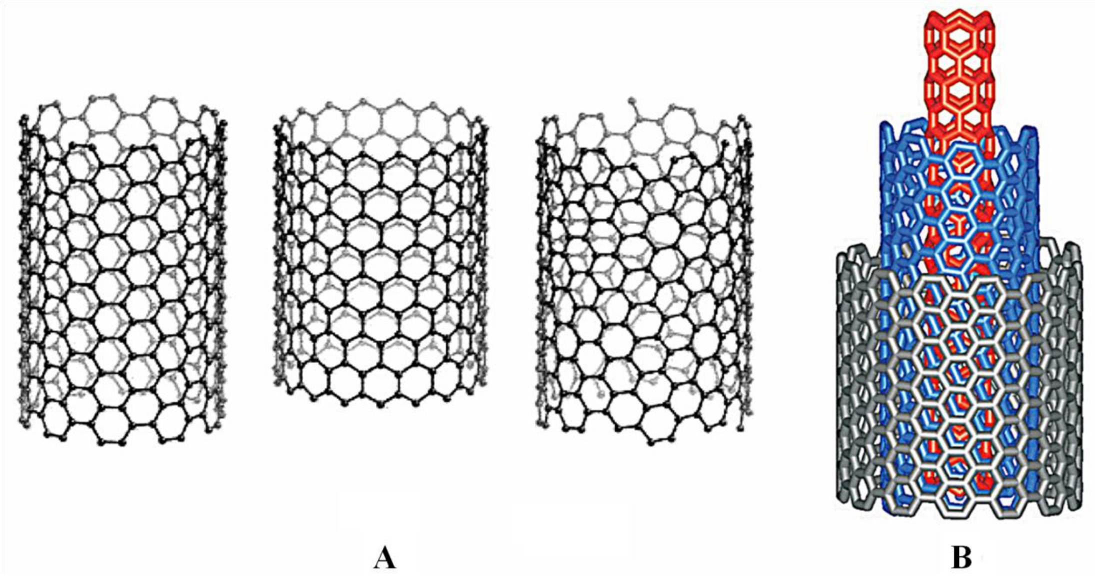

Figure 2.2: Schematic illustration of (A) three categories of SWCNTs defined by its chirality, from left to right: armchair, zigzag and chiral (Sloan et al., 2002) and (B) MWCNTs (Balasubramanian and Burghard, 2005).

The relation between *n* and *m* defines three categories of CNTs, which are armchair, zigzag and chiral types of CNTs as shown in **Figure 2.2A**. Armchair conformation of CNTs occurs when the graphene plane symmetry is parallel to the nanotube axis (n = m and $\theta = 30^o$). Whereas if the graphene plane symmetry is perpendicular to the nanotube axis, zigzag conformation of CNTs occurs (n = 0 or m = 0, $\theta = 0^o$). All other chirality ($n \neq m \neq 0$ and $0^o < \theta < 30^o$) correspond to the chiral conformation of CNTs. The diameter of SWCNTs is around 0.4 – 3 nm and several micrometers in length (Balasubramanian and Burghard, 2005).

MWCNT is a stack of graphene sheets rolled up in concentric cylinders as shown in **Figure 2.2B**. The diameters of MWCNTs are between 2 to 25 nm, whereas the distance between sheets or inter layer spacing is about 0.34 nm (Ajayan, 1999). Concentric MWCNTs are defined as having each layer of nanotube walls parallel to the central axis. Some other inner textures may be found in MWCNTs, such as the herringbone texture and bamboo texture (Rodriguez et al., 1995). The herringbone texture, in which the graphene make an angle with respect to the nanotube axis and the angle varies upon the processing conditions, is generally obtained from catalyst-enhanced thermal cracking of hydrocarbons. The bamboo texture is described as the occurrence of limited amount of graphenes oriented perpendicular to the nanotube axis and it affects both concentric MWCNTs as shown in **Figure 2.3A** and herringbone MWCNTs as shown in **Figure 2.3B**

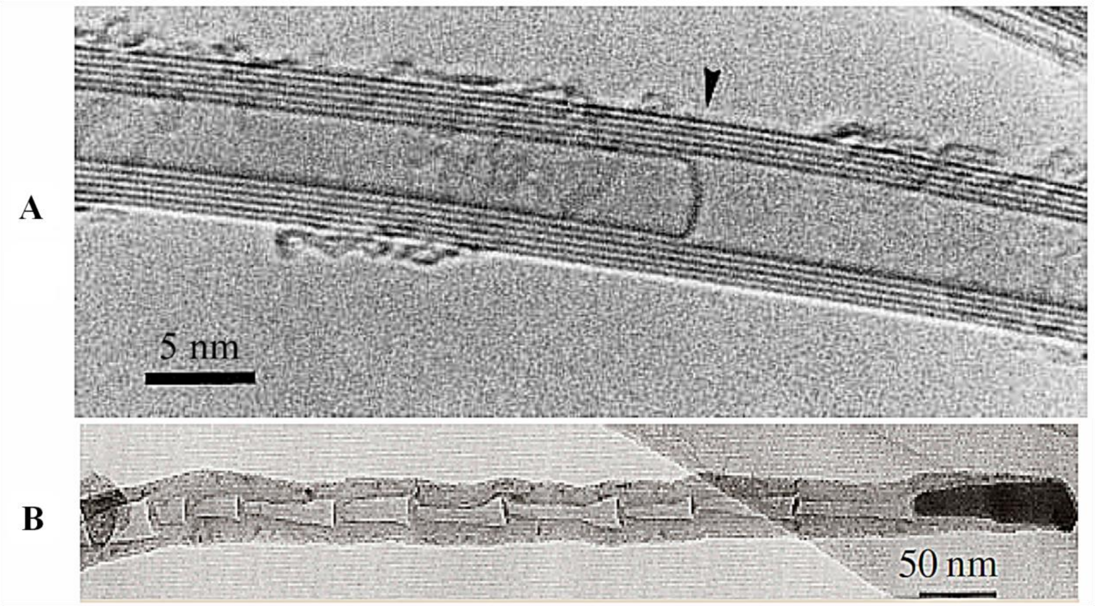

Figure 2.3: High-resolution transmission electron microscopy images of (A) concentric MWCNTs with bamboo texture (Harris, 1999) and (B) herringbone MWCNTs with bamboo texture (Saito, 1995).

Arc-discharge, laser ablation and chemical vapour decomposition are the three main methods for the synthesis of CNTs. In arc-discharge method, CNTs are synthesized through arc-vaporization of two graphite rod placed end to end, namely anode electrode and cathode electrode (Ando and Iijima, 1993). Laser ablation method involves the sublimation of graphite target and condensation of laser-vaporized carbon/metal mixtures to form CNTs (Guo et al., 1995). As for chemical vapour decomposition, it involves the catalytic decomposition of a carbon containing gas and nanotube growth on metal catalyst particles impregnated on a substrate (Che et al., 1998). Arc-discharge and laser ablation are widely employed for the synthesis of SWCNTs due to the high process temperature of 1200^{o}C and above, which favours SWCNTs formation. Chemical vapour decomposition is suited for mass production of MWCNTs as it is a simple and economic technique at lower process temperature of 500 to 1000^{o}C.

Regardless of the synthesis method applied, as-prepared CNTs usually contain a significant amount of impurities, such as amorphous carbon, carbon nanoparticles, graphitic debris, catalyst particles and fullerenes (Baughman et al., 2002). These impurities often interfere with the desired properties of CNTs and impede CNTs for detailed characterizations and advanced applications. Various purification techniques have been employed for the removal of impurities, which include flocculation, microfiltration, chromatographic, centrifugation, gas phase oxidation, liquid phase oxidation and chemical functionalization (Vairavapandian et al., 2008). Typical purification process involves combinations of these protocols, in which first stage gas phase oxidation followed with second stage liquid phase oxidation are widely employed. Gas phase oxidation is an easy and fairly effective purification step to eliminate amorphous carbon and carbon nanoparticles (Shi et al., 1999). Subsequent liquid phase oxidation usually employs an acid treatment with strong oxidizing agent or acid solution. The liquid phase oxidation not only removes the catalytic metals together with some amorphous carbon, but it causes defects and/or shortening of CNTs (Vaccarini et al., 1999, Chen et al., 1998). The oxidation purification steps introduce carboxyl group at defective sites of the open ends and the sidewall of CNTs, which are beneficial for further functionalization.

CNTs posses a framework structure of sp^2 hybridized carbon, therefore making its sidewall very hydrophobic and enriches π-stacking. These properties allow the functionalization of CNTs to prepare a wide range of nanotubes coupled with different types of materials, expanding their application range through novel nanodevices with new functions and applications. Functionalization of CNTs allows the unique properties of a nanotube to be coupled to those of other types of materials, as well as to improve the solubility and processibility of CNTs (Hirsch, 2002). Several approaches have been developed to functionalize CNTs, which include defect-group functionalization, covalent sidewall functionalization, noncovalent exohedral functionalization with either surfactants or polymers and endohedral functionalization as illustrated in **Figure 2.4**. Defect-group functionalization involves the reactive groups which lie at the defect positions along the sidewall or tube ends of CNTs, such as vacancies, Stone-Wales defect, open ends of nanotubes closed by metal catalyst particles, etc. These reactive groups are oxygenated functional groups such as carboxyl groups, which are produced during the purification of CNTs (Hamon et al., 1999). Covalent sidewall functionalization involves the covalent bond formation between the materials and CNTs with the presence of a highly reactive reagent. The sidewall functionalization of SWCNTs with organic groups is possible by reactive species such as nitrenes, carbenes and radicals (Holzinger et al., 2001).

Noncovalent exohedral functionalization involves either the aggregation of CNTs with surfactants or wrapping of CNTs with polymers. With the presence of surface active molecules, such as sodium dodecylsulphate or benzylkonium chloride, CNTs can be dispersed in the aqueous phase without purification process (Krstic et al., 1998). Aggregation of CNTs with surfactants is due to the effective π-π stacking interactions between the aromatic groups of the surfactants and the graphitic sidewall of CNTs. The suspension of purified CNTs in the presence of polymers in organic solvents will lead to the polymer wrapping around the nanotubes (Coleman et al., 2000). The properties of these polymer-functionalized nanotubes are markedly different from those of the individual components, such as higher conductivity. Endohedral functionalization involves incorporating or uptake of materials in the inner cavity or capillaries of CNTs. One example is the incorporation of fullerenes in the sidewall of CNTs, in which the encapsulated fullerenes tend to form chains that are coupled by van der Waal forces. Upon annealing, the encapsulated fullerenes coalesce into the interior of CNTs to produce fullerene peapods, a new concentric and endohedral nanotubes with diameter of 0.7 nm (Smith and Luzzi, 2000).

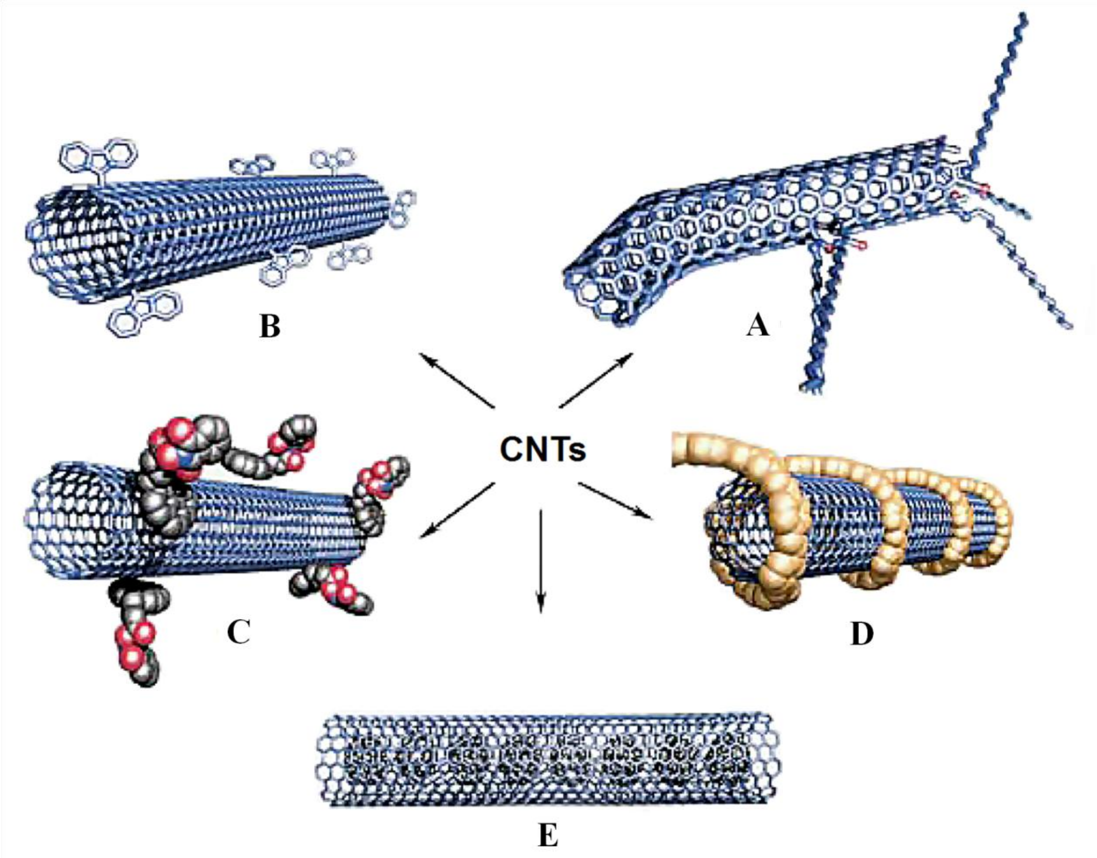

Figure 2.4: Functionalization possibilities for CNTs: (A) defect-group functionalization, (B) covalent sidewall functionalization, (C) noncovalent exohedral functionalization with surfactants, (D) noncovalent exohedral functionalization with polymers and (E) endohedral functionalization (Hirsch, 2002).

2.1.2 Physical, Electrical and Chemical Properties of CNTs

The unique physical, electrical and chemical properties of CNTs are attributed to the structural topology (chirality) and the hybridized sp^2 covalent bonding between the carbon atoms (Hu et al., 2007, Ajayan, 1999). Carbon has four valence electrons with two in the 2s subshell and two in the 2p subshell to form a ground state orbital configuration of 1s^2, 2s^2, 2p^2. Carbon forms a relatively stable excited state by promoting one of its 2s electrons into its empty 2p orbital, which allows carbon to assume different hybridization. Carbon units in the sidewall of CNTs are involved in sp^2 hybridization, in which a carbon atom is covalently bonded to three neighbouring carbon atoms via three sp^2 hybrid orbitals, whereas the remaining pure p orbital is allowed to delocalize among all carbon atoms and enriches π-stacking. Therefore, the in-plane properties of CNTs are similar to those of graphite, such as high conductivity, excellent strength and stiffness. Furthermore, the curvature of the sidewall causes distortions to the normally planar sp^2 hybridization, resulting in certain degree of sp^3 hybridization and misalignment of π-orbitals, which endows CNTs with some unique properties.

Table 2.1: Physical properties of CNTs
(Monthioux et al., 2007, Xie et al., 2005, Yu et al., 2000).

Properties	**SWCNTs**	**MWCNTs**
Specific Surface Area	400 – 900 m^2/g	200 – 400 m^2/g
Specific Gravity	0.8 g/cm^3	1.8 g/cm^3
Tensile Strength	13 – 52 GPa	11 – 63 GPA
Elastic Modulus	~ 1 TPa	~ 0.3 – 1 TPa
Thermal Conductivity	3000 Wm^{-1}K^{-1}	
Thermal Stability	> 700^{o}C in air ; 2800^{o}C in vacuum	
Magnetic Susceptibility	22 x 10^6 EMU/g (perpendicular with plane) 0.5 x 10^6 EMU/g (parallel with plane)	