CHAPTER 1

Introduction

1.1 Statement and Significance of Problem

The accurate detection and quantification of chemical and biological systems are of great significance in many areas including health care and environmental monitoring. Due to instability of the biomolecular recognition elements (antibody, enzyme, or bio-receptors) to the environment, many attempts have been put onto molecular recognition sites in conducting polymers (CPs) by molecular imprinting technique. The molecularly imprinted conducting polymers (MICPs) possess an ability to remember closely related compounds or to rebind specific template molecules via an artificial molecular lock and key process. The artificial receptor sites of the CPs can be shaped by the template molecules. Therefore, MICPs can be developed to become electrochemical sensors with superior performances such as high selectivity, low limit of detection, and good sensitivity to only the target molecules. In general, MICPs used as sensing process could be formed on the electrode surface by an electropolymerisation technique which allows the generation of the uniform MICPs film with a good adherence onto an electrode surface. In addition, the thickness and the density of the film can be adjusted by controlling polymerisation conditions, which would affect their performances of the device. Therefore, the MICPs have been extensively investigated for the development of better sensors.

1.2 Background

1.2.1 Electrochemical sensors

The electrochemistry involves charges and their transfer movements from one intermediate to others. The fundamental unit of charge is carried by electron [1]. The electrochemistry indicates the transfer of charge from an electrode to another phase, which can be found in a solid or a liquid sample. While in this process, the chemical changes take place at the charges and the electrodes is conducted through the bulk of the sample phase. Both the electrode reactions and the charge transport can be modified chemically and serve as the foundation of the sensing process. Electrochemical sensors are based on amperometry, potentiometry, or conductivity measurements. The different principles always require a specific design of the electrochemical sensors [1, 2]. The construction of electrochemical sensors is shown in Figure 1.1. Their operating and measurement principle is summarised, according to the types of the signal generations.

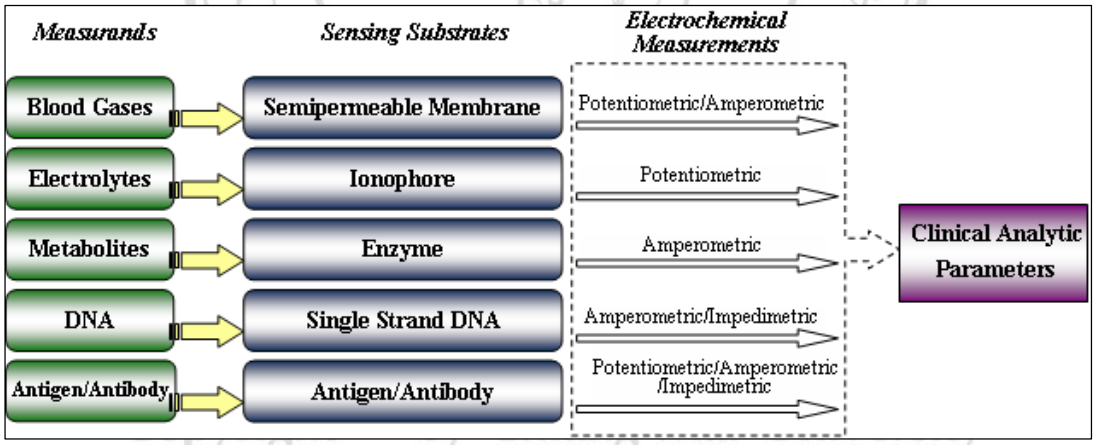

Figure1.1 Clinical analysis procedures based on electrochemical sensors [2].

The analytical information of the electrochemical sensors can be obtained from the electrical signal that resulted from the interaction of the target molecule at the electrode surface. Such sensors have advantages over conventional analytical instruments. For example, they can improve the performance of the analytical tools and operate with

shorter period of time and the use of low-cost reagents and analytical tools. Furthermore, the electrochemical sensors have been hampered by electrochemically active interferences in the sample, poor stability as well as complicated electron-transfer pathways. Nevertheless, electrochemical sensors have been used for many potential applications in environmental monitoring, clinical diagnosis and food analysis [2]. The key features of an effective sensor are that the investigation method is simple and fast, and the electrochemical sensors can be developed for parallel detection of multiple target molecules. However, in order to have high sensitivity, low limit of detection and selectivity, existing technologies need to be further developed [3].

1.2.2 Dopamine

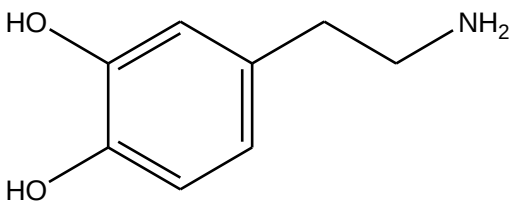

Figure 1.2 molecular structure of dopamine in urine and blood

Dopamine (DA) is a hormone and neurotransmitter classified in the catecholamine and phenethylamine species that plays a significant role in the central nervous, renal, hormonal, and cardiovascular systems. In the brain, DA is chemically released by nerve cells to send signals to other nerve cells. The brain includes several distinct DA systems, each of which contributes to reward motivated behaviour. As a part of the reward pathway, DA is manufactured in the nerve cell bodies located within the ventral tegmental area (VTA) and is released in the nucleus accumbens and the prefrontal cortex. The motor functions of DA are linked to a separate pathway, with cell bodies in the substantia nigra that manufacture and release DA into the striatum[4]. DA systhesis from the amino acid tyrosine using 2 enzyme protein. The enzyme protein are hydroxylase and dopa decarboxylase.

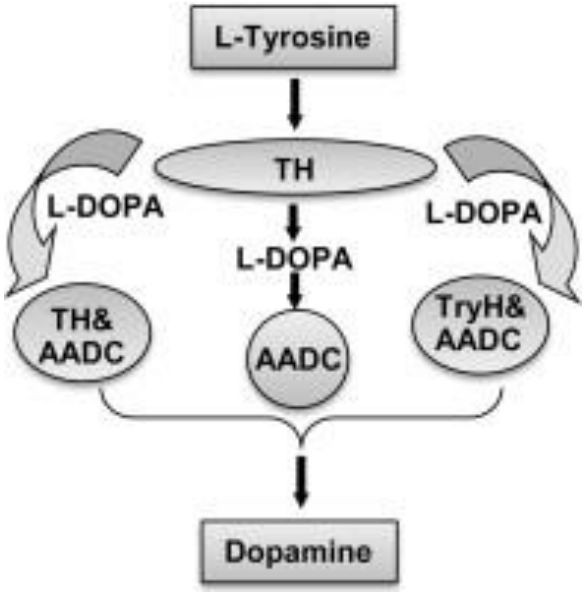

Figure 1.3 representation of dopamine synthesis by the neurons, containing only tyrosine hydroxylase, together with: (i) the neurons containing only aromatic L-amino acid decarboxylase (center); (ii) the dopaminergic neurons (left side); (iii) the serotoninergic neurons (right side). AADC, aromatic L-amino acid decarboxylase; L-DOPA, L-3,4-dihydroxyphenylalanine; TH, tyrosine hydroxylase; TryH, tryptophan hydroxylase [5].

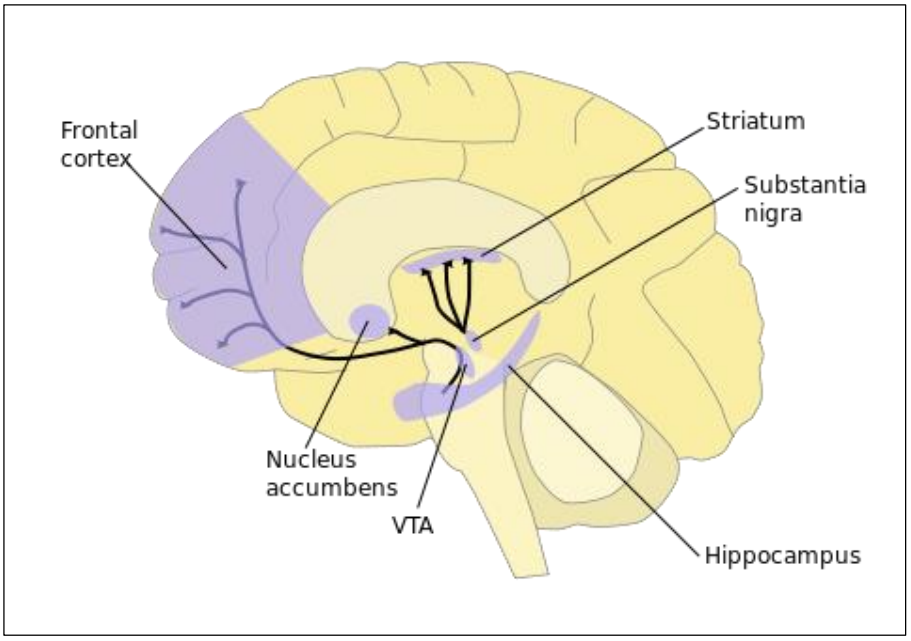

Figure 1.4 Major DA pathways [4].

Rapid, simple, and sensitive electrochemical methods have shown promising in the neurotransmission detection. However, at traditional solid electrodes, DA and its coexisting species, i.e. ascorbic acid (AA) and uric acid (UA) have an overlapping voltammetric response, resulting in rather poor sensitivity and selectivity of DA. Thus, it is a challenge to distinguish the coexistence of DA, AA, and UA in a biological environment [4].

Several research groups have investigated the determination methods and the problems of detection in order to improve the sensitivity and selectivity. These methods include fluorimetry, spectrophotometry and highly selective and sensitive determination with modified glassy carbon electrodes (GCEs). Recently, the molecular imprinting technique has been described as a versatile tool for cost-effective preparation of polymeric materials with affinity and selectivity towards rebinding of the interesting target molecules [4]. Moreover, electrochemical sensors for the determination of DA have received increasing attention for an early diagnosis of Parkinsonism, schizophrenia, and scurvy, etc. Compared to the traditional clinical methods, electrochemical methods work with simple operating processes, rapid detection and cost-effectiveness. Disease analysis would deal with monitoring the electrochemical redox properties of DA [6, 7]. Unfortunately, successful implementation of such strategies has been elusively proven and there are only a small number of reports that showed the investigation of DA electrochemical sensing in unprocessed serum samples. Typically, DA sensors can be applied to a determination of DA in serum diluted with PBS or even in pure PBS but it is difficult to demonstrate the complex serum environment for the detection. Therefore, development of good sensors that overcome the effect of serum interferences is needed. The detection in a very low DA concentration and a wide concentration range are necessary for the fabrication of better sensors. Hence, direct determination of DA on the MI material-based electrodes will perform with advantages of preconcentration at the electrode via a selective rebinding/recognising of the target analytes and then an electro-oxidation with a shorter diffusion path, potentially resulting in good performances of the sensing devices. New sensor platforms based on the MICPs would be in urgent needs for the potential applications such as disease diagnostics and medical monitoring [6, 8].

1.2.3 Graphene

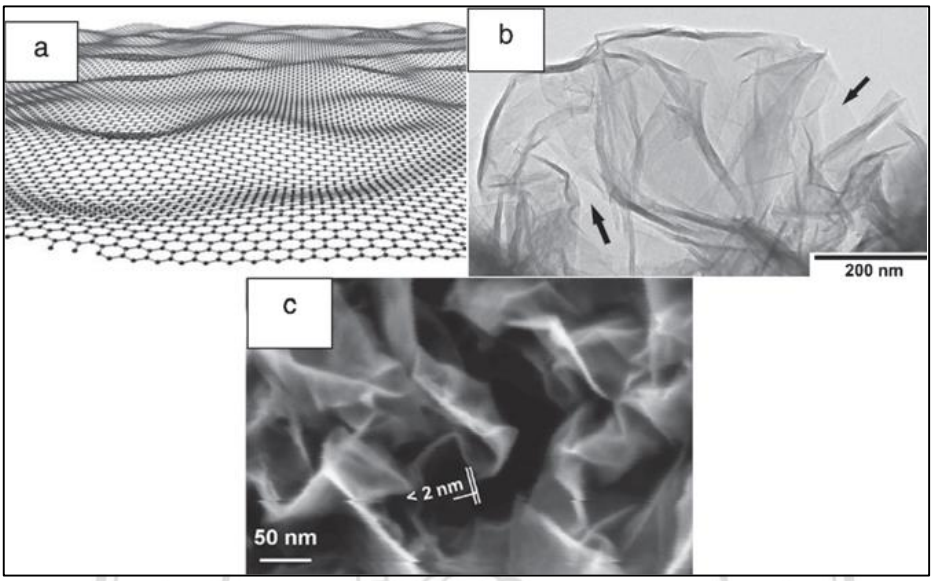

Figure 1.5 (a) Structural model of pristine GP, (b) TEM image of GP and (c) SEM image of GP. GP (b-c) is produced from chemical reduction of GPO [4].

Graphene (GP) is a monoatomic planar sheet of sp^2 carbon-based material that has attracted recent interests due to its exceptional electrical, mechanical, thermal, and sensing properties. Due to its single-atomic-layer structure, separation of GP has been recently achieved via chemical vapor deposition (CVD), sputtering, mechanical exfoliation, and chemical exfoliation. GP-based ink is of particular interest due to its advantages of being processed in solution, which facilitates the low-cost development of electronic and optical devices [9]. GP has a large theoretical specific surface area (2630 m^2g^{-1}), high intrinsic mobility (200,000 $cm^2v^{-1}s^{-1}$), thermal conductivity (~5000 $Wm^{-1}K^{-1}$) and high Young's modulus. Its optical transmittance is about 97.7%. It has good electrical conductivity which is suitable for electronic applications such as transparent conductive electrodes and printed circuits. Furthermore, GP provides essentially infinite possibilities for the modification and functionalisation of its carbon backbone [9, 10]. Thus, GP has attracted in the development of scientific and technological interests in recent years. It has exhibited great promising properties in utilisation as energy storage (supercapacitors and batteries), electronics

(bioscience/biotechnologies and electrochemical sensor) and energy conversion (solar cells and fuel cells) because of its unique physicochemical properties, excellent thermal conductivity, high surface area, good electric conductivity and strong mechanical strength. Many methods have been developed to produce GP. In 2004, Geim *et. al.* first reported a preparation of GP by mechanical exfoliation of highly oriented pyrolytic graphite. This method, which is called the Scotch-tape method, is still widely used in many laboratories to obtain pristine perfect structured GP sheets for basic scientific research and for making proof-of-concept devices. Nevertheless, it is not suitable for mass production. Another method for producing defect-free/defect-less GP is the mild exfoliation of graphite but the yield so far is very low. GP has also been prepared by thermal decay of SiC wafer under ultrahigh vacuum conditions or by CVD growth on metal substrates (ruthenium, copper, and nickel) or by substrate-free CVD process. This is a potential mass-production method with the aim of producing GP for electronics applications. Some other mass-production methods are chemical or thermal reductions of GPO. They are also considered to be the most economical way to produce GP. Most of GPs used in electrochemistry are produced by the last methods. GP from GPO reduction, which is also called functionalised GP sheets or chemically reduced GPO, usually has various structural defects and functional groups that are profitable for electrochemical applications. This section will focus on this kind of graphene.

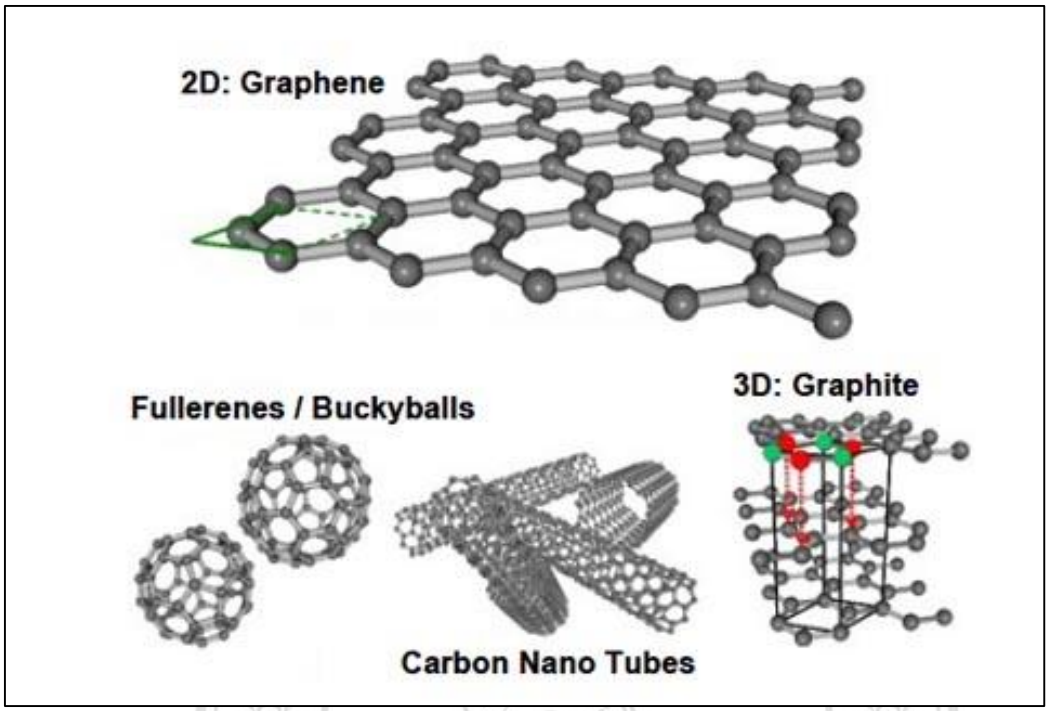

Figure 1.6 Graphenes come in a variety of structures from atom-thick sheets to Buckyballs and Nanotubes.

Carbon might be the most extensively-used material in electrocatalysis and electroanalysis. For example, CNTs have shown excellent capability in biosensors, biofuel cells, and polymer electrolyte membrane fuel cells. GP-based electrodes have shown superior performance in terms of electrocatalytic activity and macroscopic scale conductivity than carbon nanotubes based ones. The findings indicate that the opportunities in electrochemistry encountered by carbon nanotubes could be available for GP [7]. GP is the initial building block for graphitic materials of all other dimensionalities are 0D fullerenes, 1D nanotubes, 2D graphene and 3D graphite (Figure 1.6); Functionalised GP produced through reduction of GPO displays a twisted structure due to the presence of lattice defects and this is different than the rippled structure observed in pristine GP. Studying GP is expected to provide a basic insight into all carbon materials. In comparison with carbon nanotubes, GP exhibits potential advantages of high surface area, low cost, ease of preparation and safety. GP, due to its high purity (differentiation metals, Fe, Ni, etc. are absent in GP from GPO reduction,

not like carbon nanotubes), offers a good platform to study the electrocatalytic effects of carbon materials. GP is prospective to gradually contest against carbon nanotubes in many applications [10, 11].

The GP honeycomb lattice is composed of two equivalent sub-lattices of carbon atoms bonded together with σ bonds, as shown in Figure 1.7a. Each carbon atom in the lattice has a π orbital that conduce to a delocalised network of electrons. That independently suspended graphene has 'intrinsic' ripples or not has been addressed by Monte Carlo simulation and transmission electron microscopy (TEM) studies. The microscopic corrugations (Figure 1.7b.) were evaluated to have a lateral dimension of about 8 to 10 nm and a height displacement of about 0.7 to 1 nm. Sub-nanometer fluctuations in height for GP platelets deposited on SiO_2 -on-Si substrate were studied by scanning tunneling microscopy (STM). Although some STM experiments indicated a limited or negligible correlation between small (< 0.5 nm in height) corrugations and local electrical properties, evidence has been presented for strain induced local conductance modulations for bigger ripples (2–3 nm in height). Ripples can be induced, suggesting that the local electrical and optical properties of GP could be changed through 'ripple-engineering' for possible application in the devices [10].

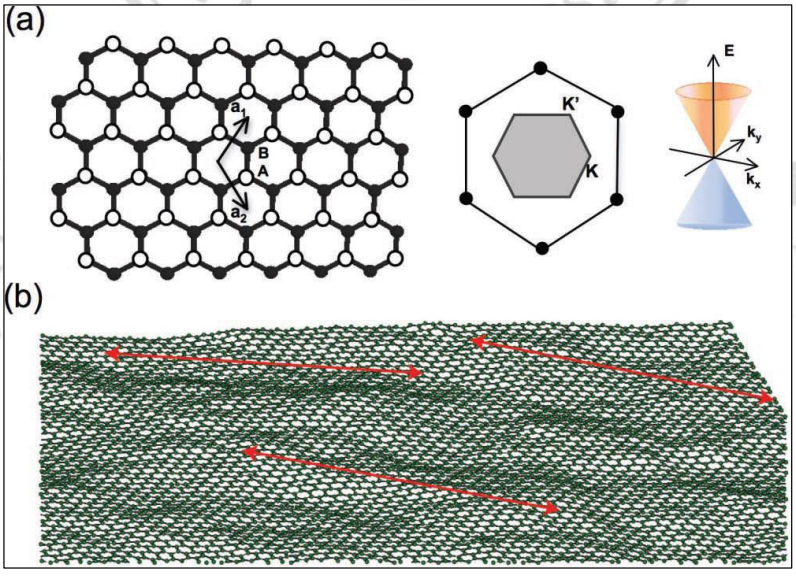

Figure 1.7 (a) Schematics of the crystal structure, Brillouin zone, and the dispersion spectrum of graphene; (b) 'Rippled graphene' from a Monte Carlo simulation. The red

arrows are ~ 8 nm long. Reproduced with permission from (b). Copyright: 2007 Nature Publishing Group (b) [10].

The excellent electrochemical behaviours of graphene indicate graphene as a promising electrodematerial in electroanalysis. Several electrochemical sensors based on graphene and graphene composites for bioanalysis and environmental analysis have been developed [4].

1.2.4 Conducting polymers

The conducting polymers (CPs), such as Polyaniline (PANI), Polypyrrole (PPy), Polythiophene (PTh) and their derivatives, have been applied as the active layers of sensors since early 1980s. In comparison with commercially usable sensors, based usually on metal oxides and operated at high temperatures, the sensors made of CPs have many enhanced characteristics. They have high sensitivities and short response time; especially, these features are ensured at room temperature. CPs are easy to be prepared through chemical or electrochemical processes, and their molecular chain structure can be modified conveniently by copolymerisation or structural derivations. In addition, CPs have good mechanical properties, which allow easy fabrication of sensors. As a result, increasing attentions have been put onto the sensors fabricated from CPs, and various related articles have been published [9]. The CPs mentioned in this section review all refer to intrinsic CPs. Their main chains consist of alternative single and double bonds, which leads to a broad p-electron conjugation. Figure 1.8 presents many typical CPs used as the active layers in gas sensors. Nevertheless, the conductivity of these pure CPs are low ($<10^{-5}$ S cm^{-1}). In order to achieve highly conductive CPs, a doping process is essential. The concept of doping is that distinguishes CPs above all other polymers. CPs can be doped by a redox reaction or a protonation, though the latter is only applicable to PANI [12].

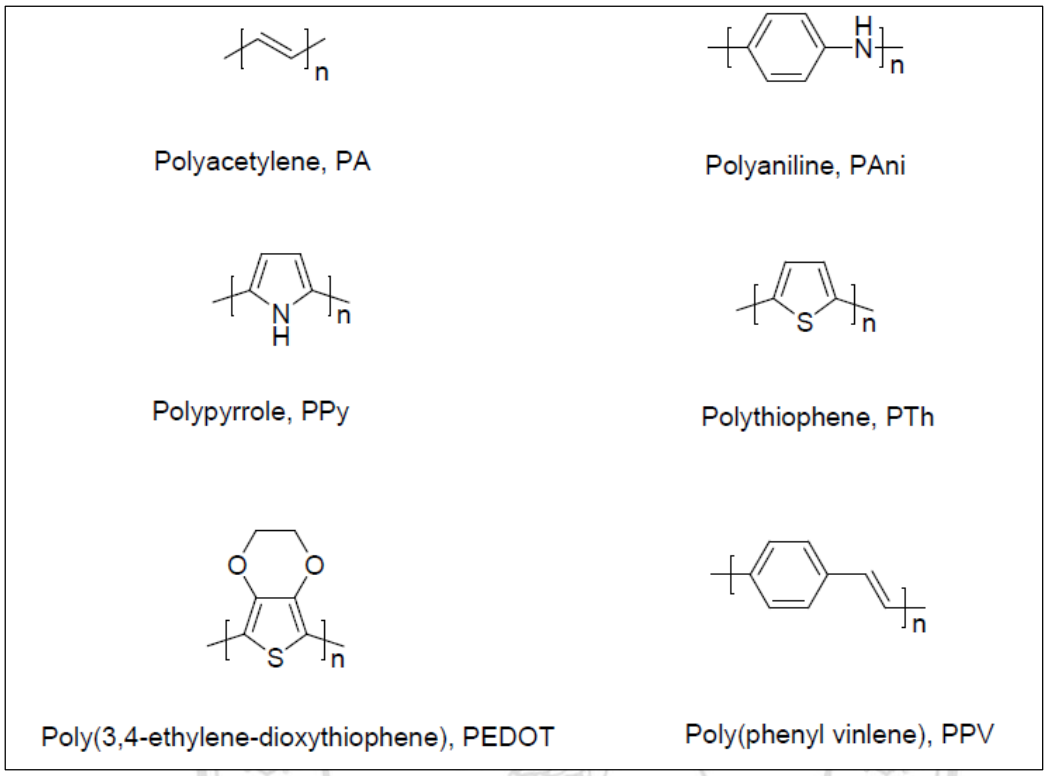

Figure 1.8 Several typical conducting polymers [13].

1.2.4.1 Preparation of conducting polymer films [12, 14]

Active layer is the most important component of a sensor. A variety of techniques have been expanded to prepare CPs films, in order to adjust to different sensing materials and other types of sensor configurations. Thus, how to deposit conducting polymer films will be discussed.

1) Electrochemical deposition Electrochemical deposition is the most suitable method to deposit conducting polymer films. The thickness of the film can be controlled by the total charge passed through the electrochemical cell during the film growing process. Moreover, the film can be deposited on electrode, which the deposition must be carried out on a conducting substrate. Nevertheless, if the insulating gap between the neighboring electrodes is close enough (~several tens of micrometer), the increasing film can cover the insulated gap and connect

the electrodes. This is significant in fabricating chemiresistors.

2) Dip-coating
3) Spin-coating
4) Langmuir-Blodgett (LB) technique
5) Layer-by-layer (LBL) self-assembly technique
6) Thermal evaporation
7) Vapor deposition polymerisation
8) Drop-coating
9) Other methods

1.2.5 Polyaniline

Polyaniline (PANI) is one of the most interesting CPs due to its, good redox reversibility, high conductivity, envirinmental stability, rapid colour change with applied potential and low cost. Consequently, PANI is extensively used for various applications in many fields. PANI is a category of forms that vary in physical and chemical properties [15]. PANI was initially known in 1835 as “aniline monomer”, a term used for any product obtained by the oxidation of aniline. The PANI are well-known for its ease of synthesis and unique acid/ base doping/dedoping and oxidation/reduction chemistry. PANI can be synthesised by chemical oxidation or electrochemical polymerisation of aniline. In its emeraldine oxidation state, PANI becomes electrically conducting when doped with a strong acid. The doping level can be tuned simply by controlling the pH of the dopant acid solution. The conductivity of PANI increases reversibly with doping from the undoped insulating base form to the fully doped, conducting salt form. Conductivity can also be controlled with chemically or electrochemically by changing the oxidation state (Figure 1.8) .The reversible conductivity achieved by doping makes PANI a promising material for many applications including in batteries, actuators, electromagnetic shielding, antistatic coatings, corrosion protection, and electro-optic, electrochromic devices and sensors [3, 16, 17].

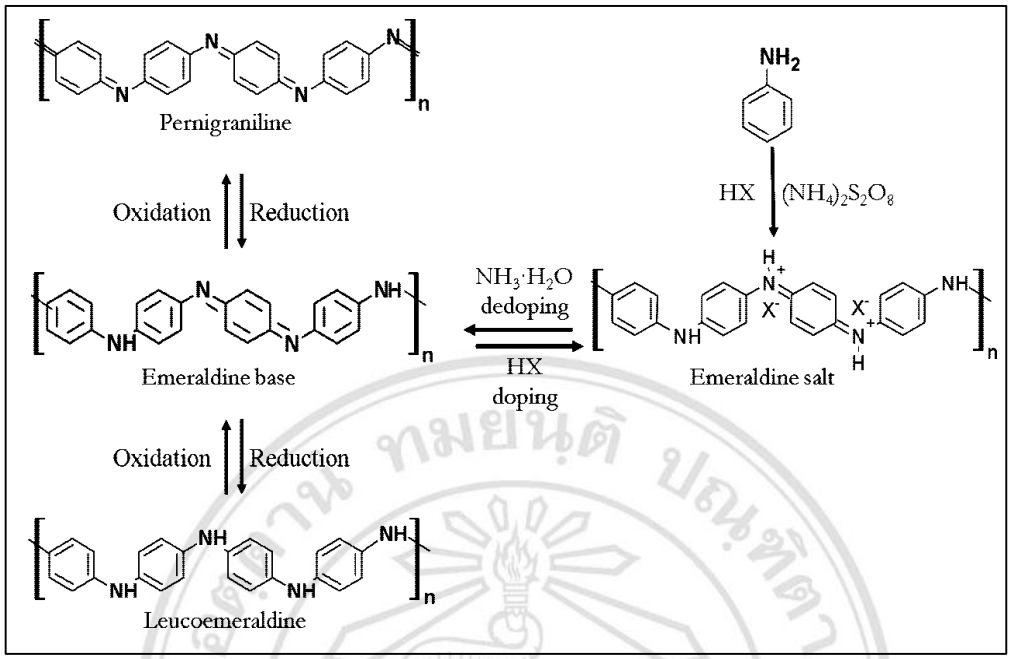

Figure 1.9 Schematic diagram showing the chemical structure, synthesis, reversible acid/base doping/dedoping, and redox chemistry of polyaniline [15].

1.2.6 Molecularly imprinted polymers

Recently, aniline based copolymer have been synthesised and applied as a molecularly imprinted polymers (MIPs). MIPs can be used applications in many fields such as sensors, antibody and enzyme. They are very useful materials for isolation and preconcentration of low concentration analytes employing the solid phase extraction (SPE) technique [6]. MIPs are synthetic materials with artificially created recognition sites able to specifically rebind a template molecule in preference to closely related compounds. These materials are obtained by polymerising cross-linking monomers and functional around a template molecule, leading to a highly cross-linked on the network polymer [18]. The monomers are chosen considering their capability to interact with the functional groups of the target molecule. Polymerisation has taken place, template molecule is removed and binding sites with size, shape and functionalities complementary to the target molecule are conformed. Therefore, the imprinted polymers are high stable, durable and resistant to a wide range of pH, temperature and solvents. Therefore, the behaviour of MIPs imitates the interactions confirmed by

receptors to selectively with remember a target molecule but without the associated stability limitations. [6, 19].

MIPs the products of molecular imprinting are generated by the polymerisation of functional monomers with crosslink polymer in the presence of a target analyte that serves as a "template" molecule. During the conventional polymerisation step (Figure 1.10), the template molecule remains in complex with the functional monomer, but after the polymerisation step is complete, the template molecule was removed. In this case, the synthesis process depends on the use of the template molecule to the formation of recognition cavities in the polymerising organic matrix. After the removed template, these cavities serve as "artificial receptor" sites intrinsic to the MIPs with size, shape, chemical functionality orientations, charge and/or hydrophobicity complementary to the template [20-22].

In general, MIPs can be using three key rules:

1. The imprinting process is achieved by preparing functional monomers around the template molecule according to non-covalent, covalent, semi-covalent, metal ion interactions.
2. The polymers are generated in a liquid solvent through a crosslinking polymerisation reaction.
3. The template is removed via a washing step, the polymer then binding sites which retain a high selective specificity for the print species.

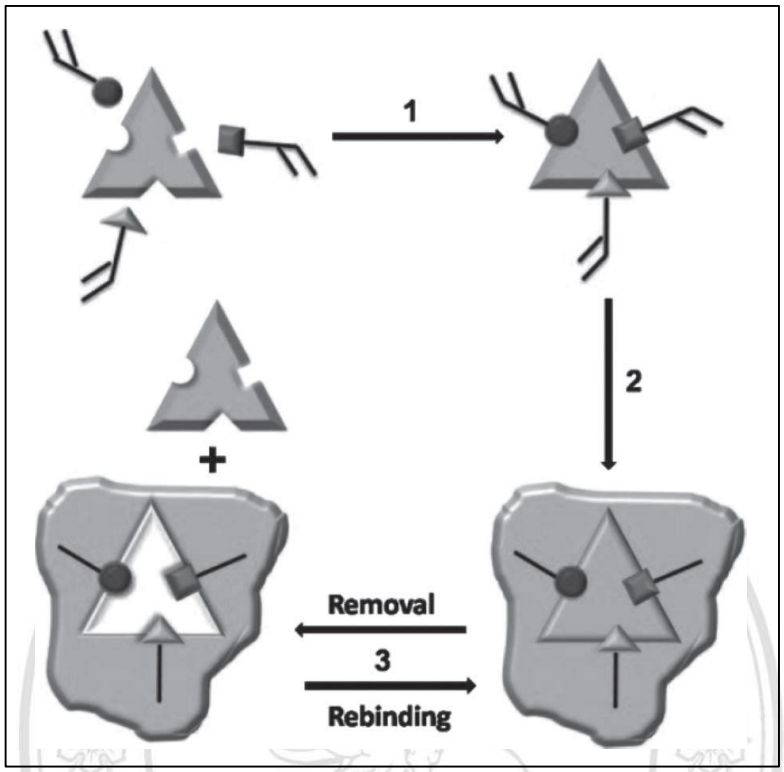

Figure 1.10 The general procedure of synthesising conventional MIPs [20].

The advantages of MIP sensor are:

1. MIPs have superior stability, low cost, and ease preparation.
2. They may replace natural receptors as the detection component of selective electrodes.
3. MIPs are made by synthesising highly crosslinked CPs in the presence of imprinted template molucule.
4. After removal of the template, the polymer can be used as a selective binding medium for the print molecule.

MIPs approaches for electrosynthesis of CPs include potentiostatic, galvanostatic and cyclic voltametric methods. These provide simple and rapid techniques for controlling the thickness of the CPs film, which can be easily grown and adhered to a transducer of any size and shape. Polymer thickness and deposition density, in turn, can be regulated by polymerisation conditions [23].

1.3 Literature review

MIPs usually have a recognition site for rebinding of target molecules. Recently, molecular imprinting technique has been employed for synthesis of CPs with the excellent recognition site. CPs possesses highly electrochemical reactivity, superior stability and excellent electronic conductivity, which is well suited for the determination of an analyte by amperometry. The imprinted materials have been considered as an interesting sensory element with specific recognition. Adding highly conductive nanocarbons onto the CPs is known to provide higher electrical conductivity and better mechanical stability, resulting in good performances in sensing devices [9, 22].

In 2008, Ozcan *et. al.* [22] studied the determination of ascorbic acid (AA) by differential pulse voltammetry using a pencil graphite electrode coated with AA-imprinted polypyrole (PPy). The imprinted electrode was prepared by electropolymerisation of PPy in a presence of AA, and then the template was removed before use. AA was chosen as a template molecule because of its attractiveness in electroactivity. The sensors based on molecularly imprinted PPy have been successfully demonstrated for the determination of AA in commercial pharmaceutical samples with high selectivity and sensitivity. In addition, the PPy electrodes showed a low response time and good mechanical stability.

In 2009, Pardieu *et. al.* [24] reported the design and the development of an electrochemical sensor based on MI conducting copolymer for a selective detection of atrazine pesticide. The copolymer, poly(3,4-ethylenedioxythiophene-co-thiophene-acetic acid) was electrochemically synthesised onto a platinum electrode by following the two steps: (i) A polymerisation of co-monomers in the presence of atrazine was associated with the acetic acid substituent through hydrogen bonding; and (ii) atrazine was then removed from the resulted polymer. The removal of atrazine left empty and highly specific recognition sites in the polymer matrix. This imprinted molecular memory on the polymer is analogous to the one that has been developed in MICPs. The voltammetric signal of the CP is changed upon a specific rebinding with atrazine by the recognition process or the sensing site–target interaction. Moreover, Li *et. al.* [23] investigated the fabrication of a highly selective and sensitive DA sensor, using an

electropolymerised MI(poly-*o*-aminophenol) as its recognition element. Electroactive DA can be used as a template molecule. The DA-imprinted sensor can be used for a selective determination of the DA molecules in the presence of high concentrations of AA. Moreover, electrochemical measurement results confirmed that the MIP membrane could rebind the related molecules selectively.

In 2010, Roy *et. al.* [25] reported a study related to MIPANI electrode for an AA detection. This electrode was fabricated by electrochemical polymerisation of ANI in the presence of AA onto indium–tin–oxide (ITO) coated glass plate, followed by a removal of the template (AA) by applying high voltage. The MIPANI electrode was studied for AA detection using DPV technique and it showed excellent reusability, selectivity and stability.

In 2011, Kan *et. al.* [26] showed that a MIPPy-based electrochemical sensor was fabricated successively by electrodepositing carboxyl-functionalised MWNTs (MWNTs-COOH) and electropolymerising pyrrole in the presence of DA onto a glassy carbon electrode (GCE) surface. The prepared sensor was characterised by scanning electron microscopy (SEM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). Under the optimised conditions, the sensor exhibited a good adsorption and a high recognition capacity for DA.

In 2012, Maouche *et. al.* [24] studied the possibility to design a simple and rapid electrochemical fabrication of films for a specific, selective and ultrasensitive electroanalysis of molecules. In this study, the electrochemical polymerisation was employed to produce thin films of DA-imprinted PPy, yielding a high-performance electrochemical sensor. The untrasensitive PPy films with artificial receptor sites had an ability to detect DA at very low concentration (picomolar) both in pure phosphate-buffered saline (PBS) and in real samples. Moreover, W. Kit-Anan *et. al.* [25] reported a low-cost and disposable point-of-care device of a new paper-based electrochemical sensor. This electrode prepared using two printing technique, i.e. screen printing for base material and inkjet-printing for modified functional material. The sensor for AA

detection by using inkjet-printing PANI modified SPCE and the sensor exhibits good sensitivity and low limit of detection.

In 2013, Jie *et. al.* [8] reported a rapid DA determination in undiluted human serum by inkjet printed Nafion/MWCNT chip. In this study, a well-dispersed Nafion/MWCNT composite ink was investigated with homogeneous double layers which increased the capability of dopamine detection, where direct electron exchange was achieved, producing a measurable current change at the underlying sensor electrode. This platform successfully demonstrated direct detection of DA concentrations in real human serum samples using DPV and amperometry methods. This direct measurement of DA in serum samples without pretreatment and dilution is reported for the first time in a Nafion/MWCNT system. Moreover, Li *et. at.* [27] reported the developed "double recognition" PANANA(polyaniline-co-anthranilic acid)-MIP electrochemical sensor which can be used to recognise DA by binding with the dial groups and the specific cavities contained in the MIP. In this study the PANANA-MIP composite possesses fast adsorption dynamics, good selectivity and high sensitivity for the DA detection in the presence of other interferences, such as norepinephrine (NE), epinephrine (EP), ascorbic acid (AA), and uric acid (UA). In addition, the PANA-MIP sensor was successfully applied to the determination of DA in DA injected in human plasma sample.

In this thesis, the preparation of high selectivity and sensitivity DA sensor will be investigated using the molecularly imprinted polyaniline (MIPANI) as an artificial recognition compound. DA was selected as a template molecule. PANI/GP composites will be developed by electrochemical polymerisation of ANI monomer on the prepared GP electrode platforms. The research will focus on the synthesis of a high quality GP by a chemical process and will investigate the fabrication of GP-modified electrodes, as well as the study of electrochemical behaviour of PANI/GP. The studies will include an optimisation and characterisation of the electrochemical polymerisation of MIPANI on electrodes for the determination of DA (target molecules) in real samples.

1.4 Research objectives

The main objectives of this research project are:

5.1 To synthesise a high quality GP by a chemical process.

5.2 To investigate the fabrication of GP-based electrodes.

5.3 To characterise the GP material and GP-based electrodes.

5.4 To develop and fabricate the novel MIPANI-GP-based electrodes via an electropolymerisation of MIPANI on the GP-based electrodes towards its electrochemical sensing properties.

5.5 To study performances and sensing properties of such MIPANI-GP-based electrodes. In comparison with non-imprinted PANI (non-PANI), the MIPANI-GP-based electrode developed would enable better recognition of target molecule such as DA (an important neurotransmitter). PANI, which will be molecularly imprinted, will be employed for the detection of DA. This molecularly imprinted PANI (MIPANI) on GP-based electrode will provide higher sensitivity and lower limit of detection.