# ENZYME-FREE GLUCOSE SENSOR BASED ON FERRITE MAGNETIC NANOPARTICLES

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# ABSTRACT

Magnetic nanoparticles have gained great interest recently due to their unique properties which stood up as the candidate constructing novel sensing devices; particularly in electrochemical sensors. The main goal of this research is to develop a sensitive enzyme-free glucose sensor based on nanocomposite comprises of magnetic nanoparticles, embedded in polymer matrix or graphene oxide. This is accomplished by preparation of four types of nanocomposites, namely polypyrrole (PPy) coated copper iron oxide (CuFe<sub>2</sub>O<sub>4</sub>/PPy), polypyrrole coated zinc iron oxide (ZnFe<sub>2</sub>O<sub>4</sub>/PPy), copper iron oxide reduced graphene oxide (CuFe<sub>2</sub>O<sub>4</sub>/rGO) and zinc iron oxide reduced graphene oxide ( $ZnFe_2O_4/rGO$ ). The morphology and surface property of coating phenomenon of prepared nanocomposites were examined by Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Fourier Transform Infrared (FTIR). In the first two mentioned composites, SEM and TEM images displayed the spherical shape of CuFe<sub>2</sub>O<sub>4</sub> and ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles with diameters ranging from 20 to 90 nm. The XRD and FTIR analyses confirmed that CuFe<sub>2</sub>O<sub>4</sub> and ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles served as the nucleation sites for the polymerization of pyrrole as there is no chemical interaction between them. For the other two nanocomposites which were modified with reduced graphene oxide, SEM and TEM images showed the dispersion of magnetic nanoparticles on the graphene nanosheets which pre-synthesized via hydrothermal method. This finding is further confirmed by XRD and FTIR which supported the reduction of GO and the presence of ZnFe<sub>2</sub>O<sub>4</sub> and CuFe<sub>2</sub>O<sub>4</sub> nanoparticles which distributed within the graphene sheets. The sensor performance based on CuFe<sub>2</sub>O<sub>4</sub>/PPy nanocomposite showed a highly active electrochemical surface area and a fascinating electro-catalytic activity for the glucose oxidation. In the amperometric detection of glucose, CuFe<sub>2</sub>O<sub>4</sub>/PPy nanocomposite modified glassy carbon electrode exhibited detection limit and sensitivity of 0.1 µM and 637.76  $\mu$ A mM<sup>-1</sup> for low concentration and 0.47  $\mu$ M and 176  $\mu$ A mM<sup>-1</sup> for high concentration of glucose respectively at a signal to noise of 3. Besides this, the modified sensor based on ZnFe<sub>2</sub>O<sub>4</sub>/PPy nanocomposite possessed good linear response in glucose concentration with an appropriate linear range up to 8.0 mM (R=0.9943) and good sensitivity to glucose (145.36  $\mu$ A mM<sup>-1</sup>) with a detection limit of 0.1 mM, at a signal to noise of 3 at room temperature. The sensitivity of ZnFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite is 110.92  $\mu$ A mM<sup>-1</sup> and 164.18  $\mu$ A mM<sup>-1</sup>, respectively. The overall results demonstrated that the CuFe<sub>2</sub>O<sub>4</sub>/PPy nanocomposite displayed the highest electro-catalytic activity towards the oxidation of glucose among all the synthesized composites.

## ABSTRAK

Kebelakangan ini nanopartikal magnet telah mendapat tumpuan hangat disebabkan oleh sifatnya yang unik membolehkan ia diguna dalam pembinaan peranti pengesan baru; khususnya dalam sensor elektrokimia tertentu. Matlamat utama kajian ini adalah untuk menghasilkan pengesan glukosa tanpa enzim yang sensitif berdasarkan nanokomposit yang mengandungi nanopartikal magnet, bersama dengan polimer dan graphene oksida. Ini dapat dicapai dengan menyediakan empat jenis nanokomposit, seperti oksida besi tembaga iaitu polypyrrole (PPy) bersalut (CuFe<sub>2</sub>O<sub>4</sub>/PPy), polypyrrole bersalut besi zink oksida (ZnFe<sub>2</sub>O<sub>4</sub>/PPy), besi tembaga oksida dengan penurunan graphene oksida (CuFe<sub>2</sub>O<sub>4</sub>/rGO) dan oksida besi zink dengan penurunan graphene oksida (ZnFe<sub>2</sub>O<sub>4</sub>/rGO). Morfologi dan ciri permukaan fenomena salutan nanokomposit yang dihasilkan telah dikaji dengan menggunakan Transmisi Elektron Mikroskopi (TEM), Mikroskop Imbasan Elektron (SEM), pembelauan X-ray (XRD) dan Fourier Transform Infrared (FTIR). Berdasarkan dua komposit pertama yang dinyatakan imej SEM dan TEM menunjukkan bentuk sfera bagi nanopartikal CuFe<sub>2</sub>O<sub>4</sub> dan ZnFe<sub>2</sub>O<sub>4</sub> dengan diameter antara 20-90 nm. Analisis XRD dan FTIR mengesahkan bahawa nanopartikal CuFe<sub>2</sub>O<sub>4</sub> dan ZnFe<sub>2</sub>O<sub>4</sub> bertidak sebagai tapak penukleusan bagi pempolimeran *pyrrole* kerana tiada interaksi kimia antara mereka. Untuk kedua-dua nanokomposit lain yang diubahsuai dengan menggunakan penurunan graphene oksida, imej SEM dan TEM menunjukkan penyebaran nanopartikel magnet di atas kepingan nano graphene yang pra-sintesis melalui kaedah hidroterma. Penemuan ini seterusnya disahkan oleh XRD dan FTIR yang menyokong penurunan GO dan kehadiran nanopartikal ZnFe<sub>2</sub>O<sub>4</sub> dan CuFe<sub>2</sub>O<sub>4</sub> yang disebarkan dalam kepingan graphene. Prestasi sensor berasaskan nanokomposit CuFe<sub>2</sub>O<sub>4</sub>/PPy menunjukkan kawasan permukaan elektrokimia yang sangat aktif dan aktiviti pemangkin electron menarik untuk pengoksidaan glukosa. Dalam pengesanan amperometrik glukosa, Karbon elektrode berkaca yang diubahsuai dengan nanocomposit CuFe<sub>2</sub>O<sub>4</sub>/PPy menunjukkan had pengesanan dan sensitiviti 0.1  $\mu$ M dan 637.76  $\mu$ A mM<sup>-1</sup> untuk kepekatan glukosa yang rendah, manakala 0.47  $\mu$ M dan 176  $\mu$ A mM<sup>-1</sup> untuk kepekatan glukosa yang tinggi pada nisbah 3. Selain itu, sensor diubahsuai berdasarkan nanokomposit ZnFe<sub>2</sub>O<sub>4</sub>/PPy mempunyai tindak balas linear yang baik dalam kepekatan glukosa dengan linear yang sesuai berukuran sehingga 8.0 mM (R = 0.9943) dan had kepekaan yang baik kepada glukosa (145.36  $\mu$ A mM<sup>-1</sup>) dengan takat pengesanan 0.1 mM, pada nisbah 3 dalam suhu bilik. Kepekaan nanokomposit ZnFe<sub>2</sub>O<sub>4</sub>/rGO dan CuFe<sub>2</sub>O<sub>4</sub>/rGO masing-masing adalah 110.92  $\mu$ A mM<sup>-1</sup> dan 164.18  $\mu$ A mM<sup>-1</sup>. Keputusan keseluruhan menunjukkan bahawa nanokomposit CuFe<sub>2</sub>O<sub>4</sub>/PPy memaparkan aktiviti pemangkin elektro yang paling tinggi terhadap pengoksidaan glukosa di kalangan semua komposit yang telah disintesis.

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# LIST OF SYMBOLS AND ABBREVIATIONS

| AA                               | :  | Ascorbic acid                            |
|----------------------------------|----|--|
| b                                | :  | Slope of the calibration curve           |
| BSE                              | :  | Backscattered electrons                  |
| CE                               | :  | Counter electrode                        |
| CTAB                             | :  | Cetyltrimethyl ammonium bromide          |
| CuFe <sub>2</sub> O <sub>4</sub> | :  | Copper iron oxide or copper ferrite      |
| CV                               | :  | The cyclic voltammetry                   |
| Eg                               | :  | Band gap                                 |
| EIS                              | :  | Electrochemical impedance spectroscopy   |
| FTIR                             | :  | Fourier Transform Infrared               |
| GCE                              | :  | Glassy carbon electrode                  |
| GDH                              | :  | Glucose dehydrogenase                    |
| GO                               | :  | Graphene oxide                           |
| GOx                              | :  | Glucose oxidase                          |
| LOD                              | :  | Limit of detection                       |
| MNPs                             | :  | Metallic nanoparticles                   |
| MWCN7                            | Γ: | Multi-walled carbon nanotubes            |
| PPy                              | :  | Polypyrrole                              |
| PVP                              | :  | Poly(vinyl pyrrolidone)                  |
| RE                               | :  | Reference electrode                      |
| rGO                              | :  | Reduced graphene oxide                   |
| SB                               | :  | Standard deviation of the blank solution |
| SCE                              | :  | Saturated calomel electrode              |
| SE                               | :  | Secondary electrons                      |
| SEM                              | :  | Scanning Electron Microscopy             |

TEM : Transmission Electron Microscopy

TDMAPP: Tetra (p-dimethylaminophenyl) porphyrin

TPP : 5,10,15,20-tetraphenylporphyrin

TPPP : Tetra (N-phenylpyrazole) porphyrin

- UA : Uric acid
- UMMC : University Malaya Medical Centre
- V : Voltage
- WE : Working electrode
- XRD : X-ray Diffraction
- $ZnFe_2O_4$ : Zinc iron oxide or zinc ferrite

#### **CHAPTER 1 : INTRODUCTION**

## 1.1 Study background

As diabetes is a worldwide public health problem, the quick and tight monitoring of glucose level in the human body is required in the market due to increase in the number of diabetes patient every year (Association, 2014). Providing this reliable control and fast determination of glucose is the interest of many researches. It needs highly sensitive glucose sensors and many efforts have been done to develop effective methods for glucose measurement. Sensitive and selective glucose sensors are used in blood sugar monitoring, food industry, bio-processing and also in the development of renewable and sustainable fuel cells (Kumary *et al.*, 2013; Prilutsky *et al.*, 2010).

Although glucose oxidase (GOx) and glucose dehydrogenase (GDH)-based biosensors have been widely used in the determination of blood glucose since 1962 but they shared common disadvantages (Santhosh *et al.*, (2009); Wang *et al.*, 2013). The intrinsic nature of enzymes is the main drawback and enzyme-based sensors suffer from instability problem and easily being affected during fabrication, storage or use by the environmental factors such as temperature, humidity, pH values and toxic chemicals (Sim *et al.*, 2012; Zhang *et al.*, 2014). Furthermore, glucose oxidase immobilization which included adsorption, cross-linking and electro-polymerization, is a complicated and expensive process. Thus, enzyme-free glucose sensors have started to catch the scientist's attention (Huang *et al.*, 2013; Li *et al.*, 2014; Qiu & Huang, 2010). The benefits of long term stability, reproducibility, resistance to thermal implications with low cost, simple fabrication method and being free from oxygen limitation are the strengths behind this category of sensors (Huang *et al.*, 2013; Park *et al.*, 2006; Qiu & Huang, 2010).

Metallic nanoparticles (MNPs) can play a significant role in modifying an electrode surface by increasing the surface area, high catalytic efficiency and enhancing the mass transport (Shi & Ma, 2010). Over the past decade, magnetic nanoparticles have proven their uniqueness by having the ability to promote faster electron transfer kinetics between electrodes, large surface-to-volume ratio and provide active site for the biomolecules (Lata *et al.*, 2012; Song *et al.*, 2007; Xu *et al.*, 2009; Zhang *et al.*, 2009). Recently, Fe<sub>3</sub>O<sub>4</sub> nanoparticles have been investigated for immunology sensor applications and also as glucose sensor in which both have shown good performance in terms of high detection limit and accuracy (Kaushik *et al.*, 2008; Singh *et al.*, 2011). These special nanoparticles have been found on various important applications in nanotechnology and nanomedicine (Kaushik *et al.*, 2008; Sandhu *et al.*, 2010; Vijayalakshmi *et al.*, 2008; Zhao *et al.*, 2006).

Spinel ferrites, with the general formula of MFe<sub>2</sub>O<sub>4</sub>, are an important class of magnetic materials where oxygen forms the face-centered cubic close packing, whereas  $M^{2+}$  and Fe<sup>3+</sup> occupy either tetrahedral or octahedral interstitial sites (Naseri *et al.*, 2011). They possess attractive properties to use in many applications such as catalysis, medical diagnostics, drug delivery and environmental remediation as well as in technological application and fundamental studies (Wang *et al.*, 2008; Zhang *et al.*, 2009). Their conductivity is due to charge hopping of carriers between cations which occupy the octahedral sites (Gul *et al.*, 2008). Some efforts showed that this kind of material can be used as sensor as well (Covaliu *et al.*, 2013; Luo *et al.*, 2010; Pita *et al.*, 2008; Zhang *et al.*, 2012).

Agglomeration is a big problem associated with magnetic particles as they tend to reduce the energy associated with the high surface area to volume ratio of the nanoparticles. Protecting the magnetic nanoparticles by various types of coatings is a solution to overcome this problem (Si & Samulski, 2008).

Polypyrrole (PPy) is one of the most extensively used conducting polymers for construction of bio-analytical sensors and as supporting matrix in electrochemical systems, due to its executive physical and electrical properties and biocompatibility (Liu *et al.*, 2011; Sekine *et al.*, 2010). Moreover, PPy can support good dispersion of metal nanoparticles due to the intrinsic existence of functional groups and long carbon chains (Bai *et al.*, 2011; Correa-Duarte *et al.*, 2004). Covering magnetic nanoparticles with an external shell using polypyrrole can improve the properties of these particles due to the strong electronic interaction between the MNPs and the polymer matrices (Li *et al.*, 2009; Tian *et al.*, 2004; Xu *et al.*, 2008). Core-shell structured materials are promising for biological applications as their offer high dispersibility, better thermal and chemical stability with less cytotoxicity (Chatterjee *et al.*, 2014; Fumioshi, 1984; Gomez-Lopera *et al.*, 2001; Law *et al.*, 2008; Sounderya & Zhang, 2008). Using polypyrrole as shell in magnetic nanocomposite has also provided a strict barrier between nanoparticles and reduced the magnetic-coupling effect between them (Liu *et al.*, 2008).

Reduced graphene oxide (rGO), an excellent electron-transporting material in the photocatalytic process, is a single layer of two-dimensional  $sp^2$  hybridized carbon nanosheet with great thermal conductivity, large surface area, excellent electron mobility, high transparency and with mechanical strength flexibility (Pei & Cheng, 2012). Integration of graphene nanosheets with metal nanoparticles to make graphene-metal hybrids has intensively developed a wide variety of applications in catalysis, surface enhanced raman scattering, targeted at drug delivery and removal of organic pollutants (Chung *et al.*, 2013; Kumary *et al.*, 2013; Yang *et al.*, 2009). Graphene acts as a separator to prevent the particles aggregating and the synergetic effects between graphene and the second components improves hybrids functionalities (Si & Samulski, 2008).

To my best knowledge, this is the first time that polypyrrole-ZnFe<sub>2</sub>O<sub>4</sub> magnetic nanocomposite and polypyrrole-CuFe<sub>2</sub>O<sub>4</sub> magnetic nanocomposite were applied in glucose sensors. The shell thickness in these core-shell nanoparticles is adjustable by controlling the amount of pyrrole monomers. To take advantages on the benefits of graphene oxide,  $ZnFe_2O_4/rGO$  magnetic nanocomposite and  $CuFe_2O_4/rGO$  magnetic nanocomposite were fabricated as enzyme-free glucose sensor as well.

## **1.2** Thesis outline

The work presented in this thesis deals with the potential application of magnetic nanocomposites as an enzymless sensor for detection of glucose in human blood.

**Chapter 1** presents the general introduction on research background about magnetic nanoparticles, conductive polymers, graphene oxide and their application in electrochemical sensors, as well as the scope and objectives of this thesis.

**Chapter 2** describes the literature review on nanomaterials, electrochemical glucose sensors, chemical modified electrodes and electrochemical techniques.

**Chapter 3** discusses chemicals, materials and techniques that are applied to synthesize and characterize four types of nanocomposites as well as a general procedure for fabrication of reported electrochemical sensors.

Chapter 4 reports the characterization and performance of each fabricated electrochemical sensor.

Part 1 illustrates a novel glucose electrochemical sensor based on polypyrrole coated copper ferrite oxide (CuFe<sub>2</sub>O<sub>4</sub>/PPy) nanocomposite (This work has been published in Journal of Solid State Electrochemistry, April 2015, Volume 19, Issue 4, pp 1223-1233, doi:10.1007/s10008-015-2738-6). Part 2 demonstrates the synthesized polypyrrole coated zinc ferrite oxide nanocomposite (ZnFe<sub>2</sub>O<sub>4</sub>/PPy) for glucose sensing (This work has been published in Journal of Applied Surface Science, Volume 317, pp 622-629,

doi:10.1016/j.apsusc.2014.08.194). Part 3 reveals the performance of copper ferrite oxide reduced graphene oxide nanocomposite (CuFe<sub>2</sub>O<sub>4</sub>/rGO) for oxidation of glucose where it shows that the hydrothermal synthesis graphene oxide was used to improve the sensitivity of glucose sensor. (This work has been published in Journal of Ceramics International doi:10.1016/j.ceramint.2015.06.103). Part 4 shows the ability of zinc ferrite oxide reduced graphene oxide nanocomposite (ZnFe<sub>2</sub>O<sub>4</sub>/rGO) for glucose sensing. (This work has been submitted to Journal of Applied Surface Science). All nanocomposites were characterized by X-ray diffraction technique (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Chapter 5 draws the thesis summary and proposed future works.

# 1.3 Objectives

The aim of this research is to synthesise and characterise new class of magnetic nanocomposites to develop new sensing materials for detecting glucose concentrations in blood serum.

The goals of this thesis are as follow:

- To synthesize polypyrrole/magnetic nanocomposite with core-shell structure and reduced graphene oxide/ magnetic nanocomposite.
- To demonstrate the synthesized nanocomposite as electrochemical sensor.
- To optimize the performance of the electrochemical sensor as an enzyme-free sensor for detection of glucose.

#### **CHAPTER 2 : LITERATURE REVIEW**

In this section, a literature review of the relevant subjects is presented, including a brief description of the needs and techniques used to monitor the blood glucose level, and review of magnetic nanoparticles, conducting polymers and graphene which are the subject of this work.

#### 2.1 Diabetes

Diabetes mellitus is becoming more widespread serious disease; there are about 387 million people have diabetes, while the number is expected to multiply by 1.54 folds by the end of 2030 (Kernt et al., 2014; Shaw et al., 2010). Diabetes is not curable but manageable and accurate blood glucose monitoring of a diabetic patient is an unavoidable activity. The blood glucose level must be maintained within the normal range (4.4 - 6.6 mM) which is vital for the healthcare of diabetics (Wang, 2008). Glucose is the most common tested analyte since many of the patients test their level of glucose from blood daily. Via a finger prick, a small sample of blood is placed onto a sensor test strip and a handheld electronic reader reports the glucose concentration without the need for laboratory analysis (Ginsberg, 2009). However, there are limitations can alter the accuracy of blood glucose strips including low stability of enzymatic sensor test strip and interfering substances. Strips have a finite lifetime and storing strips at high temperature or high humidity can shorten the life of the strips. There is usually a great excess of enzyme, lack of enzyme or enzyme failure is the cause of inaccuracy. Interfering substances in the blood can confound the accuracy of glucose meters, thus using sensors which can negate effect of interferences substances is very important (Bode, 2007; Cash & Clark, 2010). Improving the clinical use of blood glucose monitoring is a need which can be done possibly by developing easier and more accurate sensor in the future.

## 2.2 Analyte

An analyte refers as substance or chemical constituent which its properties is measured in an analytical procedure. The main analyte that is determined by the developed electrochemical enzyme-free sensor in this thesis is glucose; some sugars such as fructose and sucrose were tested as interference studies. Interferences are molecules which co-exist with glucose in blood and continue to disturb signals during blood glucose monitoring in diabetes. An important factor in developing glucose sensor is to eliminate or minimize the effect of interferences on glucose detection in the electrochemical sensor; as a result ascorbic acid and uric acid (blood species) were studied as part of the analyte in this work.

#### 2.2.1 Glucose

Glucose (Figure 2.1) is a simple hydrocarbon which is known as grape sugar, blood sugar or corn sugar. This monosaccharide is the major cellular carbohydrate source and a common medical analyte measured in blood samples. Liver cell glycogen gets converted to glucose and returns to the blood when insulin level is low or absence. The concentration of glucose in the blood is regulated by the insulin and other mechanisms. High blood sugar level is a symptom of pre-diabetic and diabetic conditions. As a primary source of energy for the brain, any decline in glucose concentration will damage psychological processes such as self-control and decisionmaking (Berg *et al.*, 2002).



Figure 2.1: Glucose molecule structure.

# 2.2.2 Fructose

Fructose (Figure 2.2) is a simple hydrocarbon that is known as fruit sugar and naturally exists in many plants such as vine fruits, flowers, berries and root vegetables. This sugar is only metabolized in the liver by fructokinase and has a different metabolic pathway (Bray, 2010). Dry and pure fructose is very sweet and exists in crystalline solid but cannot be the preferred energy source for muscles or the brain. The 6-carbon polyhydroxyketone fructose is an isomer of glucose where both are having the same molecular formula ( $C_6H_{12}O_6$ ) but differ structurally.



Figure 2.2: Fructose molecule structure.

# 2.2.3 Sucrose

The structure of sucrose is shown in Figure 2.3. Sucrose which is obtained from sugar cane or sugar beets is known as table sugar. This odorless, white and crystalline powder sugar with a sweet taste can be found in fruits and vegetables. After consumption, this sugar would be separated into glucose and fructose units by beta-fructosidase. The glucose will be used as main body energy source, and if not in need, it will be poured into fat synthesis, which is stimulated by the insulin released in response to glucose (Wind *et al.*, 2010).



Figure 2.3: Sucrose molecule structure.

# 2.2.4 Uric acid

The determination of uric acid (Figure 2.4) exists as the last product of purine metabolism, is a clinically valuable diagnostic indicator (Raj & Ohsaka, 2003). The normal levels of uric acid in men and women are less than 420  $\mu$ M L<sup>-1</sup> and 330-360  $\mu$ M L<sup>-1</sup> respectively (Johnson *et al.*, 1999). Elevated level of uric acid in body fluids could be a sign of diseases such as gout, hyperuricemia, obesity, diabetes, high cholesterol, kidney disease and cardiovascular diseases. Uric acid is one of the species which coexist with glucose in human serum. To secure the precise measurement of glucose level in blood serum, selectivity of the sensor is one of the most important analytical factors for an amperometric sensor.



Figure 2.4: Uric acid molecule structure.

# 2.2.5 Ascorbic acid

Ascorbic acid (Figure 2.5) is an unsaturated lactone which has important biological functions such as the preservation and maturation of fibroblasts, elaboration of hydroxyproline and hydroxylysine, metabolism of phenylalanine, tyrosine and dihydrophenylalanine (Zaeslein & Körner, 1982), which facilitates the absorption of

iron by keeping it in the reduced form and also quenching of free radicals (hydroxyl, singlet oxygen, superoxide). Premature babies, neonates, women during pregnancy and lactation or during treatment such as anti-microbial and hemodialysis require high amount of ascorbic acid. Similarly, higher doses are prescribed for stimulating wound healing, improving iron absorption and to patients with lower blood pressure, respiratory symptoms and cancer.



Figure 2.5: Ascorbic acid molecule structure.

#### 2.3 Chemical sensors

Sensors are devices which have an active sensing material and a signal transducer where it transmits the signal without any changes in a reaction (Wilson & Gifford, 2005). Sensors produce electrical, thermal or optical output signals which can be converted into digital signals that can be read by an observer or instrument. A chemical sensor which is an essential component of an analyzer is a small device that transforms chemical or biochemical information into an analytically useful signal. The analyzer is an essential part of an automated system and contains devices to perform the sampling, sample transport, signal and data processing. The analyzer working according to a sampling plan as a function of time acts as a monitor. An insight about the chemical composition of the system in real-time is obtained in chemical sensing process. The interaction between some chemical species and the sensor produces an amplified electrical signal. Transduction in chemical sensing consisting of two basic steps: recognition and amplification. One common example is the measurement of pH with a glass electrode. The recognition (selectivity) is provided by some chemical interaction, whereas the amplification must be provided by some physical transducer. Enzymatic reactions can be considered as exceptions in which the high selectivity of the enzyme combined with the catalytic properties of the enzyme, represents an amplification step in itself. As the coupling of the chemically selective layer to the physical part of the sensor has a great effect on the overall performance of the sensor, the use of an improper transduction mechanism can destroy the highly selective primary interaction. The response of a sensor is generated by the change in some physical parameter, as a result of some chemical stimulation (Janata, 2010).

# 2.4 Types of chemical sensors

Chemical sensors can be classified into the following depending on the transducer types:

- i. Optical sensor
- ii. Mass sensitive sensor
- iii. Heat sensitive sensor
- iv. Electrochemical sensor

# 2.4.1 Optical sensors

The principles of an optical sensor are based on classical spectroscopy except in the elements of the experiment and its arrangement. In an optical sensor, the sample is placed in a well-defined path of the light beam in a spectrophotometer and the emerging radiation is captured by the detector. The light after interaction with the sample is reintroduced into the spectrophotometer for further processing. The materials and the applications in an optical sensor are chosen based on the necessity of guiding and manipulating the light over a distance dictates. The development and use of optical sensors are informed by the knowledge base of spectroscopy. Yang synthesized three porphyrin compounds including, 5,10,15,20-tetraphenylporphyrin (TPP), tetra(*p*-dimethylaminophenyl)porphyrin (TDMAPP) and tetra(N-phenylpyrazole) porphyrin (TPPP). These compounds were studied as mercury ions (Hg<sup>2+</sup>) optical sensor. Among them TDMAPP showed the best performance for detecting Hg<sup>2+</sup> ions with a linear range covering from  $4.0 \times 10^{-8}$  mol L<sup>-1</sup> to  $4.0 \times 10^{-6}$  mol L<sup>-1</sup> with a detection limit of  $8.0 \times 10^{-9}$  mol L<sup>-1</sup> (Yang, 2009). In another work by Pandey and co-workers, silver nanoparticles were synthesized by a very simple method to be used for ammonia measurement. In this green method, guar gum acted as reducing agent to reduce silver nitrate salts. The proposed optical sensor showed the response time of 2-3 s and the detection limit of 1 ppm at room temperature (Pandey *et al.*, 2012).

# 2.4.2 Mass sensitive sensors

This sensor relies on a change in mass on the surface of an oscillating crystal which shifts the frequency of oscillation. The extent of the frequency shift is a function of the amount of material absorbed on the surface (Gründler, 2007). In this sensor the piezoelectric effect is important and is particularly useful as gas sensors. Among several types of materials that exhibit the piezoelectric effect, quartz is one the best because of its properties such as inexpensive and relatively strong piezoelectric coefficient. A mass-sensitive pH sensor was described by Ruan *et al.* This sensor was based on the poly(acrylic acid-co-isooctyl acrylate) hydrogel, with an acrylic acid (80%) and N,N-methylenebis(acrylamide) cross linker which enhanced the sensitivity by increasing the acrylic acid fraction in the poly(acrylic acid-co-isooctyl) acrylate copolymer (Ruan *et al.*, 2003). In another work, Mujahid *et al.* reported a mass-sensitive sensor for detection of organic solvent vapours for both polar (methanol and ethanol) and non-polar

(chloroform and tetrachloroethylene) substances by using pure cholesteric liquid crystals and in a polymer matrix. The results showed that the mass effect for tetrachloroethylene was about six times higher than chloroform and combining cholesteric liquid crystals with imprinted polymers improved the mechanical stability (Mujahid *et al.*, 2010).

## 2.4.3 Thermal sensors

Since the thermal sensors employed kinetic selectivity, some catalysis form is always involved. The batch calorimetry can provide important information for thermal chemical sensors as these sensors are in situ microcalorimeters. Heat is non-specific and also it cannot be contained. The optimal design of a thermal sensor is based on these two unique properties of heat. The heat of a chemical reaction involving the analyte is monitored either as the change in temperature of the sensing element or as the heat flux through the sensing element with transducers such as thermistor or a platinum thermometer. They are often called as calorimetric sensors (Fürjes et al., 2005; Yao et al., 2011). In 2014, Xiang and his team prepared a novel room-temperature hydrogen sensor based on palladium nanoparticles doped titanium dioxide nanotube film. The combination of palladium nanoparticles with titanium dioxide nanotubes enhanced the sensitivity and selectivity of this composite and made it capable to be applied as a high performance hydrogen sensor (Xiang et al., 2014). Another low temperature hydrogen sensor was designed by Gupta et al. using thin films of palladium nanoparticles on glass substrates. This thermal sensor showed good performance in the temperature range, 35-75 °C and in different hydrogen concentrations (0.1-1%). The optimum response was obtained at 50 °C with response time (t<sub>90</sub>) of 3 s in 1000 ppm hydrogen in nitrogen (Gupta *et al.*, 2014).

## 2.4.4 Electrochemical sensors

In electrochemical sensor, the electrode serves as the signal transducer and the measurable response is an electrical current. This kind of sensor is designed to detect and responds to an analyte in all states. In comparison with optical, thermal and mass sensors, electrochemical sensors are growing fast because of their outstanding simplicity, detectability, low-cost and reproducibility (Bakker & Qin, 2006; Janata, 2001). There are three major types of electrochemical sensors which include voltammetric, potentiometric and amperometric sensors (Janata, 1992). In voltammetry, the current and the potential are measured and recorded. The position of peak current is related to the specific chemical and the peak current density is proportional to the concentration of the corresponding species. Low noise and simultaneous detection of multiple analytes are the advantages of this technique (Su et al., 2011). Potentiometric sensor interface has a local equilibrium where either the membrane or electrode potential is measured, and information about the composition of a sample is obtained from the potential difference between two electrodes (Skoog et al.). In this technique a gas-sensing electrode or an ion-selective electrode is the transducer. Although a potentiometric biosensor has high sensitivity and selectivity due to the species-selective working electrode used in the system, but some required conditions such as a highly stable and accurate reference electrode may potentially limit its application in microbial biosensors (Su et al., 2011). In amperometric sensors, the current at the working electrode is measured which is produced by an applicable voltage at the interface of electrode and solution (Wang, 2006). The information from this kind of sensor is obtained from the current-concentration relationship. The working electrode can be a cathode or anode and it depends on added electrons or withdrawn electrons from the sample. The current is the resultant of electrochemical oxidation or reduction of the electroactive compound. By applying steady state convection, a constant current is

measured as the concentration of electroactive species is uniform (Kellner *et al.*, 2004). Sensitivity of an amperometric sensor is better than the potentiometric sensor and thus it is used in high-performance liquid chromatography. The signal-transduction mechanism is frequently used for biosensors. In this class of sensors, the high degree of electrodes reproducibility eliminates the cumbersome requirement for repeated calibration and an *in situ* measurement is possible. Based on advantages of amperometric sensors, this kind of sensor was selected for determination of glucose level in this work.

# 2.5 Electrochemical glucose sensors

An electrochemical glucose sensor mainly consists of two- or three-electrodes which are called working, reference and auxiliary (or counter) electrodes. Working electrode is coated by active materials to react specifically with the glucose molecules. In this configuration the working electrode potential can be measured against the reference electrode without compromising the stability of the reference electrode by passing current over it. The analyte diffuses into the sensor where it is oxidized or reduced, thereby generating the change of electric signal, which then passes through the external circuit comprising of amplifiers and other signal processing devices. The electrical signal is then converted to the analyte detection signal and displayed as concentration value (Zhang & Li, 2004). In an electrochemical sensor, an electrode serves as the signal transducer and the measurable response is an electrical current. The choice of molecular–recognition element depends on the analyte. Overall, the electrochemical glucose sensor can be divided into two main categories; enzymatic glucose sensor and enzyme-free glucose sensor.
#### 2.5.1 Enzymatic glucose sensors

After the first enzyme electrode proposed by Clark and Lyons, the developed and improved glucose sensors have been the subjects of investigational studies for decades. The timeline of glucose sensor development can be divided into three primary generations. In the first generation (Figure 2.6), oxygen reduces to  $H_2O_2$  by GOx when it plays a role of electron mediator between glucose oxidase and electrode surface. Since the glucose concentration is proportional to the rate of  $O_2$ , increase of the  $H_2O_2$  level or reduction of  $O_2$  concentration can be measured. Oxygen dependence and interference by redox-active species are among the disadvantages of first generation glucose sensors (Wang, 2008; Zhu *et al.*, 2012).



Figure 2.6: The first generation of the enzymatic glucose sensors (Liu & Wang, 2001).

An artificial mediator is another solution to eliminate oxygen limitation under low pressure of oxygen in second generation of enzymatic glucose sensors (Figure 2.7). The quick electron transfer occurs between the enzyme and electrode by the electron mediators (Tian *et al.*, 2014; Toghill & Compton, 2010). The redox system needs to improve its design to develop the efficiency of sensor due to the existing competition between oxygen (redox active species) and the mediators.



Figure 2.7: The second generation of enzyme glucose sensors (Cash & Clark, 2010).

In the third generation of glucose sensors, the electrons travel to the electrode surface. The electrical connection between electrode and the active redox sites of the enzyme facilitate the electron transfer as amperometric signal directly which it is not influence by concentration of oxygen or redox mediators. The elimination of possible interferences can be considered as the most important advantage in this design (Palmisano *et al.*, 2002; Rahman *et al.*, 2010).

The enzymatic glucose sensors dominated the market; however, they suffer various drawbacks. High oxygen dependence in first generation sensors made them unsuitable for practical and reliable analytical use. Moreover, the presence of other electroactive interferences in the sample hinders the enzymatic sensors ability. In second generation sensors, the synthetic mediators could overcome oxygen dependence with lower amperometric potential to avoid some electroactive interferences. In the third generation sensors which are still in their infancy, stability is a big issue in development and application of enzymatic glucose sensors (Bao *et al.*, 2008), GOx is still affected by pH ranges of 2-8, high temperatures and humidity levels (Sim *et al.*, 2012; Zhang *et al.*, 2014) which make the sensor to be deformed, denatured or inactivated. The stability of enzyme immobilization and mediator electrodes requires high attentions which include fabrication processes, covalent cross-linking and sol-gel entrapment. These processes are complicated and time consuming which may decrease the activity of the GOx since the sensitivity of these glucose sensors is highly depend on the activity of the

immobilized enzymes (Li & Lin, 2007b). On the other hand, the high selectivity of the enzyme towards glucose is one of the considerable advantages of enzymatic glucose sensors but still it is essential to develop stable sensing applications in high temperatures and under aggressive environment conditions. The problems associated with enzyme based glucose sensors have steered researchers to explore enzyme-free detection. These sensors allow glucose to be oxidized directly on the electrode surface and have led to the development of the fourth generation of glucose sensor technology. A considerable amount of research is on-going all around the world regards to enzyme-free free sensors and the number of publications has increased over recent years (Tian *et al.*, 2014).

## 2.5.2 Enzyme-free glucose sensors

The use of enzyme-free electrode as glucose sensor is an ideal system which facilitates glucose oxidization directly in the sample instead of the needs of a fragile and relatively difficult enzyme immobilization. Fabrication of glucose enzymless sensor is still an attractive subject which can overcome the limitations of enzymatic glucose sensor. Various metal based enzyme-free sensors have been investigated to improve the electrocatalytic activity and selectivity toward the glucose oxidation by using: inert metals, metal alloys and metal-dispersed carbon nanotubes (Meng *et al.*, 2009; Sun *et al.*, 2001; Wang *et al.*, 2008; Zhu *et al.*, 2009). Kim *et al.* developed an enzyme-free glucose sensor based on nanoporous platinum thin films which exhibited a sensitivity of 10  $\mu$ A mM<sup>-1</sup> cm<sup>-2</sup> and a detection limit of 50  $\mu$ M (Kim *et al.*, 2013). An enzyme-free glucose sensor was prepared by Kurniawan *et al.* using Au nanoparticles immobilized on thin Au electrode, grown by a layer-by-layer deposition method with the detection limit of below 500  $\mu$ M and the sensitivity of about 160  $\mu$ A mM<sup>-1</sup> cm<sup>-2</sup> (Kurniawan *et al.*, 2006). However these materials exhibited unsatisfied sensitivity and selectivity to

glucose, high costing and quick loss of activity by adsorption and accumulation of intermediates or chloride ions (Sun *et al.*, 2001; Wang *et al.*, 2008). Besides low sensitivity, various suggested enzyme-less glucose sensors are lack in glucose recognition units and distinguishing of glucose from other interferents. To enhance the sensitivity and selectivity of sensors, a lot of efforts were concentrated on the modification of working electrodes by new nanomaterials composites. The extensive research into the enzyme-free approach actually coincided with enzymatic development. Direct enzyme-free electro-oxidation of glucose is non-diffusion controlled process (Park *et al.*, 2006) and considerably depends on the electrode material used. By considering these aspects, the enzyme-free glucose sensor based on nano-sized particles is an attractive alternative technique. The enzyme-free glucose sensor offers the following advantages;

**Stability:** Improvement of enzymatic glucose sensor drawbacks has been the subject of numerous studies during the past few years. These sensors suffer from insufficient stability of the enzymes which could be deformed easily by high temperature or chemicals. Enzyme-less glucose sensors have overcome this issue and shown excellent sensitivity and selectivity towards detection of glucose even after exposure to sodium hydroxide or sulphuric acid solution (Toghill & Compton, 2010).

**Simplicity and reproducibility:** Direct adsorption, sol–gel entrapment, cross-linking are the processes of enzyme immobilization. Most of the sensitivity of enzymatic glucose sensor depends on the immobilized enzymes activity (Cosnier, 2003). In spite of attractive immobilization methods, reproducibility and the sorts of the enzymes immobilized are still a critical issue in all kinds of enzyme electrodes. The enzymless sensor is an effective alternative to eliminate these issues (Park *et al.*, 2006).

**Oxygen free:** Although oxygen dependence is one of the main targets of enzymatic glucose sensor but they still suffer from oxygen effect. This affection is due to the

competition between electron-mediating sites and dissolved oxygen in the solution (Mano & Heller, 2005). Oxygen limitation is not an issue in enzyme-free glucose sensors since oxygen in these sensors have been eliminated at appropriate potential for oxygen effect.

## 2.6 Nanomaterials

Nanomaterial possesses the size between 1 to 100 nm and the large ratio between surface atoms to inner atoms (Daniel & Astruc, 2004; Siegel & Fougere, 1995). Chemical and physical properties of nanoparticles are very different from their bulk counterparts and it can be tuned by changing the size and shape (Schmidt et al., 1998). Nanoparticles are very useful for many applications due to their shape and size dependent properties. These properties help to increase in surface to volume ratio of nanoparticles and quantum size (Singh, 2011). The size at which the nanoparticle behaves like its bulk depends on the type of materials. In metals, compared to semiconductors, a few tens of atoms are adequate to make the nanoparticles behave. With improvement of characterization and synthesis techniques on the nanometre scale, nanotechnology has developed significantly in the last ten years. Nowadays, many efforts have been done to shift from nanomaterials and investigation of their physicochemical properties to the use of these properties in several applications. Biomedical is one of the research fields that can vary benefit from the advancement in nanotechnology (Gaffet, 2011). The great advantages of nanomaterials in the biomedical research field lies in its ability to operate on the same small scale as all the intimate biochemical functions involved in the growth, development and ageing of the human body. One of the disadvantages of nanomaterials is certainly related to long-term safety for in vitro and in vivo applications. Both toxicology and risk assessment and management need to be defined for the use of nanoparticles in medical applications.

Nanomaterials are supposed to revolutionize human life in the future and have a great impact on development of biosensors (Singh, 2011; Yousaf & Ali, 2008). Lei's group constructed two biosensors based on transferred ZnO and grown ZnO nanoparticles by two different immobilization approaches to study their performance for glucose detection. The finding showed that sensitivity of the grown biosensor is higher than that of the transferred biosensor because the grown zinc oxide have higher specific surface area and more glucose oxidase can be immobilized on them (Lei *et al.*, 2011). Periasamy and co-workers proposed a novel nanocomposite based on bismuth oxide nanoparticles ( $Bi_2O_3$ ) and multi-walled carbon nanotubes (MWCNT) to develop a  $H_2O_2$ biosensor. A thin layer of 1% nafion solution was coated as a binder to anchor the horseradish peroxidase molecules onto  $Bi_2O_3$ –MWCNT matrix. The nanocomposite film possesses good biocompatibility and showed excellent electro-catalytic activity towards  $H_2O_2$  with high sensitivity and selectivity (Periasamy *et al.*, 2011).

## 2.7 Magnetic nanoparticles

Magnetism is, to a large extent, a nanoscale phenomenon and the atomic exchange interaction that defines ferromagnetism is typically on the length scale of 10 nm for most materials (Lin & Samia, 2006; Skomski, 2003). In magnetic nanoparticles, the difference between a massive (bulk) material and a nanomaterial is especially pronounced. Magnetic nanoparticles show unique magnetic attributes such as superparamagnetic behaviour, high coercivity, low curie temperatures, high magnetic susceptibility and appropriate physico-chemistry properties (Indira & Lakshmi, 2010; Majewski & Thierry, 2007). Magnetic characteristics of the material can be extended by changing the nanoparticle size, shape, composition and structure. However, these factors cannot always be controlled during the synthesis of nanoparticles nearly equal in size and chemical composition; therefore, the properties of nanomaterials of the same type can be markedly different. Magnetic nanomaterials have attracted the attention of scientific community as potential materials for various applications. They are used in information recording and storage systems (Chernyshov *et al.*, 2013), new permanent magnets (Balamurugan *et al.*, 2012), magnetic cooling systems (Franco *et al.*, 2012). Magnetic nanoparticles can take the advantage of specific binding to detect or purify the biological entities after being modified by biomolecules. The unique property of magnetic nanoparticles which is response to a magnetic field, exhibit two features, specificity and magnetism and they are being actively pursued for potential biomedical applications such as drug delivery (Knežević & Lin, 2013), hyperthermia (Jiang *et al.*, 2014), magnetic resonance imaging (Yallapu *et al.*, 2011). Due to high number of potential applications for high quality magnetite nanoparticles in recent years, efficient methods for the preparation and stabilization of magnetic nanoparticles have been developed considerably.

Brownian motion of bare nanoparticles enhanced by Van der Waals and magnetic dipole-dipole interaction leads to irreversible aggregation of the nanoparticles and can affect the magnetic properties. For example, aggregation of magnetic nanoparticles in catalysis decrease the number of accessible reactive groups and leads to less specific surface areas which the whole catalytic process can be blocked. This unwanted phenomenon can be minimized by producing colloidal stable magnetic nanoparticles which can be gained by electrostatic stabilization or steric stabilization or combination of both. Steric stabilization is provided by organic molecules, such as surfactants, polymers, and designed ligand or by inorganic coating like silica, gold, silver and carbon. Polymers are ideal candidates for magnetic nanoparticles coating and they can also enhance stability via ionic interactions. Pyrrole with heterocyclic structure gives easy access for polypyrrole to cover magnetite NPs, which form core-shell structures (Karsten *et al.*, 2012; Sharma *et al.*, 2005).

#### 2.8 Synthesis of magnetic nanoparticles

The great interest in magnetic nanoparticles has provided many ways of preparation. Magnetic nanoparticles can be synthesized via following methods; such as template-directed, thermal decomposition, deposition method and self-assembly.

## 2.8.1 Thermal decomposition

In thermal decomposition method, organometallic compounds such as acetylacetonates in organic solvents (benzyl ether, ethylenediamine and carbonyls) with surfactants such as oleic acid, oleylamine, poly(vinyl pyrrolidone) (PVP), cetyltrimethyl ammonium bromide (CTAB) and hexadecylamine are used. The ratio of precursors and time are two important factors which can affect morphology (spherical particles, cubes) and particles size in this method. Short decomposition duration resulted in spherical and longer duration resulted in cubic morphology. Hyeon *et al.* synthesized cobalt nanodisks from the thermal decomposition of dicobalt octacarbonyl in the presence of two surfactants mixture (Hyeon, 2003). The novel core/shell magnetic nickel nanoparticles were developed via thermal decomposition by Zhang *et al.* The oleic acid acted as surfactant and non-coordinating reagent in the preparation of Ni nanoparticles with a narrow size distribution. It is expected to have a significant potential for biomolecule separation, magnetic imaging, and optoelectronics (Zhang *et al.*, 2009).

## **2.8.2. Template assisted fabrication**

The template-assisted fabrication is based on growth of the nuclei at the holes of the template to yield the desired morphology of the nanostructures. The size and shape of the magnetic nanoparticles can be controlled via this method. Although this technique has advantages such as determination of the final size and morphology of the nanostructures with full control but the synthesis method is a multi-step process which needs the fabrication based templates before any depositions (Khan *et al.*). Hurst and co-workers demonstrated the synthesis of two components rod structure that was made by deposition of hydrophilic Au block and hydrophobic polypyrrole block on anodic alumina oxide template. Hydrophilic Au and hydrophobic polypyrrole were successfully assembled in a unique shape which is attributed to differences in their diameter (Hurst *et al.*, 2006). Zhang *et al.* reported that nickel ferrite nanorods were successfully synthesized by the thermal treatment of the rod-like precursor that were fabricated by a coprecipitation of Ni<sup>2+</sup>, Fe<sup>2+</sup> and C<sub>2</sub>O<sub>4</sub><sup>2-</sup> ions in a microemulsion solution. Small subunits of nickel ferrite nanorods made it capable to exhibite higher discharge than that of the sample with bigger building blocks and using this method increased proportion of the total number of atoms lies on the surface with decreasing particle size to improve the electrochemical performances (Zhang *et al.*, 2009).

## 2.8.3 Self-assembly of magnetic nanostructures

The self-assembly method is based on the thermodynamically atomic arrangement of magnetic nanoparticles into arrays of complex shape via nanoscale forces. The relative strong dipole forces of magnetic nanoparticles can form the linear, branch chains and close packed arrays of magnetic nanostructures. Perez *et al.* reported monodisperse magnetic nanoparticles conjugated with virus-surface-specific antibodies self-assemble in the presence of specific viral particles to create supramolecular structures with enhanced magnetic properties (Perez *et al.*, 2003). Another monodispersed, stabilized cobalt nanocrystals were produced by this method. These particles have been observed to produce two-dimensional self-assemblies when evaporated at low rates under the controlled atmosphere (Puntes *et al.*, 2001).

### **2.8.4 Hydrothermal synthesis**

Hydrothermal synthesis is a technique for synthesis of magnetic nanoparticles from high boiling point aqueous solution at high vapour pressure which gives great control over the size and shape of the magnetic nanoparticles. This chemical technique involves the use of liquid-solid-solution (LSS) reaction (Li et al., 2010). A novel roomtemperature ferromagnetic Mn-doped ZnO nanocrystal was synthesized by hydrothermal method under high magnetic field. It was found that ferromagnetism is strongly related to defects and pulsed high magnetic field improved the grain size to be uniform and enhanced the saturation magnetization of Mn-doped ZnO nanocrystal (Yang et al., 2010). A novel Fe<sub>3</sub>O<sub>4</sub> nanoprism was prepared by a hydrothermal process. In this work, Zeng et al. used oleylamine as surfactant and reducing agent and proved that it plays key role in the formation of different planes of Fe<sub>3</sub>O<sub>4</sub> nanoprism due to presence of amine group which absorb at certain planes that lead to different morphology (Zeng et al., 2010). Wu' group synthesized nanocubes of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> by one step facile hydrothermal method. They investigated the effect of volume ratio of oleylamine and acetylacetone for the fabrication of  $\alpha$ - Fe<sub>2</sub>O<sub>3</sub> and used these magnetic nanoparticles for photocatalytic degradation of organic dye. The synthesized nanoparticles showed higher photocatalytic degradation activity as compared to oblique nanocubes which is attributed to the exposure of high-index planes (Wu et al., 2013). Since the hydrothermal synthesis offers advantages such as uniformity of nucleation, growth nanoparticles and reduced aggregation levels, which is not possible with many other synthesis processes, this method was chosen in this thesis.

## 2.9 Application of magnetic nanoparticles

Magnetic nanoparticles have been used widely in various applications such as storage devices, magnetic information storage, magnetic refrigeration, water splitting, gas sensors and biomedical applications.

### 2.9.1 Gas sensing

Researchers reported that copper ferrite (CuFe<sub>2</sub>O<sub>4</sub>), zinc ferrite (ZnFe<sub>2</sub>O<sub>4</sub>) and nickel ferrite (NiFe<sub>2</sub>O<sub>4</sub>) possessed great response for hydrogen sulfide and chlorine gas sensing. Reddy et al. revealed that virgin cobalt and zinc ferrites can detect hydrogen sulfide among all the studied gases. Moreover, it was indicated that nickel ferrite was suited for chlorine gas sensing (Reddy et al., 2000). In similar work, Liu et al. reported the preparation of NiFe<sub>2</sub>O<sub>4</sub> nanopowder doped with Au, Pd and Pt by impregnation technique as gas sensor. Au, Pd and Pt with different concentration were incorporated into NiFe<sub>2</sub>O<sub>4</sub> nanoparticles. The results showed that NiFe<sub>2</sub>O<sub>4</sub> is a p-type semiconductor with superior response and selectivity to H<sub>2</sub>S gas (Liu et al., 2004). An ethanol and H<sub>2</sub>S gas sensor were defined by Liu et al. by a convenient and efficient solid-state reaction. This sensor was based on MgFe<sub>2</sub>O<sub>4</sub> nanomaterial with the grain size of about 15-30 nm and experimental results showed excellent sensitivity of these nanomaterials to ethanol and H<sub>2</sub>S gas at different operating temperatures. The optimum performance to ethanol and H<sub>2</sub>S gas was obtained at 335 °C and 160 °C respectively (Liu et al., 2005). In another research in 2009, magnesium ferrite was prepared via a co-precipitation method by Hankare et al. The gas sensing of this compound was measured towards gases like hydrogen sulfide, liquefied petroleum gas, ethanol vapors, H<sub>2</sub>, NH<sub>3</sub>, methanol, acetone and petrol. The results showed the sensor exhibited various responses towards these gases at different operating temperatures. Furthermore; the MgFe<sub>2</sub>O<sub>4</sub> based sensor exhibited a fast response and a good recovery towards petrol at temperature 250 °C (Hankare *et al.*, 2009).

#### **2.9.2** Water treatment

Magnetic nanomaterials have been used in removal of metals in wastewater treatment. They are widely applied for purification of small molecule pollutants in water due to their unique properties as adsorbents that can be separated and recovered from complex multiphase by an external magnetic field. Zhang *et al.* demonstrated superparamagnetic  $Fe_3O_4$  mesoporous carbon capsules can be used as absorbents for highly efficient removal of pollutants from the wastewater. Experiments indicated that the magnetic nanocomposite exhibited high adsorption rates and excellent removal capacity of organic pollutants (Zhang *et al.*, 2011). Humic acid coated  $Fe_3O_4$ nanoparticles for the removal of toxic Hg(II), Pb(II), Cd(II) and Cu(II) from water by co-precipitation were proposed by Liu *et al.* The nanocomposite was stable in tap water, natural waters and acidic/basic solutions and was able to remove over 99% of Hg(II) and Pb(II) and over 95% of Cu(II) and Cd(II) in natural and tap water at optimized pH (Liu *et al.*, 2008).

# **2.9.3 Biomedical**

In the last few years many efforts were directed towards the development of diagnostic tools in order to improve their performance in sensitivity of the response and to reduce the time and labour required for analysis (Baby & Ramaprabhu, 2010; Yang *et al.*, 2009). Since many biosensors take several successive steps to generate results, an obvious necessity for devices to operate in a short time is needed. Such devices can have a major impact on the diagnosis of several diseases by allowing at-risk patients to check tell-tale signs of proteins or other biomolecules by simply testing a small droplet

of blood or serum. Kaushik et al. demonstrated a new urea sensor by synthesis of nanobiocomposite of CH and superparamagnetic  $Fe_3O_4$ , (magnetisation: 60.8 emu/g) via immobilization of urease and glutamate dehydrogenase (Kaushik et al., 2009). Teymourian et al. developed Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles/reduced graphene oxide nanosheets modified glassy carbon (Fe<sub>3</sub>O<sub>4</sub>/rGO/GCE) as a novel system for the preparation of electrochemical sensing platform (Teymourian et al., 2012). Jimenez et al. fabricated a magneto-assisted formation of conducting nanowires upon selfassembling of Au-shell/CoFe<sub>2</sub>O<sub>4</sub>-magnetic-core nanoparticles ( $18 \pm 3$  nm diameter) on Au electrode surface by application of an external magnetic field to study bioelectrocatalytic oxidation of glucose in the presence of soluble glucose oxidase (Jimenez et al., 2008). In the case of sensors on a substrate, this nanoparticlebiomolecule reacts with "probes" molecules on the surface of the magnetic sensor after making complex. An output signal will be produced by the magnetic nanoparticles. In the case of label-free biosensors, the nanoparticle-biomolecule complexes are directly detected by probing changes in magnetic properties of the nanoparticles after the binding events. These kind of label-free biosensors are extremely promising, especially for point of care applications, where the assay should be simple, requiring no or minimum preparation. The possibility of analyte detection directly in biological samples will lead to more economic, simple to use, versatile and flexible sensors.

#### 2.10 Selected magnetic nanoparticles

Ferrites are important group of magnetic nanoparticles that have general formula  $AB_2O_4$  where A is a transition metal such as Fe, Mn, Cu or Zn and B is the Fe ions. They have a typical spinel lattice with a cubic close-packed arrangement, thus forming two different types of sites, tetrahedral (A) and octahedral (B) (Figure 2.8). The spinal structure is derived from MgAl<sub>2</sub>O<sub>4</sub> or MgO.Al<sub>2</sub>O<sub>3</sub>, by Bragg in 1915. Spinel ferrites are

very stable attributed to its crystal structure; they are predominantly ionic. Spinel ferrites can be divided into three categories-normal, inverse and random spinel ferrites (Szotek et al., 2006). With the growing need for high specific applications, a lot of research has been conducted to improve novel electrode materials for sensors development. Nanostructured transition metal oxides (e.g.  $Fe_3O_4$ , CuO, CdFe<sub>2</sub>O<sub>4</sub>, and CoFe<sub>2</sub>O<sub>4</sub>) have been used in many biomedical applications such as drug delivery, diagnosis and magnetic mediated hyperthermia. Spinel ferrite nanoparticles present different properties than their bulk counterparts and this makes them the focus for new material. The varieties of transition metal cations have provided wide range of applications. Sathiwaitayaku and his team investigated the gas-sensing properties of orthorhombic and spinel ferrites. These ferrites were synthesized by self-propagating high-temperature synthesis and were tested against a wide range of environmentally important gases (ethanol, ethane, ethene, ammonia, propane and CO) at a range of different operating temperatures. Good gas response behavior was found with excellent selectivity towards ethanol, particularly in the case of the LaFeO<sub>3</sub> sensor (Sathiwitayakul et al., 2015). Mahmoodi studied the photocatalytic degradation of manganese ferrite nanoparticle and finding showed formate, acetate and oxalate anions were detected as dominant aliphatic intermediate and inorganic anions (nitrate and sulfate) were detected as the mineralization products of dyes during the degradation processes. These results confirmed the role of manganese ferrite nanoparticle as a magnetic catalyst to degrade reactive dyes from wastewater (Mahmoodi, 2015). In this thesis, zinc ferrite and copper ferrite were chosen to develop new nanomaterials for glucose detection. So far, only a few research has been carried out on zinc ferrite and copper ferrite in medical sensing and there is not any report about the glucose sensing ability of these magnetic nanoparticles. In this work the ability of these magnetic nanoparticles in glucose detection has been studied.

## 2.10.1 Zinc ferrite (ZnFe<sub>2</sub>O<sub>4</sub>) magnetic nanoparticles

Zinc ferrite is found to be one of the most interesting spinel systems as its magnetic behaviour depends on its particle size (Stewart et al., 2007). ZnFe<sub>2</sub>O<sub>4</sub> nanomaterials have two transition elements with a relatively narrow band gap of 1.9 eV, which provides the possibility to tune the energy density and working voltage (Xu et al., 2009). Spinel ZnFe<sub>2</sub>O<sub>4</sub> might be a promising candidate attributed to their high specific surface-area, low-resistance, fascinating electrochemical and optical properties. Haetge et al. showed that by using the poly(omega-hydroxypoly(ethylene-co-butylene)-copoly(ethylene oxide)), ZnFe<sub>2</sub>O<sub>4</sub> can be templated to produce high quality thin films and exhibit reasonable levels of pseudocapacitive charge storage which demonstrates that the electrochemical properties are largely determined by surfaces and interfaces and not by bulk behaviour. Yao et al. synthesized ferromagnetic zinc ferrite nanocrystals at ambient temperature and demonstrated that magnetic properties of these particles can be largely modified by just changing their sizes, which might be a useful way to design novel magnetic materials (Haetge et al., 2010; Yao et al., 2007). Zinc ferrites are also technologically significant doped nanomaterials due to their exceptional mechanical, electrical, thermal and magnetic characteristics (Yang et al., 2006). Their semiconducting and ferrimagnetic properties have made them a good subject in gas sensing field in many research. Darshane and co-workers developed a gas sensor by synthesizing single-phase zinc ferrite nanoparticles having crystallite size in the range of 15-20 nm. This sensor exhibited great sensitivity toward 200 ppm of H<sub>2</sub>S at the operating temperature of 250 °C which is a great achievement in gas sensing (Darshane et al., 2008). Ikenaga et al. prepared a H<sub>2</sub>S absorbent in coal gasification using zinc ferrite in the presence of carbon materials. Carbon material-supported ZnFe<sub>2</sub>O<sub>4</sub> removed H<sub>2</sub>S from 4000 ppm levels in a simulated coal gasification gas to less than 1 ppm at 500  $^{\circ}$ C. The regenerated ferrite can be used for repeated absorption of H<sub>2</sub>S with a very slight decrease in the absorption capacity (Ikenaga *et al.*, 2004). Non-stoichiometric  $ZnFe_2O_4$  powders were synthesized by Sutka and his group to study optical and visible light photocatalytic activity of these ferrite nanoparticles (Sutka *et al.*, 2012).  $ZnFe_2O_4$  magnetic nanomaterials have also been a major focus of research in medicine, medical diagnostics and drug delivery. Mixed spinel hydrophobic  $Zn_xFe_{1-x}O.Fe_2O_3$  (up to x= 0.34) nanoparticles encapsulated in polymeric micelles synthesized, Bárcena *et al.* have developed a highly sensitive magnetic resonance probes for molecular imaging applications (Bárcena *et al.*, 2008). Combining all the special features possesed by zinc ferrite magnetic nanoparticles, it makes a good candidate to explore its potential in glucose sensor application.



**Figure 2.8:** Zinc ferrite (ZnFe<sub>2</sub>O<sub>4</sub>); a) in powder; b) chemical structure.

## 2.10.2 Copper ferrite (CuFe<sub>2</sub>O<sub>4</sub>) magnetic nanoparticles

Copper ferrite,  $CuFe_2O_4$  can be described as a cubic close-packed arrangement of oxygen ions, with  $Cu^{2+}$  and  $Fe^{3+}$  ions at two different crystallographic sites (Krupicka *et al.*, 1982). Local symmetries of the two sites are different which are tetrahedral and octahedral (Jiang *et al.*, 1999). The development of gas sensors in detection of toxic gas pollutants based on these magnetic nanoparticles has been the subject of many fundamental and applied researches. In an excellent work by Singh *et al.*, copper ferrite was successfully synthesized via co-precipitation for liquefied petroleum gas sensing at room temperature. The band gap of copper ferrite were 3.09 and 2.81 eV, respectively

for nanospheres/nanocubes and nanorods. The authors found that the mixed shaped of CuFe<sub>2</sub>O<sub>4</sub> improved the sensing performance over the CuFe<sub>2</sub>O<sub>4</sub> nanorods (Singh *et al.*, 2011). In a similar work, Kumar' groups reported manganese substituted copper ferrite nanoparticles in gas sensor application. These nanoparticles were prepared by evaporation method using metal nitrates and egg white. Conductance response of Mn/Cu ferrite nanomaterial was measured by exposing the material to reducing gas like liquefied petroleum gas which showed a sensor response of 0.2 at an optimum operating temperature of 250 °C (Kumar et al., 2014). Mesoporous copper ferrite nanoparticles were synthesized through the nanocasting strategy with high surface area and large pore size by Wang *et al.* The meso-copper ferrite presented excellent catalytic activity for the degradation of imidacloprid, achieving almost complete removal of 10 mg  $L^{-1}$ imidacloprid after 5 h at the reaction conditions of 0.3 g  $L^{-1}$  catalyst and 40 mM H<sub>2</sub>O<sub>2</sub> This magnetic catalyst provides a potential advantage in organic pollutant removal (Wang et al., 2014). In another application, dye removal ability of the surface modified copper ferrite nanoparticle from single system was investigated by Mahmoodi and his team. By increasing surfactant concentration and copper ferrite nanoparticle dosage, dye removal increases. It is obvious that higher the initial dye concentration, the lower the percentage of dye adsorbed. The dye removal does not change when the pH changes. All results confirmed that copper ferrite magnetic nanoparticle might be a suitable alternative to remove dyes from colored aqueous solutions (Mahmoodi et al., 2013).



**Figure 2.9:** Copper ferrite (CuFe<sub>2</sub>O<sub>4</sub>); a) in powder; b) chemical structure.

#### 2.11 Conducting polymer

Polymer is a Greek word which means "many part'. Macromolecular and electrical transport properties are the main polymer characteristics (Brady et al., 2005). Due to their insulating properties, polymers have been used noticeably in the electronics and packaging industries (Seanor, 2013). Conducting polymers have  $\pi$ -conjugation polymer backbone; polyaniline, polypyrrole, polythiophene across the and polyacetylene and their derivatives are typical conducting polymers. In year 1985, for the first time, Bredas and Street proposed the band theory of solids to determine the conductivity classification of conducting polymers (Bredas & Street, 1985). According the electrical conductivity, materials are summarized into three groups: to nonconductors/insulators, semiconductors and conductors. There are two energy bands, the first one is valence band which relates to the electronic energy levels where they are occupied and the second one is conductance band which is the unoccupied energy levels (Figure 2.10). The band gap,  $E_g$ , is the difference in energy between the top of the outermost valence band and the bottom of the conduction band. Conductivity occurs when the electrons travel from the valence band to the conductance band. The valence band in conductors overlaps with the conduction band, *i.e.*,  $E_g \approx 0$  eV, and the electrons fill up the conduction band partially. The gap between the valence and conduction bands is small in semiconductors, where  $E_g \approx 1.0$  eV; therefore the electrons have this ability to be excited from the valence band into the conduction band at room temperature. However, for insulators, the gap between the valence and conduction bands is large, where  $E_g \ge 10$  eV, and as a result the excitation of the electrons from the valence band into the conduction band is very difficult. However conducting polymers in the region of 1.0 eV were considered as semiconductors according to the Bredas and Street' establishment but this band theory model fails to explain the conductivity associated with conducting polymers. It is now generally accepted that the conducting nature of the polymers arises from the formation of various redox states upon oxidation of the conjugated backbone. This is due to the formation of mobile charge carriers, which are termed polarons and bipolarons (Dai, 2004; Molapo *et al.*, 2012).



Figure 2.10: A band gap energy model for insulators, semiconductors and conductors.

The synthesis of conducting polymers can be carried out by both electrochemical and chemical oxidative polymerization. A chemical route is recommended for large amounts of polymer. In chemical synthesis which typically carried out in solution, a relatively strong oxidising agent such as ammonium peroxydisulphate permanganate or dichromate anions, ferric ions or hydrogen peroxide will need to be used to oxidise a monomer. The concentration of oxidant and monomer, the reaction temperature and surface treating are the factors which affect the rate of polymerization. Electrochemical polymerization is generally employed by galvanostatic, potentiostatic or potentiodynamic methods. Electrochemical polymerization is simple, reproducible and more preferable, especially if the polymeric product is intended for use as a polymer film electrode, thin-layer sensor or in microtechnology because potential control is a prerequisite for the production of good-quality material and the formation of the polymer film at the desired spot in order to serve as an anode during synthesis (Inzelt, 2012; Lange *et al.*, 2008).

### **2.11.1 Synthesis of polypyrrole (PPy)**

Polypyrrole is an organic material consists of carbon, hydrogen and nitrogen atoms which formed by pyrrole repeating units (Figure 2.11). Polypyrrole was synthesized for the first time in 1916, when a report on the oxidation of pyrrole with hydrogen peroxide gives an amorphous black powder was published. Since then a large variety of PPy films and PPy derivatives have been successfully synthesized on different electrode substrates, using various polymerizing solutions. In 2003, Fenelon and Breslin reported the successful electropolymerization of pyrrole on CuNi electrode. method, presence of the copper cations facilitated the pyrrole In this electropolymerization to generate a homogenous and adherent polypyrrole film (Fenelon & Breslin, 2003). Cadierno et al. reported a simple and highly efficient method for the preparation of fully substituted pyrroles using readily accessible secondary propargylic alcohols, 1,3-dicarbonyl compounds and primary amines. The one-pot multicomponent reaction, which involves initial propargylation of the 1,3dicarbonyl compound promoted by  $CF_3CO_2H$  and subsequent condensation between the resulting  $\gamma$ -keto alkyne and the primary amine to afford a propargylated  $\beta$ -enamino ester or ketone, which undergoes a ruthenium-catalysed 5-exo-dig annulation to form the final pyrrole (Cadierno et al., 2007). Rakshit's group reported a conceptually novel pyrrole synthesis by a novel rhodium catalyzed  $sp^3$  C-H bond activation of enamines and successive coupling with unactivated alkynes (Rakshit et al., 2010). Li's group described an efficient method for the synthesis of 1, 3, 4-trisubstituted or 3, 4disubstituted pyrroles. The authors used AgOAc-mediated oxidative coupling reaction in a one-pot manner. In this report, the pyrroles were synthesized directly from aldehydes and amines (anilines) as starting materials (Li, Q. et al., 2010). Wang and Domling designed a new reaction for synthesis of 2-amino-5-ketoaryl pyrroles by reacting aminoacetophenone sulfonamides, (hetero) aromatic aldehydes and malonodinitrile or cyanoacetic acid derivatives in one-pot manner. This unprecedented reaction gave an efficient access to the new scaffold class of 2-amino-5-ketoarylpyrroles (Wang & Dömling, 2010). A three-component reaction for the synthesis of 2,3,4,5tetrasubstituted pyrroles has been developed by Tamaddon and co-workers. They studied the reaction among ammonium acetate, 1,3-dicarbonyl compounds and benzoin derivatives under acidic conditions, using silica sulfuric acid as catalyst which was carried out under solvent-free conditions and the catalyst could be recovered (Tamaddon et al., 2012).



**Figure 2.11:** The structure of PPy where (a) is the neutral PPy and (b) is the oxidized PPy.

## 2.11.2 Application of polypyrrole

Numerous properties of this polymer such as redox activity, ion-exchange and ion discrimination capacities, catalytic activity, corrosion protection and easy

electrochemical surface deposition have made it one of the most extensively used conducting polymers in various applications (Raudsepp *et al.*, 2014; Zhang *et al.*, 2014).

High electrical conductivity and mechanical flexibility of pyrrole make it suitable for the construction of electronic devices. Guo *et al.* reported an organic/inorganic p-n junction nanowire consisting of polypyrrole and CdS fabricated using an Al<sub>2</sub>O<sub>3</sub> template to convert light energy into electricity. The organic/inorganic semiconductor nanowire exhibits a power conversion efficiency of 0.018% under an illumination intensity of 6.05 mW/cm<sup>2</sup> (Guo *et al.*, 2009). Liu *et al.* demonstrated that Au/polypyrrole nanofiber using aluminum anodic oxide membrane as template, rectifying behavior, and might have been used for further application as nano-rectifiers (Liu *et al.*, 2006).

The electrical and optical properties of PPy make this polymer to be explored as chemical sensor, optical sensor and biosensor. Jin et al. reported the electrochemical growth of conducting polymer filaments across screen-printed carbon track arrays and this polypyrrole/polyvinyl sulfonic acid sodium salt filament sensor possess great sensitivity to ethanol vapour which was three times more than a broad sensor (Jin et al., 2004). Li et al. fabricated a novel biosensor based on nano-gold/overoxidized polypyrrole composite. This nanocomposite had strong catalytic activity toward the oxidation of epinephrine, uric acid and ascorbic acid. The results showed that the modified electrode can selectively determine epinephrine and uric acid in the coexistence of a large amount of ascorbic acid. Modifying electrodes with overoxidized polypyrrole improved the selectivity of the sensor and eliminate the effects of interferences (Li & Lin, 2007a). Buar et al. presented a reagentless DNA sensor which was constructed based on an electropolymerized poly-pyrrole-nitrilotriacetic acid film (poly(pyrrole-NTA)) for the first time. This sensor combined with  $Cu^{2+}$  and histidine derivatives mimics the biological avidin-biotin interactions by replacing the bulky avidin with a copper cation. Findings revealed an extremely sensitive detection limit for

the hybridization event without a labeling step for the DNA. The polypyrrole derivatives provide the stable and reproducible immobilization of biological macromolecules on a surface with complete retention of their biological activity (Baur et al., 2009). In another work, Baba et al. developed glucose biosensor based on watersoluble N-alkylaminated polypyrrole/glucose oxidase (GOx) multilayer thin. The electrochemical and optical signals were simultaneously obtained from the composite by the electro-activity and electrochromic property of the polypyrrole layers (Baba et al., 2010). Shi et al. presented a non-enzymatic glucose sensor based on modified palladium/silicon microchannel plate array electrode by over-oxidized polypyrrole. The excellent performance of this sensor is attributed to a combination of the larger electroactive surface area resulting from 3D structure of silicon microchannel plate and permselectivity due to the over-oxidized polypyrrole film (Shi et al., 2011). Xing et al. designed a facile strategy for the preparation of polypyrrole/platinum nanocomposite. This nanocomposite was fabricated using direct deposition of Pt nanoparticles on polypyrrole through an ultrafast microwave-assisted polyol process. Since this nanocomposite possess a good electro-catalytic activity toward the reduction of  $H_2O_2$ , a selective, stable, repeatable and reproducible non-enzymatic electrochemical sensor of  $H_2O_2$  based on the nanocomposite was constructed (Xing *et al.*, 2015). During last decades, considerable progress has been made in synthesis of conducting polymer and they have displayed an impressive applicative potential from biochemical sensing to electronic devices. Hybrid nanostructures which are based on conductive polymer can enhance their properties and addressing the challenges in their applications. In this work, PPy was used to improve the sensor performance due to its great properties and its ability which can lead to a substantial increase in surface area and excellent ionic and electronic conductivity.

### 2.12 Graphene and its applications

Graphene (Figure 2.12) is a one-atom-thick two-dimensional structure of  $sp^2$ bonded carbon with unique mechanical, electronic and optical properties (Balandin et al., 2008; Geim & Novoselov, 2007). High surface area (Park & Ruoff, 2009; Rao et al., 2009), great stability, low cost, ease of processing and strong mechanical strength (Lee et al., 2008; Shao et al., 2010) have made graphene an interesting material in studies since its discovery in 2004. In recent years graphene exhibits a significant potential for many applications and researchers have developed novel electronic materials including transparent conductors, ultrafast transistors, high-performance energy generate devices. One of the potential applications of graphene is in field emission displays. The electrons are emitted from a material under the application of high electric field. This field can be created by field enhancement at the tip of a sharp object and by erected the graphene sheets on the substrates; higher field enhancement will be approached. Eda et al. prepared field emission cathodes from graphene, synthesized from graphite oxide, dissolved in polystyrene by spin coating it onto silicon substrates. This method provides a route for the deposition of graphene based thin film on different substrates, opening up avenues for a variety of applications (Eda et al., 2008). Malesevic et al. synthesized few-layer graphene in the absence of any metallic catalyst by microwave plasma enhanced chemical vapor deposition with gas mixtures of methane and hydrogen to study the electric field emission behavior of vertically aligned few-layer graphene. They found out that the few-layer graphene can be a good field emitter and samples grown on titanium show lower turn-on field values and higher amplification factors when compared to samples grown on silicon (Malesevic et al., 2008).

Reversibility and reasonable specific capacity of graphene make it a good choice in Li-ion battery and supercapacitor. Paek *et al.* prepared graphene nanosheets decorated with  $SnO_2$  nanoparticles by dispersing reduced graphene nanosheets in the ethylene glycol and reassembling in presence of  $SnO_2$  nanoparticles. The obtained  $SnO_2$ /graphene exhibits a reversible capacity of 810 mA h/g and its cycling performance is drastically enhanced in comparison with that of the bare  $SnO_2$  nanoparticle (Paek *et al.*, 2008). Zhang *et al.* designed supercapacitor electrodes by preparing graphene/polyaniline nanofiber composites with uniform structure. The capacitor showed high specific capacitance and good cycling stability which can be related to doping chemically modified graphenes with polyaniline or by doping the bulky polyaniline with graphene/graphene oxide (Zhang *et al.*, 2010). Yang *et al.* reported the preparation of chitosan and graphene nanocomposites by self-assembly of both components in aqueous media for electrochemical applications. Incorporation of 1.0 wt% graphene oxide sheets prefer to disperse well within the nanocomposites (Yang *et al.*, 2010).

Another application of graphene is in sensors and biosensors. The change of graphene's electrical conductivity ( $\sigma$ ) plays main role which due to adsorption of molecules as donors or acceptors on graphene surface (Lee *et al.*, 2008). Some properties of graphene aid to increase its sensitivity; for instance graphene is a two-dimensional (2D) material and all carbon atoms are exposed to the analyte (Schedin *et al.*, 2007). Graphene is also highly conductive and a little change in carrier concentration can cause a notable variation of electrical conductivity. Furthermore, graphene has very few crystal defects ensuring a low level of noise caused by thermal switching (Geim & Novoselov, 2007; Novoselov *et al.*, 2006). Schedin *et al.* developed a gas sensor by using graphene which showed good sensing properties towards NO<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>O and CO. It was demonstrated that graphene sensing properties are recoverable after analyte exposure and chemical doping of graphene by both holes and electrons, in high concentration, did not affect the mobility of graphene (Schedin *et al.*, 2007)

2007). In the work by Sundaram *et al.* the electrodeposition of Pd nanoparticles on graphene surface was modified to detect  $H_2$  gas. The finding showed Pd has good affinity towards  $H_2$  detection and combining with graphene improved the response of sensor to  $H_2$  detection (Sundaram *et al.*, 2008).



Figure 2.12: The structure of graphene.

In addition to gas sensing, the biomedical application of graphene is a relative new area. Bai *et al.* used graphene oxide sheets to demonstrate the graphene oxide/polyvinyl alcohol hydrogels for loading and selectively releasing drugs at physiological pH (Bai *et al.*, 2010). Kong *et al.* developed an enzyme-free glucose sensor based on high-density gold nanoparticles using thionine functionalized graphene oxide as a supporting material with a wide linear range between 0.2 to 13.4 mM, and a lower detection limit of 0.05  $\mu$ M (Kong *et al.*, 2012). Luo *et al.* reported an enzyme-free glucose sensor by synthesis of metallic Cu nanoparticles on graphene sheets. Cugraphene sheets electrode shows much better electro-catalytic properties for glucose oxidation and detection compared to the unmodified graphene sheets electrode (Luo *et al.*, 2012). Zeng *et al.* reported palladium nanoparticle/chitosan-grafted graphene nanocomposites for construction of a glucose biosensor. The sensor showed great electro-catalytic activity toward  $H_2O_2$  with a high sensitivity of 31.2  $\mu$ A·mM<sup>-1</sup>·cm<sup>-2</sup> for glucose was obtained with a wide linear range from 1.0  $\mu$ M to 1.0 mM. Although graphene-based biomedical applications are growing fast but they are still in their initial stage. Some challenging issues for development of an efficient graphene-based biosensor must be overcome. For instance, graphene derivatives must keep their unique properties under physiological conditions, or understanding the graphene-cell interactions especially the cellular uptake mechanism (Shen *et al.*, 2012). In this thesis, graphene oxide has been used to modify the magnetic nanoparticles to improve an enzyme-free glucose detector.

### **CHAPTER 3 : METHODOLOGY**

This chapter emphasizes on the materials, techniques and experimental methods that were used in this research to fabricate and characterise the sensors. They include electrochemical techniques (cyclic voltammetry, amperometry and electrochemical impedance spectroscopy), microscopic (transmission electron microscopy, scanning electron microscopy), Fourier transform infrared spectroscopy and X-ray diffraction. The measurements were performed in three-electrodes system. For electrochemical studies, depending on the system under investigation, different composites served as the working electrode, a coiled platinum wire was used as the auxiliary (counter) electrode and the reference electrode used was saturated calomel electrode (SCE), unless mentioned elsewhere.

## 3.1. Reagents & materials

In this thesis, AR grade chemicals with high purity were used and were listed in Table 3.1. Deionized water (resistivity of 18.2 M $\Omega$ .cm at 25 °C) was used throughout the studies.

| Tuble offer List of enerineuis used. |                                  |               |            |  |
|--------------------------------------|----------------------------------|---------------|------------|--|
| Chemical compounds                   | Molecular Formula                | Brand         | Purity (%) |  |
| Zinc iron oxide                      | ZnFe <sub>2</sub> O <sub>4</sub> | Sigma Aldrich | ≥99        |  |
| Copper iron oxide                    | CuFe <sub>2</sub> O <sub>4</sub> | Sigma Aldrich | >98.5      |  |
| Ammonium persulfate                  | $(NH_4)_2S_2O_8$                 | Sigma Aldrich | ≥98        |  |
| Ethanol                              | $C_2H_6O$                        | Sigma Aldrich | >99.8      |  |
| Pyrrole                              | $C_4H_5N$                        | Sigma Aldrich | >98        |  |
| Sodium nitrate                       | NaNO <sub>3</sub>                | Sigma Aldrich | ≥99        |  |
| Phosphoric acid                      | $H_3PO_4$                        | Sigma Aldrich | ≥85        |  |
| Potassium permanganate               | KMnO <sub>4</sub>                | Sigma Aldrich | ≥99        |  |
| Sodium hydroxide                     | NaOH                             | Sigma Aldrich | ≥98        |  |
| Hydrochloric acid                    | HCl                              | Fluka         | ≥99        |  |
| Zinc nitrate hexahydrate             | $Zn(NO_3)_2.6H_2O$               | Sigma Aldrich | ≥98        |  |
| Iron(III) nitrate                    | $Fe(NO_3)_3 \cdot 9H_2O$         | Sigma Aldrich | ≥98        |  |
| nonahydrate                          |                                  |               |            |  |

Table 3.1: List of chemicals used

| Copper(II) nitrate   | $Cu(NO_3)_2.3H_2O$               | Sigma Aldrich | ≥98      |
|----------------------|----------------------------------|---------------|----------|
| trihydrate           |                                  |               |          |
| Sucrose              | $C_{12}H_{22}O_{11}$             | Sigma         | ≥99.5    |
| Fructose             | $C_{6}H_{12}O_{6}$               | Sigma         | ≥99.5    |
| Uric acid            | $C_6H_{14}O_6$                   | Sigma         | >99      |
| Ascorbic acid        | HNO <sub>3</sub>                 | Sigma         | >99      |
| Sulfuric acid        | $H_2SO_4$                        | Fluka         | ≥99      |
| Glucose              | $C_{6}H_{12}O_{6}$               | Sigma         | >88      |
| Potassium chloride   | $H_3PO_4$                        | Fluka         | ≥97.5    |
| Disodium phosphate   | Na <sub>2</sub> HPO <sub>4</sub> | Fluka         | ≥97.5    |
| Monosodium phosphate | NaH <sub>2</sub> PO <sub>4</sub> | Sigma Aldrich | ≥99      |
| Potassium            | $K_3Fe(CN)_6$                    | Sigma Aldrich | ≥99      |
| ferricyanide(III)    |                                  | -             |          |
| Potassium chloride   | KCl                              | Sigma Aldrich | ≥99      |
| Graphite flakes      | С                                | Sigma Aldrich |          |
| Hydrogen peroxide    | $H_2O_2$                         | Sigma Aldrich | 30%(w/w) |

# **3.2** Experimental set-up

A three-electrodes system (Figure 3.1) including a working electrode, a reference electrode and a counter electrode were used for the electrochemical measurements. These electrodes were immersed in a mixture solution of electrolyte and analyte. The presence of counter electrode is essential to minimize the amount of the uncompensated ohmic drop ( $\Delta E_{ohmic}$ =IR) between the working electrode and reference electrode.



Figure 3.1: Diagram of three-electrodes electrochemical system.

Figure 3.2 shows the conventional three-electrodes electrochemical cell setup in which a glassy carbon (3 mm diameter) (GCE), a saturated calomel electrode (SCE) and a coiled platinum wire are used as working, reference and counter electrode respectively. These electrodes were purchased from BASi Company. The cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and amperometric measurements were carried out using Autolab model PGSTAT 302N.



Figure 3.2: Three-electrodes electrochemical system.

## **3.3** Preparation of conducting polymer–magnetic nanocomposites

Nanomaterials possess unique properties such as geometric, mechanical, electronic, chemical and small size effect which have greatly prompted a broad range of applications of nanomaterials in medicine, electronics, biomaterials, environmental

science, energy production and biosensors (Aillon *et al.*, 2009). A variety of nanomaterials have been synthesized and characterized for the electrochemical sensing due to the huge specific surface area for the immobilization of more functional molecules on the electrodes by nanomaterial-modified electrodes. Conducting polymers are organic polymers with metallic conductivity or semiconductors properties. Many conducting polymer–nanomaterials modified electrodes have been prepared for the analysis of bioanalytes. The preparation of this modified electrode is often realized via *in situ* electropolymerization from monomer solution. They act as promoters of electrochemical communications, accelerating the electron transfer rate between an analyte and electrodes. In this part of work, core-shell magnetic nanoparticles were synthesized by combining the advantages of superparamagnetism of  $CuFe_2O_4$  and  $ZnFe_2O_4$  with highly conductive PPy.  $CuFe_2O_4$ /PPy core-shell nanoparticles and  $ZnFe_2O_4$ /PPy core-shell nanoparticles have been prepared to develop a novel amperometric sensor for glucose determination.

## 3.3.1 CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

CuFe<sub>2</sub>O<sub>4</sub> nanoparticles (0.1 g, 0.43 mmol) were dispersed in deionized (200 ml) water ultrasonically. Then pyrrole monomer (1.0 ml, 14 mmol) was injected into the mixture. After stirring for 30 min,  $(NH_4)_2S_2O_8$  (1.0 ml, 28 mmol, 0.01 mM) aqueous solution (for oxidation of pyrrole) was dropped slowly into the mixture. The polymerization was performed under 80 °C for 8 hours with constant mechanical stirring. After several times washing with distilled water and ethanol, the core-shell composite was dried at 50 °C for 6 hours in the air. The influence of pyrrole has also been studied by deposited different amount of pyrrole (1.0, 2.0 and 4.0 ml) onto the composite. The sample was denoted as CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles.

## 3.3.2 ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

After weighing ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles (0.1 grams, 0.41 mmol) and dispersed in deionized water (200 ml) ultrasonically, pyrrole monomer (1.0 ml, 14 mmol) was injected into this mixture. To complete the polymerization,  $(NH_4)_2S_2O_8$  (1.0 ml, 28 mmol, 0.01 mM) aqueous solution was added into the mixture. The obtained core-shell nanoparticles were washed several times with distilled water followed by ethanol and then was dried at 50 °C for 6 hours in the air. To evaluate the influences of pyrrole thickness on the sensor performance, different amount of pyrrole (1.0, 2.0 and 4.0 ml) was used to synthesize different composites. The sample was denoted as  $ZnFe_2O_4/PPy$  core-shell nanoparticles.

## 3.4 Preparation of graphene-magnetic nanocomposites

In this work a simple strategy is demonstrated to synthesize MFe<sub>2</sub>O<sub>4</sub>-graphene nanocomposites (M= Zn and Fe) with different graphene content (10 wt% - 40 wt%) via a one-step hydrothermal method. In the as-obtained composites, the excellent magnetic properties of CuFe<sub>2</sub>O<sub>4</sub> and ZnFe<sub>2</sub>O<sub>4</sub> were maintained in the composite and could be separated easily by an external magnetic field.

## **3.4.1 Graphene oxide (GO)**

Graphene oxide (GO) was synthesized from graphite according to the modified Hummer method (Barroso-Bujans *et al.*, 2010; Gao *et al.*, 2012). In brief, graphite flakes (5.0 g, 420 mmol) and NaNO<sub>3</sub> (2.5 g, 30 mmol) were mixed together followed by the addition of concentrated  $H_2SO_4$  (108 ml, 2.0 mol) and 12 ml  $H_3PO_4$  (231 mmol). After 10 min stirring in an ice bath, KMnO<sub>4</sub> (15.0 g, 950 mmol) was slowly added. Temperature of the mixture was kept below 5 °C to prevent overheating and explosion. The mixture was stirred at 35 °C for 12 h and the resulting solution was diluted by adding water (400 ml) under vigorous stirring. To ensure the completion of reaction with KMnO<sub>4</sub>,  $H_2O_2$  (15.0 ml, 30% (w/w), 880 mmol) was added to the mixture. The reaction product was centrifuged and washed with deionized water and 5% HCl solution repeatedly. Finally, the product was dried at 60 °C (Figure 3.3).



Figure 3.3: Preparation of graphene oxide (GO).

## 3.4.2 CuFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide magnetic nanocomposite

CuFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide (rGO) with different graphene contents (10, 20, 30 and 40 wt%) were synthesized by hydrothermal method. A typical experiment procedure for the synthesis of CuFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide with 30 wt% graphene content is as follows: GO (96 mg) was dispersed into ethanol (72 ml) with sonication for 1 h. Then, an aqueous solution contains Cu(NO<sub>3</sub>)<sub>2</sub>,3H<sub>2</sub>O (0.29 g, 1.55 mmol) and Fe(NO<sub>3</sub>)<sub>3</sub>,9H<sub>2</sub>O (0.9696 g, 2.4 mmol) were added to ethanol (36 ml) with stirring for 30 min at room temperature. The above two solutions were then mixed together and stirred for 30 min. After that, the mixture was adjusted to a pH of 10.0 with NaOH solution (6.0 M) and stirred for 30 min, yielding a stable homogeneous emulsion. The resulting mixture was transferred into a 200 mL Teflon-lined stainless steel autoclave and heated to 180 °C for 24 h. The reaction mixture was allowed to cool to room temperature, and the precipitate was filtered, washed with distilled water five times, and dried in a

vacuum oven at 60 °C for 12 h. The product was labelled as  $CuFe_2O_4/rGO$  (30 wt%).  $CuFe_2O_4$  nanoparticles were synthesized by the same method without adding in rGO.

### 3.4.3 ZnFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide magnetic nanocomposite

The ZnFe<sub>2</sub>O<sub>4</sub>-reduced graphene oxide nanocomposite with different graphene content (10, 20, 30 and 40 wt%) were synthesized and the method for the synthesis of ZnFe<sub>2</sub>O<sub>4</sub>-reduced graphene nanocomposite with 30 wt% graphene oxide content, is as follows: GO (96 mg) was dispersed into absolute ethanol (72 ml) with sonication for 1 h. Zn(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O (0.357 g, 1.2 mmol) was dissolved in water and then was mixed with a solution of Fe(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O (0.9696 g, 2.4 mmol). This mixture was added to absolute ethanol (24 ml) with stirring for 30 min at room temperature. The above two systems were then mixed together, and stirred for 30 min, yielding a stable homogeneous emulsion and then transferred into a 200 ml Teflon-lined stainless autoclave and heated to 180 °C for 12 h under autogenously pressure. The obtained precipitate was filtered, washed with distilled water several times and dried in a vacuum oven at 60 °C for 12 h. The product was labelled as ZnFe<sub>2</sub>O<sub>4</sub>/rGO (30 wt%). For comparison, the same method was used to synthesize ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles without rGO.

## **3.5** Preparation of phosphate buffer

0.1 M phosphate buffer solution (PBS) was prepared from 1.0 M monosodium phosphate ( $NaH_2PO_4$ ) and 1.0 M disodium phosphate ( $Na_2HPO_4$ ) in deionized water making a solution of approximately pH 7.4 which is the same level of human blood pH.

## 3.6 Preparation of real sample

For determination of glucose in human blood serum, the serum sample was collected from University Malaya Medical Hospital (UMMC) to prepare a standard solution for comparison of the hospital results and the results shown by the prepared sensor. For this purpose 1.0 ml of the real serum samples were respectively added into 9.0 mL of 0.1 M PBS to determine the glucose level (Li *et al.*, 2015).

## 3.7 Fabrication of modified electrode

The polished working electrode was fabricated by using various amounts of magnetic nanoparticles, polypyrrole and graphene oxide for its sensor performance testing.

## **3.7.1 Pre-treatment of the electrode**

Prior to fabrication, the surface of bare glassy carbon electrode (3.0 mm diameter) must be polished to eliminate any traces which affect the rate of electron transfer. The most common method is via mechanical polishing in which pads are used with 1.0 and 0.3  $\mu$ m alumina polish powder. The electrode is held in a vertical position while making figure-8 motions on the polishing pad (Figure 3.4). The electrode surface is rinsed with distilled water to remove all traces of the polishing material and then is sonicated in distilled water for a few minutes to ensure complete removal of the alumina particles.



Figure 3.4: Electrode polishing process.

# 3.7.2 CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

The CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles were mixed with deionized water and ultrasonically treated to form a homogeneous dispersion. Then, 10  $\mu$ L of the dispersion was dropped onto the surface of a clean GCE electrode and dried at room temperature prior to the electrochemical experiments (Figure 3.5).



Figure 3.5: Fabrication of CuFe<sub>2</sub>O<sub>4</sub>/PPy glassy carbon modified electrode.
## 3.7.3 ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

The  $ZnFe_2O_4/PPy$  core-shell nanoparticles were mixed with deionized water and ultrasonically treated to form a homogeneous dispersion. Then, 10 µl of the dispersion was dropped onto the surface of a clean GCE and dried at room temperature before the electrochemical experiments (Figure 3.6).



Figure 3.6: Fabrication of ZnFe<sub>2</sub>O<sub>4</sub>/PPy glassy carbon modified electrode.

## 3.7.4 CuFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide magnetic nanocomposite

1.0 mg of CuFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide composite was mixed with 1.0 ml deionized water and ultrasonically treated to form a homogeneous dispersion. Then, 10  $\mu$ l of the dispersion was dropped onto the surface of a clean GCE and dried at room temperature before the electrochemical experiments (Figure 3.7).



Figure 3.7: Fabrication of CuFe<sub>2</sub>O<sub>4</sub>/rGO glassy carbon modified electrode.

#### 3.7.5 ZnFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide magnetic nanocomposite

To obtain a homogeneous dispersion, a mixture of 1.0 mg of  $ZnFe_2O_4$ /reduced graphene oxide composite and 1.0 ml deionized water were ultrasonically treated. 10 µl

of the dispersion was dropped onto the surface of a clean GCE and dried at room temperature before the electrochemical experiments (Figure 3.8).



**Figure 3.8:** Fabrication of ZnFe<sub>2</sub>O<sub>4</sub>/rGO glassy carbon modified electrode.

#### 3.8 Characterization of modified electrode

Various characterization techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and amperometric have been used for modified electrode characterization. This section is devoted to explain the basic principles and the involved techniques.

#### 3.8.1 Scanning electron microscopy (SEM)

The scanning electron microscopy (SEM) is an extremely useful tool for the surface study of the samples which offers a better resolution of surface samples images than the optical microscopy. This powerful tool is used widely in materials science field for examining and interpreting materials structure. The filament (electron gun) is heated by a current to generate an electron beam and this beam is collimated by electromagnetic condenser lenses and scanned across the surface of the sample by electromagnetic detection coils. Secondary electrons (SE) and backscattered electrons (BSE) signals are mostly used to generate SEM images (Goldstein *et al.*, 2012). The SEM measurements were done on a Hitachi SU 8000 model instrument.

#### **3.8.2** Transmission electron microscopy (TEM)

In this technique a thin sample is imaged by an electron beam which is irradiated through the sample at uniform current density. Electrons being charged in nature can be easily deflected using an external electric or magnetic field and can be accelerated using external potential. As the electrons travel through the sample, they are either scattered or transmitted unaffected through the sample. The probability of scattering is described in terms of the interaction cross-section and can be elastic or inelastic. This results into a non-uniform distribution of electrons in the beam that comes out of the sample, which contains all the structural information of the sample (Wen, 2014). TEM has been used for analysing the spherical shape and size of CuFe<sub>2</sub>O<sub>4</sub> and ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles and also for indication of the hydrothermal dispersion of magnetic nanoparticles on the graphene nanosheets. To prepare for TEM screening, a small amount of sample was suspended in a solvent (water or ethanol). Then, it was droped and spread out over the mesh to provide a very thin layer of film for TEM analysis. The measurements were done on a Hitachi SU 8000 model instrument.

#### **3.8.3** Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is an analytical technique based on the vibrations of the atoms within a molecule and measures the absorption of various infrared light wavelengths by the material of interest to specify molecular components and structures. An interferogram will collect the infrared spectra of a sample and measures all the infrared frequencies simultaneously. FTIR spectrometer acquires and digitizes the interferogram, performs the FT function and outputs the spectrum. Sensitivity is one of the advantages of FTIR which makes the identification of even the smallest of contaminants possible. The very little possibility of mechanical breakdown is another advantage of this technique. These advantages, along with several others, make this tool a very reliable, extremely accurate and reproducible technique. In this work, FTIR spectra of the samples were recorded on a Perkin-Elmer RX1FT-IR spectrometer with a wave-number resolution of 2 cm<sup>-1</sup> as potassium bromide (KBr) pellets at a weight ratio in the 4000 – 400 cm<sup>-1</sup> region.

# 3.8.4 X-ray diffraction technique (XRD)

The powder X-ray diffraction technique is based on the measurement of fluorescence, absorption and scattering which is widely used to investigate the characterization of composites and structures of matters. In this method, the composite to be examined is reduced to a very fine powder and placed in a beam of monochromatic X-rays. This technique is a well-established tool to confirm the formation of solid state reaction, presence of impurity phases, determination of lattice constants, interplanar distances, octahedral and tetrahedral site radii (Suryanarayana & Norton, 1998). In current experiments, X-ray diffraction (model: Siemens D5000) was performed using Cu K $\alpha$  radiation to analyze the nanoparticles structures and synthesized magnetic nanocomposites.

#### 3.8.5 Cyclic voltammetry (CV)

CV is one of the important and sensitive electroanalytical methods to study the redox processes, understanding reaction intermediates and obtaining stability of reaction products. Cyclic voltammetry provides crucial information about the thermodynamics and kinetics of redox processes based on varying the applied potential in both forward and reverse directions while monitoring the current. The peak potentials and peak currents of the cathodic and anodic peaks are two important parameters in a cyclic voltammogram. If the electron transfer process is fast when compared to other processes (such as diffusion), the reaction is said to be electrochemically reversible and the peak separation is:

$$\Delta E p = E pa - E pc = 2.303 RT / nF \qquad (Eq. 3.1)$$

In this work, the electrochemical performance of modified electrodes based on different composites was examined using cyclic voltammetry method in the presence of glucose in 0.1 M PBS (pH 7.4) and 0.1 M NaOH solution using Autolab PGSTAT 302N.

#### **3.8.6 Electrochemical impedance spectroscopy (EIS)**

The electrochemical impedance spectroscopy is a more general concept of resistance and has become very popular nowadays as a complementary technique for the characterization of electrode processes at complex interfaces. Electrochemical impedance spectroscopy is measured by applying AC potential with small amplitude (5 to 10 mV) to an electrochemical cell and measuring the current flowing through the working electrode. An electrode-solution interface undergoing an electrochemical reaction is treated as an electronic circuit consisting of a combination of resistors and

capacitors (Tlili *et al.*, 2006). By using this useful technique, the study of any intrinsic material property or specific processes that could influence the conductivity/resistivity or capacitivity of an electrochemical system is possible. For electrochemical sensing, impedance techniques are useful to observe changes in electrical properties arising from biorecognition events at the surfaces of modified electrodes. For example, changes in the conductance of the electrode can be measured as a result of protein immobilization and antibody-antigen reactions on the electrode surface (Bakker, 2004; Janata, 2002). In this work, this technique was used to analyse the different modified electrodes with a frequency ranging from 0.1 to  $1 \times 10^5$  Hz in 0.1 M KCl solution containing 1.0 mM Fe[(CN)<sub>6</sub>]<sup>3-/4-</sup>(1:1) and 0.1 M NaOH solution.

#### 3.8.7 Amperometry

In this technique a constant potential is applied to a working electrode and the current is measured as a function of time. The applied potential is usually chosen (based on the CV experiments) such that the resulting current is mass transport limited, thus at steady state, it represents a concentration of the electro-active species, which is the analyte of interest or can be correlated to its concentration. Amperometry is based on study of the sensor response to a change of substrate concentration which is referred as titration. It involves the current measurements of a sensor under constant polarisation immersed in a buffer solution, while changing the analyte concentration (stepwise). The results are plotted on a current versus time curve. The time between the changes of analyte concentration is determined by the properties of the sensor, namely by the time required for the current to reach equilibrium state. In this study, during the amperometric measurements, a constant potential at +0.2 V vs. SCE in 0.1 M phosphate buffer solution (pH 7.4) and +0.85 V vs. SCE in 0.1 M NaOH was applied at the working electrode to record the response of sensor to successive additions of glucose.

Solution was stirred to provide faster convective transport of the analyte to the electrode surface.

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#### **CHAPTER 4 : RESULTS AND DISCUSSION**

This chapter reports the fabrication of four novel enzyme-free glucose sensors and associated characterization in order to develop the new electrochemical sensors. There are four main sections in this chapter begin with fabrication of  $CuFe_2O_4/PPy$  nanoparticles. The effect of different shell thickness on the performance of sensor is investigated. ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles are synthesized for the development of enzyme-free glucose sensor together with its characterization. Third part of this thesis focuses on development of sensor based on magnetic nanocomposite of CuFe<sub>2</sub>O<sub>4</sub>/rGO. Lastly, a new approach based on magnetic nanocomposite of ZnFe<sub>2</sub>O<sub>4</sub>/rGO which has been synthesized via hydrothermal method, and applied as the active materials for high-performance enzyme-free glucose sensor.

# Part 1: Core-shell $CuFe_2O_4/PPy$ nanoparticles enzyme-free sensor for glucose detection

#### 4.1 Introduction

CuFe<sub>2</sub>O<sub>4</sub> is a spinal ferrite which has two crystallographic spinal structures and attracted great attention due to its unique optical, electrical and magnetic properties. The intrinsic existence of functional groups and long carbon chains of PPy will improve the properties of CuFe<sub>2</sub>O<sub>4</sub> particles due to the strong electronic interaction between the nanoparticles and the polymer matrices. Here in this study, an enzyme-free sensor for detection of glucose based on chemical oxidative polymerization of pyrrole monomers on the surface of CuFe<sub>2</sub>O<sub>4</sub> nanoparticles is demonstrated. The morphology and surface property of coating phenomenon of CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles were examined by TEM, SEM and XRD. The electro-catalytic activity of CuFe<sub>2</sub>O<sub>4</sub>/PPy towards glucose oxidation was investigated using cyclic voltammetry and

chronoamperometry under alkaline conditions. CuFe<sub>2</sub>O<sub>4</sub>/PPy core shells with different shell thickness by varying the amount of pyrrole monomers incorporated were synthesized and its influence on the morphology and sensing of sensor were also examined.

# 4.2 Characterization of CuFe<sub>2</sub>O<sub>4</sub> and CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

# 4.2.1 Fourier transform infrared spectroscopy (FTIR)

FTIR spectrophotometer was used to characterize  $\text{CuFe}_2\text{O}_4$  and core-shell structured  $\text{CuFe}_2\text{O}_4/\text{PPy}$  nanoparticles. Figure 4.1 indicates the FTIR spectra in the 4000-400 cm<sup>-1</sup> region of CuFe<sub>2</sub>O<sub>4</sub> and core-shell structured CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles prepared with 4.0 ml pyrrole. The peak (curve b) at 1285 cm<sup>-1</sup> corresponds to =C–H inplane vibration, while C–C out-of-plane ring deformation vibration is found at 1596 cm<sup>-1</sup> (Jing *et al.*, 2007). The appearance of peak at 1694 cm<sup>-1</sup> is attributed to the over oxidization of PPy (Lu *et al.*, 2006). Additionally, a strong band at 570 cm<sup>-1</sup> appears in the spectrum of CuFe<sub>2</sub>O<sub>4</sub> (curve a), which is assigned to the Fe–O stretching vibration mode. The fundamental vibration of PPy ring (curve b), compared with pure PPy has shifted to higher wavenumber at 1694 cm<sup>-1</sup> (Blinova *et al.*, 2007). This happened because of the skeletal vibrations and delocalizing the  $\pi$ -electrons of PPy. In addition to these vibrational bands, a broad signal due to water symmetric stretching and antisymmetric stretching in the range of 3385-3448 cm<sup>-1</sup> were observed (Bamzai *et al.*, 2013). The FTIR spectrum from the CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles prepared with 1.0 and 2.0 ml pyrrole showed the similar results as the reported spectra.



**Figure 4.1:** FTIR spectra of (a) CuFe<sub>2</sub>O<sub>4</sub> nanoparticles and (b) core-shell structured CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles prepared by using 4.0 ml of pyrrole at 80 °C for 8 h.

#### 4.2.2 X-ray diffraction (XRD)

Figure 4.2 shows the XRD pattern of the CuFe<sub>2</sub>O<sub>4</sub> and CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles. The two stronger peaks corresponding to (211) and (103) reflections at  $2\theta \approx 34.7^{\circ}$  and  $35.9^{\circ}$ . These peaks merged into a single broad peak, indistinguishable from the strongest reflection of the cubic CuFe<sub>2</sub>O<sub>4</sub> phase ( $2\theta \approx 35.9^{\circ}$ ). The Bragg plane corresponds to tetrahedral structure of CuFe<sub>2</sub>O<sub>4</sub> nanoparticles at  $2\theta \approx 54^{\circ}$  appears in all of the XRD patterns. There are obvious similarities between labeled diffraction peaks of CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles and pure CuFe<sub>2</sub>O<sub>4</sub> nanoparticles. Amorphous form of the polymer in CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles makes the diffraction strength of CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles weaker than the pure CuFe<sub>2</sub>O<sub>4</sub> nanoparticles. It can conclude that  $CuFe_2O_4$  nanoparticles only served as the nucleation sites for the polymerization of pyrrole because there is no chemical interaction between  $CuFe_2O_4$  and PPy in the composites through the XRD and FTIR analyses. The similar patterns were observed for core-shell structured  $CuFe_2O_4$ /PPy nanoparticles by using 1.0 ml and 2.0 ml of pyrrole.



**Figure 4.2:** XRD patterns of (a) CuFe<sub>2</sub>O<sub>4</sub> and (b) core-shell structured CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles by using 4.0 ml of pyrrole.

#### 4.2.3 Surface morphology study

The morphology and shell thickness of  $CuFe_2O_4$  and the  $CuFe_2O_4/PPy$  core-shell nanoparticles were characterized by SEM and TEM (Figure 4.3). These images showed the spherical shape of  $CuFe_2O_4$  nanoparticles with diameters ranging from 20 to 90 nm. Agglomeration of some particles can be attributed to magnetic dipole interactions between particles. The influence of the amount of pyrrole monomers on the morphology and shell thickness of core-shell nanoparticles was investigated in the experiment. Coating shell with different thicknesses which were obtained using pyrrole monomers at 80 °C have been examined. From Figure 4.3(c), (d) and (e) it can obviously be observed that the  $CuFe_2O_4$  cores are surrounded by polypyrrole shells with the diameter of 10-25 nm. TEM images of  $CuFe_2O_4/PPy$  core-shell nanoparticles obtained by using 1.0, 2.0 and 4.0 ml of pyrrol respectively, in which, the coating phenomenon can be clearly observed. The PPy shell thickness varies from 10 to 25 nm with the increasing volumes of pyrrole monomers.



**Figure 4.3:** The SEM images of (a)  $CuFe_2O_4$  and TEM images of (b)  $CuFe_2O_4$ , (c) core-shell structured  $CuFe_2O_4$ /PPy nanoparticles prepared by 1.0 ml (d) 2.0 ml and (e) 4.0 ml of PPy.

The Scherrer equation, in X-ray diffraction and crystallography, is a formula that relates the size of sub-micrometre particles or crystallites in a solid to the broadening of a peak in a diffraction pattern. It is used in the determination of size of particles of crystals in the form of powder. The Scherrer equation can be written as:

$$\tau = \frac{K\lambda}{\beta\cos\theta}$$
(Eq. 4.1)

where:

- τ is the mean size of the ordered (crystalline) domains, which may be smaller or equal to the grain size;
- *K* is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite;
- $\lambda$  is the X-ray wavelength;
- β is the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, in radians. This quantity is also sometimes denoted as Δ(2θ);

•  $\theta$  is the Bragg angle.

Table 4.1 shows the comparison of crystallite size of  $CuFe_2O_4$  nanoparticles and  $CuFe_2O_4/PPy$  nanoparticles with different shell thickness, by XRD and TEM techniques.

| samples  | Crystallite size (nm) | Crystallite size (nm) |
|--|-----------------------|-----------------------|
|  | by                    | by                    |
|  | XRD                   | TEM                   |
| CuFe <sub>2</sub> O <sub>4</sub> nanoparticles | 19-87                 | 20-90                 |
| CuFe <sub>2</sub> O <sub>4</sub> /PPy (1 ml)   | 9.4                   | 10                    |
| CuFe <sub>2</sub> O <sub>4</sub> /PPy (2 ml)   | 14                    | 15                    |
| CuFe <sub>2</sub> O <sub>4</sub> /PPy (4 ml)   | 24                    | 25                    |

**Table 4.1:** Comparison of the crystallite size from the XRD and TEM results.

#### 4.2.4 Optimization of the sensor

In order to improve the performance of the sensor, the influence of factors which may affect the response of the sensor were studied.

#### 4.2.4.1 Polypyrrole shell thickness

The influence of the shell thickness on the response of the sensor was investigated; the effect of the PPy shell thickness on the response current of the sensor is illustrated in Figure 4.4. There are two reasons for using the selected amount of PPy, firstly, main focus of this work was on  $CuFe_2O_4$  nanoparticles and to study the effects of these magnetic particles on glucose oxidation. After the discovery of the significant sensitivity of these ferrites, the results were improved by using polypyrrole as conducting polymer. Secondly, in spite of all advantages of conducting polymers, the poor cycling stability is one of the major drawbacks of conducting polymers during the charging process. This failure is due to the formation of cracks in the polymer chains attributed to continuous swelling/shrinkage of polymer backbone during the charge/discharge cycles (Lu et al., 2014; Yang et al., 2013). To minimize this drawback and also gain advantages from the electrical conductivity of PPy, small amount of PPy were applied compared to concentration of ferrite nanoparticles. The figure 4.4 indicates that the oxidation peak currents of CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell obtained by using 1.0, 2.0 and 4.0 ml of pyrrol respectively. Upon addition of glucose (0.5 to 2.0 mM), the oxidation peak was changed considerably and by increasing amount of PPy, the changes became more significant. All the results have proven that the electro-catalytic activity towards glucose of CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell electrode was greatly improved which may be due to the synergic effect between magnetic nanoparticles and PPy and the electrocatalytic activity.



**Figure 4.4:** Cyclic voltammogram of CuFe<sub>2</sub>O<sub>4</sub>/PPy /GCE by (a) 1.0 ml of PPy (b) 2.0 mM of PPy (c) 4.0 ml of PPy in (i) 0.5 mM, (ii) 1.0 mM and (iii) 2.0 mM glucose in 0.1 M NaOH at the scan rate of 10 mV s<sup>-1</sup>.

#### 4.2.4.2 Optimization of potential for glucose oxidation

The relationship between the applied potential in chronoamperometry and the oxidation current of glucose was examined. From Figure 4.5, the dependence of the amperometric response on the applied potential of the CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell coated GCE under the batch conditions was evaluated over the range of 0.7 V to 1.0 V. The current response was increased when the applied potential was changed from 0.7 V to 0.85 V *vs.* SCE, which suggests that the oxidation of glucose was achieved at low potential. However, when the potential was more positive than 0.85 V, the response current is decreased slightly. Since a suitable working potential should be chosen based on the least potential to achieve good selectivity, thus, 0.85 V was selected as the optimized condition.



**Figure 4.5:** Effect of the applied potential on the current response of  $CuFe_2O_4/PPy/GCE$  (4.0 ml of PPy) in the presence of 2.0 mM glucose at the scan rate of 10 mV s<sup>-1</sup> in 0.1 M NaOH.

#### 4.2.5 Cyclic voltammetry studies

The cyclic voltammetry response of the bare GCE, PPy/GCE, CuFe<sub>2</sub>O<sub>4</sub>/GCE and CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE with presence of 2.0 mM glucose at a scan rate of 10 mV s<sup>-1</sup> was studied and compared. From Figure 4.6, it can be seen that the single broad oxidative peak of CuFe<sub>2</sub>O<sub>4</sub> modified electrode, corresponding to the irreversible glucose oxidation, is much larger than those of the bare GCE and PPy, which confirms that there is a strong electro-catalytic function of CuFe<sub>2</sub>O<sub>4</sub> nanoparticles towards glucose. This is presumably due to the surface of  $Cu^{2+}$  ions and  $Cu^{3+}$  ions that act as an electron transfer mediator in the oxidation of glucose (Farrell & Breslin, 2004; Kang, X. et al., 2007). CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shells modified electrode reveals significant effect on the oxidation of glucose. The oxidation current of the CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE starts to rise rapidly at approximately +0.70 V (vs. SCE) with a peak potential at +0.85 V (vs. SCE) and it is greater than those of CuFe<sub>2</sub>O<sub>4</sub>. These results can be attributed to the excellent properties of polypyrrole which possesses good electrical conductivity and its presence can facilitate the electron transfer rate and decreased the formal potential of  $Cu^{2+}$  ions. Previous studies revealed that present of PPy in different morphologies may lead to an increase in surface area, excellent ionic and electronic conductivity. In this study the performance of CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles was furtur enhanced by core-shell morphology of obtained nanoparticles, attributed to the polymer matrices where it brings to the improvement in terms of better conjugation with magnetic nanoparticles and increased thermal and chemical stability.



**Figure 4.6:** Cyclic voltammograms of (a) bare GCE, (b) PPy/GCE, (c) CuFe<sub>2</sub>O<sub>4</sub>/GCE, (d) CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE by 4.0 ml of PPy in presence of 2.0 mM glucose in 0.1 M NaOH at the scan rate of 10 mV s<sup>-1</sup>.

In order to verify sensor ability to detect the glucose in different pH near to blood serum pH, the oxidation current of the CuFe<sub>2</sub>O<sub>4</sub> nanoparticles modified electrode and CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell modified electrode was studied by cyclic voltammetry (CV) method in 0.1 M phosphate buffer solution at pH 7.4 in the presence of 2.0 mM glucose at a scan rate of 10 mV s<sup>-1</sup>. The results (Figure 4.7) exhibited a great performance of these nanoparticles with high oxidation current in 0.1 M phosphate buffer solution. The oxidation current of glucose is less than those recorded in 0.1 M NaOH solution where highest activity of modified electrode towards the oxidation of glucose is expected under influence of alkaline solution. It can be concluded that the proposed sensor in this work has the potential to be used for blood glucose sensing.



**Figure 4.7:** Cyclic voltammograms of (a)  $CuFe_2O_4/GCE$  at pH 7.4 without glucose (b) in presence of glucose (c)  $CuFe_2O_4/PPy/GCE$  (4.0 ml of PPy) at pH 7.4 without glucose (d) in the presence of 2.0 mM glucose at the scan rate of 10 mV s<sup>-1</sup>.

#### 4.2.6 Electrochemical impedance spectroscopy (EIS) studies

EIS is an effective method for probing the features of surface and understanding chemical transformations of modified electrodes. The electron transfer resistance ( $R_{ct}$ ) of the electrode surface controls the electron transfer kinetics of the redox probe which is equal to the semicircle diameter of the Nyquist plot. Figure 4.8 presents the Nyquist plots (Z'' vs Z') of the impedance spectroscopy of CuFe<sub>2</sub>O<sub>4</sub> and CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles (1.0 and 4.0 ml pyrrole) in 0.1 M KCl solution containing 1.0 mM Fe[(CN)<sub>6</sub>]<sup>3-/4-</sup> (1:1) in the frequency range of 0.01–10<sup>5</sup> Hz. Fe(CN)<sub>6</sub><sup>3-/4-</sup> is a wellknown redox couple that has been used to characterize the properties of electrode surfaces or electrolyte solutions. In aqueous electrolyte solutions, the one-electron redox reactions, Fe(CN)<sub>6</sub><sup>3-</sup> + e  $\leftrightarrow$  Fe(CN)<sub>6</sub><sup>4-</sup>, is considered reversible and diffusion-controlled processes (Fox *et al.*, 2013). The Nyquist semicircles of the different modified electrodes show significant differences. Compared to the bare GCE and the CuFe<sub>2</sub>O<sub>4</sub> nanoparticles was cast on the GCE surface, possibly due to the great conductivity of PPy. In fact, electron transfer ability of  $CuFe_2O_4$  nanoparticles was improved by PPy shells and also the electron transfer resistance has decreased with increasing of PPy shell thickness. These shells were proven to improve the speed of electron transfer rate.



**Figure 4.8:** EIS of (a) bare GCE, (b) CuFe<sub>2</sub>O<sub>4</sub>/GCE, (c) CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE using 1.0 ml of PPy, (d) CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE using 4.0 ml of PPy, in 0.1 M KCl solution containing 1.0 mM  $Fe[(CN)_6]^{3-/4-}$  (1:1). The frequency range was from 0.1 to  $1 \times 10^5$  Hz; right inset: the  $R_s(CPE[R_{ct}W])$  equivalent circuit model.

#### 4.2.7 Amperometric detection of glucose on CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

The amperometric response of the CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell modified electrode to successive additions of glucose into 0.1 M NaOH is depicted in Figure 4.9. A typical calibration curve for the sensor can be prepared from the amperometric response (left inset). As the concentration of glucose has been changed, the sensor electrode responded rapidly and showed a linear steady-state amperometric response up to 5.6 mM of glucose under the applied potential of +0.85 V *vs.* SCE. A quick increase in the current after each glucose addition was observed and the amperometric signal displayed linear correlation to glucose concentration in the range from 20.0  $\mu$ M to 5.6 mM. The

limit of detection (LOD) of CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell coated GCE was calculated using the following equation (Krull & Swartz, 1998).

$$LOD = 3S_{\rm B}/b \tag{Eq. 4.2}$$

Where  $S_{\rm B}$  is the standard deviation of the blank solution and *b* is the slope of the analytical curve. The proposed sensor displays a linear response ( $R^2 = 0.9945$ ) to glucose for low concentrations (20.0 µM to 0.6 mM) and ( $R^2 = 0.9919$ ) for high glucose concentrations (0.6 mM to 5.6 mM) with a sensitivity of 637.76 and 176.0 µA mM<sup>-1</sup> respectively. The sensitivity and detection limit of this modified electrode is comparable and better than those obtained by using other modified electrodes based on metals or metal oxide nanoparticles (Table 4.2).



Figure 4.9: The typical current–time dynamic response of the (a)  $CuFe_2O_4/PPy/GCE$  (4.0 ml of PPy) modified GCE towards various concentrations of glucose; left inset: the calibration curve for glucose detection. The calibration curves for glucose detection (b) in low concentration, (c) in high concentration.

| gideose sensor with other gideose sensors based on eu materials. |             |           |           |                    |         |                    |
|--|-------------|-----------|-----------|--------------------|---------|--------------------|
| Electrode  | Electrolyte | Detection | Detection | Sensitivity        | Linear  | Ref.               |
|  |             | potential | limit     | $(\mu A m M^{-1})$ | range   |                    |
|  |             | (vs. SCE) |           |                    |         |                    |
| GE/CuO/GOx/  | pH= 7.4     | 0.54 V    | 1.37 μM   | 47.19              | 0.01-10 | (Umar              |
| Nafion   | PBS         |           | -         |                    | mM      | et al.,            |
|  |             |           |           |                    |         | 2009)              |
| Nafion/CuO   | 0.1 M       | 0.56 V    | 1.0 µM    | 404.53             | 0-2.55  | (Reitz et          |
|  | NaOH        |           |           |                    | mM      | al.,               |
|  |             |           |           |                    |         | 2008)              |
| CuO nanorod  | 0.1 M       | 0.56 V    | 4.0µM     | 371.43             | 4 -8    | (Wang,             |
|  | NaOH        |           |           |                    | mM      | X. et al.,         |
|  |             |           |           |                    |         | 2010)              |
| CuO nanorod  | 0.1 M       | 0.6 V     | 1.2 μM    | 450                | Up to 1 | (Batchel           |
|  | NaOH        |           |           |                    | mM      | or <i>et al</i> ., |
|  |             |           |           |                    |         | 2008)              |
| CuFe <sub>2</sub> O <sub>4</sub> /PPy                            | 0.1 M       | 0.85 V    | 0.1 µM    | 637.76             | 0.6-5.6 | This               |
| core-shell   | NaOH        |           |           |                    | mM      | work               |
| nanoparticles  |             |           |           |                    |         |                    |

**Table 4.2:** Comparison of the present CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles enzyme-free glucose sensor with other glucose sensors based on Cu materials.

#### 4.2.8 Interference study

It is well-known that some interfering species co-exist with glucose in human serum such as uric acid (UA) and ascorbic acid (AA) which influence the performance of sensor during catalytic oxidation of glucose; therefore, selectivity of the sensor to target the analyte is one of the most important analytical factors for an amperometric sensor. Figure 4.10 indicates the amperometric response of the sensor by successive injection of 1.0 mM glucose and blood interfering species (1.0 mM UA and 1.0 mM AA) into the solution containing 0.1 M NaOH to study the anti-interference ability of the fabricated glucose sensor. Furthermore, the performance of the sensor to differentiate glucose from the other sugars like sucrose and fructose was demonstrated. The concentration of glucose in human blood is between 4.4 and 6.6 mM (Wang, 2008) while the other interferences are present at levels as low as 0.1 mM (Safavi *et al.*, 2009) and as a result small amount of AA, UA, fructose and sucrose can be neglected when CuFe<sub>2</sub>O<sub>4</sub>/PPy exhibits high selectivity for glucose sensing. All the above figures imply

that the CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles modified electrode have a good selectivity toward glucose detection.



Figure 4.10: Interference test of the sensor in 0.1 M NaOH with 0.1 mM glucose and other interferes as indicated.

#### 4.2.9 Reproducibility and stability of the sensor

CuFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles sensor had an acceptable repeatability. Four electrodes were prepared under the same conditions and relative standard deviation (RSD) of the current response towards 0.1 mM glucose was found to be 4.2%. In addition, the stability of the CuFe<sub>2</sub>O<sub>4</sub>/PPy (4.0 ml of PPy) core-shell nanoparticles electrode was tested by measuring responses for longer than two weeks. Electrode performance was investigated every two days (electrode was placed in ambient condition) and as it can be seen from Figure 4.11 the sensor retains around 88.4% of initial response after two weeks ( $I_0$  and I are the response current in the first and later days). The good long term stability could be attributed to the great composites compatibility. The overall performance shows that this sensor displays a good superiority in terms of sensitivity, selectivity and linear calibration.



Figure 4.11: Long-term stability of a CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE measured in more than two weeks.

### **4.2.10 Detection of real samples**

Real serum samples were utilized to verify the applicability of the sensor for determination of glucose in blood serum. The serum sample (0.2 ml) was added to 10.0 ml of 0.1 M NaOH as testing solution. Table 4.3 shows the data composition of glucose level obtained via hospital standard method and the prepared electrochemical sensor. The low RSD value indicates that there is a high possibility to use  $CuFe_2O_4/PPy$  coreshell nanoparticles modified electrode in future for clinical diagnostics.

| Table 4.5. Determination of glucose in real sample of blood serum. |                   |                    |                   |  |
|--|-------------------|--------------------|-------------------|--|
| Blood serum  | Glucose           | Glucose            | Relative standard |  |
| samples  | concentration     | concentration      | deviation         |  |
|  | measured by       | measured by sensor | measured by       |  |
|  | University Malaya | (mM)               | sensor            |  |
|  | Medical Centre    |                    | (%)               |  |
|  | (mM)              |                    |                   |  |
| 1  | 5.56              | 5.72               | 3.88              |  |
| 2  | 8.22              | 8.51               | 4.24              |  |
| 3  | 7.62              | 7.88               | 3.82              |  |
| 4  | 9.44              | 10.25              | 4.64              |  |

Table 4.3: Determination of glucose in real sample of blood serum.

#### 4.3 Electro-oxidation mechanism of glucose on CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE

A mechanism for the electro-oxidation of glucose on CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles modified electrode is proposed in Scheme 4.1. The glucose detection is performed in the alkaline electrolyte condition where the occurrence of glucose oxidation is at the highest rate. Enediol is the oxidized glucose intermediate which makes oxidation of glucose easier in alkaline media (Qian et al., 2013). As can be seen in Scheme 4.1, glucose loses one of its protons to form the enediol structure in alkaline solution, which is the intermediate form of glucose under electro-oxidation. CuFe<sub>2</sub>O<sub>4</sub> nanoparticles only serve as the nucleation sites for the polymerization of pyrrole without any chemical interaction between CuFe<sub>2</sub>O<sub>4</sub> and PPy in the composites. Therefore, the mechanism of glucose electro-oxidation on CuFe<sub>2</sub>O<sub>4</sub> and CuFe<sub>2</sub>O<sub>4</sub>/PPy is similar, but the peak current is different. A broad oxidation peak at about 0.85 V vs. SCE with a peak current of 700.00  $\mu$ A is observed for CuFe<sub>2</sub>O<sub>4</sub>/PPy, which is about 7 times of that obtained at the CuFe<sub>2</sub>O<sub>4</sub>. All these results indicated that the electro-catalytic activity toward glucose oxidation on CuFe<sub>2</sub>O<sub>4</sub>/PPy is highly improved. This enhanced electrochemical behavior is attributed to the special property of PPy which can provide better catalytic activity as well as facilitate the electron transfer rate compared to CuFe<sub>2</sub>O<sub>4</sub> (Qian *et al.*, 2013).



Scheme 4.1: Electro-oxidation mechanism of glucose on CuFe<sub>2</sub>O<sub>4</sub>/PPy/GCE.

#### 4.4 Conclusion

In this work, a novel core-shell CuFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles and its electrochemical properties for the determination of glucose is presented. The sensor showed a very high electrochemical active surface area and electro-catalytic activity for the glucose electro-oxidation, a lower detection limit of 0.1  $\mu$ M and high sensitivity of 637.76  $\mu$ A mM<sup>-1</sup> cm<sup>-2</sup>. All these advantageous features hold the promise for the development of an accurate enzyme-free glucose sensor.

# Part 2: Polypyrrole-ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles with core-shell structure for glucose sensing

#### 4.5 Introduction

Among the magnetic nanoparticles, zinc ferrite (ZnFe<sub>2</sub>O<sub>4</sub>) nanoparticles are occupying an important place for their unusual properties such as narrow band gaps, excellent visible-light response, good photochemical stability and favorable magnetism. Using polypyrrole as shell in polypyrrole-ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles has provided a strict barrier between nanoparticles and reduced the magnetic-coupling effect between them. An amperometric enzyme-free glucose sensor using core-shell nanoparticles based on chemical oxidative polymerization of pyrrole on ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles surface was investigated in this study. The electrochemical performance of the modified electrodes was investigated by cyclic voltammetry method. The morphology and surface property of coating phenomenon of ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles were examined by TEM, SEM and XRD.

#### 4.6 Characterization of ZnFe<sub>2</sub>O<sub>4</sub> and ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

#### **4.6.1** Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectrophotometer was used to characterize ZnFe<sub>2</sub>O<sub>4</sub> and core-shell structured ZnFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles. The FTIR spectra of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles (Figure 4.12) indicates the peak at 1289 cm<sup>-1</sup> corresponded to =C–H inplane vibration, while C–C out- of- plane ring deformation vibration is found at 931 cm<sup>-1</sup> (Jing *et al.*, 2007). 1694 cm<sup>-1</sup> Peak is due to the over oxidization of PPy (Lu *et al.*, 2006). The fundamental vibration of pyrrole ring compared with 1543 cm<sup>-1</sup> of pure PPy has shifted to higher wavenumbers at 1569 cm<sup>-1</sup> which is due to the influence of the skeletal vibrations and consequently delocalizing the  $\pi$ -electrons (Blinova *et al.*, 2007). Additionally, a strong band at 572 cm<sup>-1</sup> and a broad signal at 3400 cm<sup>-1</sup> are assigned to the Fe–O stretching vibration mode and water symmetric and antisymmetric stretching were observed respectively.



Figure 4.12: FTIR spectra of (a)  $ZnFe_2O_4$  and (b) core-shell structured  $ZnFe_2O_4$ /PPy nanoparticles.

#### 4.6.2 X-ray diffraction (XRD)

Figure 4.13 shows the XRD pattern of the  $ZnFe_2O_4$  and  $ZnFe_2O_4/PPy$  core-shell nanoparticles. The two stronger peaks are corresponding to (220) and (311) reflections

at  $2\theta \approx 30^{\circ}$  and  $35.5^{\circ}$ . These peaks merged into a peak (400), which is the reflection of the cubic ZnFe<sub>2</sub>O<sub>4</sub> phase  $2\theta \approx 37.18^{\circ}$ . The Bragg planes of (422) and (440) were corresponded to the tetrahedral structure of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles ( $2\theta \approx 57^{\circ}$ -  $62^{\circ}$ ). There are obvious similarities between labeled diffraction peaks of ZnFe<sub>2</sub>O<sub>4</sub>/PPy coreshell nanoparticles and pure ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles, which show the presence of ZnFe<sub>2</sub>O<sub>4</sub> in the ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles. The observed broad reflection planes can be due to the ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles and amorphous form of the polymer in ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles which makes the diffraction strength of ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles only served as the nucleation sites for the polymerization of pyrrole because there is no chemical interaction between ZnFe<sub>2</sub>O<sub>4</sub> and PPy in the composites through the XRD and FTIR analyses.



Figure 4.13: XRD patterns of (a)  $ZnFe_2O_4$  and (b) core-shell structured  $ZnFe_2O_4/PPy$  nanoparticles.

#### 4.6.3 Surface morphology study

The morphologies of the prepared ZnFe<sub>2</sub>O<sub>4</sub> in the ZnFe<sub>2</sub>O<sub>4</sub>/PPy electrodes were investigated by scanning electron microscopy (SEM) analysis and transmission electron microscopy (TEM). Coating shell with different thicknesses were obtained by using pyrrole monomers at 80 °C and the influence of different PPy loadings on the morphology of nanoparticles was studied in the experiment. Figure 4.14(a) and (b) present the SEM and TEM images of  $ZnFe_2O_4$  nanoparticles. Figure 4.14(c), (b) and (e) showed the ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles with diameters ranging from 5.0 to 20.0 nm. Magnetic dipole interactions between particles can contribute to agglomeration of some particles. In Figure 4.14(c) it can be obviously seen that the ZnFe<sub>2</sub>O<sub>4</sub> cores are surrounded by polypyrrole shells with the average diameter of 5.0 nm. The coating phenomenon can also be observed clearly in Figure 4.14(d) and (e) that  $ZnFe_2O_4/PPy$  core-shell nanoparticles were obtained by using 2.0 and 4.0 ml of pyrrol respectively. The PPy shell thickness reaches to 20.0 nm with the increasing volumes of pyrrole monomers. Table 4.4 shows the comparison of crystallite size of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles and ZnFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles with different shell thickness, by XRD and TEM techniques.

| samples  | Crystallite size (nm) | Crystallite size (nm) |
|--|-----------------------|-----------------------|
|  | by                    | by                    |
|  | XRD                   | TEM                   |
| ZnFe <sub>2</sub> O <sub>4</sub> nanoparticles | 20-86                 | 20-90                 |
| ZnFe <sub>2</sub> O <sub>4</sub> /PPy (1 ml)   | 4.8                   | 5                     |
| ZnFe <sub>2</sub> O <sub>4</sub> /PPy (2 ml)   | 9.5                   | 10                    |
| ZnFe <sub>2</sub> O <sub>4</sub> /PPy (4 ml)   | 20                    | 20                    |

**Table 4.4:** Comparison of the crystallite size from the XRD and TEM results.



**Figure 4.14:** The SEM images of (a)  $ZnFe_2O_4$  and TEM images of (b)  $ZnFe_2O_4$ , (c)  $ZnFe_2O_4$ /PPy core-shell nanoparticles prepared by 1.0 ml, (d) 2.0 ml and (e) 4.0 ml of PPy.

# 4.6.4 Optimization of the sensor

The relationship of PPy shell thickness and the oxidation peak current were investigated to improve the performance of the sensor. The effect of the PPy shell thickness on the sensor response current is demonstrated in Figure 4.15 which shows the oxidation peak currents of  $ZnFe_2O_4/PPy$  core-shell obtained by using 1.0, 2.0 and 4.0 ml of pyrrol respectively. The oxidation peak was increased greatly by the addition of glucose (0.5 to 2.0 mM) which corresponds to the amount of PPy added. All the

results have proven that synergic effect between  $ZnFe_2O_4$  nanoparticles and PPy has greatly improved the electro-catalytic activity towards glucose of  $ZnFe_2O_4/PPy$  modified electrode.



**Figure 4.15:** Cyclic voltammograms of  $ZnFe_2O_4/PPy/GCE$  by (a) 4.0 ml of PPy (b) 2.0 mM of PPy (c) 1.0 ml of PPy in (i) 0.5 mM, (ii) 1.0 mM and (iii) 2.0 mM glucose in 0.1 M NaOH at the scan rate of 10 mV s<sup>-1</sup>.

#### 4.6.5 Cyclic voltammetry studies

The electro-catalytic activity of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles and ZnFe<sub>2</sub>O<sub>4</sub>/PPy coreshell were investigated to detect their response to the oxidation of glucose in 0.1 M NaOH solution. Figure 4.16 gave the CV responses of bare GCE, PPy/GCE, ZnFe<sub>2</sub>O<sub>4</sub>/GCE and ZnFe<sub>2</sub>O<sub>4</sub>/PPy/GCE with the presence of 2.0 mM glucose at scan rate 10 mV s<sup>-1</sup>. The oxidation peak current of ZnFe<sub>2</sub>O<sub>4</sub>-modified electrode (Figure 4.16c) is much larger than those of bare GCE (Figure 4.16) and PPy/GCE (Figure 4.16b) which confirms a good performance of these nanoparticles in terms of high detection of glucose. The CV curve of the ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell modified electrode changed significantly with an increase of oxidation current, revealing an obvious electrocatalytic behavior to the oxidation of glucose. The composite exhibited better electrocatalytic activity towards glucose than the ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles and it can be due to the presence of polypyrrole which possess good electrical conductivity and facilitate the electron transfer along the polymer framework.



**Figure 4.16:** Cyclic voltammograms of (a) bare GCE, (b) PPy/GCE, (c) ZnFe<sub>2</sub>O<sub>4</sub>/GCE, (d) ZnFe<sub>2</sub>O<sub>4</sub>/PPy/GCE by 4.0 ml of PPy in presence of 2.0 mM glucose in 0.1 M NaOH at the scan rate of 10 mV s<sup>-1</sup>.

For measurement of sensor ability in pH near to blood serum, the response currents of the  $ZnFe_2O_4$  nanoparticles and the  $ZnFe_2O_4/PPy$  core-shell modified electrodes were obtained in 0.1 M phosphate buffer solution in the presence of 2.0 mM glucose. These nanoparticles exhibit high oxidation current in pH 7.4, however, it can be seen also that the oxidation peak currents of these nanoparticles in buffer solution were lower than those in NaOH as electrolyte, which may attribute to the same reason as mentioned earlier (See section 4.2.5, Part 1).



**Figure 4.17:** Cyclic voltammograms of (a) ZnFe<sub>2</sub>O<sub>4</sub>/GCE at pH 7.4 without glucose (c) in presence of glucose (b) ZnFe<sub>2</sub>O<sub>4</sub>/PPy/GCE (4.0 ml of PPy) at pH 7.4 without glucose (d) in the presence of 2.0 mM glucose at the scan rate of 10 mV s<sup>-1</sup>.

#### 4.6.6 Electrochemical impedance spectroscopy (EIS) studies

Interfacial properties of the different modified electrodes were characterized by electrochemical impedance spectroscopy (EIS) which is an effective method for probing the features of the surface and understanding chemical transformations of modified electrodes. Figure 4.18 displays the Nyquist plots (Z'' vs Z') of modified electrodes in the presence of 0.1 M KCl solution containing 1.0 mM Fe[(CN)<sub>6</sub>]<sup>3-/4-</sup> (1:1) in the frequency range from 1× 10<sup>-2</sup>-1×10<sup>5</sup> Hz. The semicircular part at higher frequencies

corresponds to the electron transfer limited process, and its diameter is equivalent to the electron transfer resistance ( $R_{ct}$ ). The ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles cast on GCE had shown a clear decline of  $R_{ct}$  (b=580  $\Omega$ ) compared to the bare GCE (a=1.31 K $\Omega$ ). Moreover, modification of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles with polypyrrole leads to a smaller semicircle and decreased  $R_{ct}$ , which reduces further with increasing PPy shell thickness (c=375  $\Omega$ ) and (d=188  $\Omega$ ). This can be attributed to the conductivity of PPy which improved the electron transfer ability of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles.



**Figure 4.18:** EIS of (a) bare GCE, (b)  $ZnFe_2O_4/GCE$ , (c)  $ZnFe_2O_4/PPy/GCE$  using 1.0 ml of PPy, (d)  $ZnFe_2O_4/PPy/GCE$  using 4.0 ml of PPy, in 0.1 M KCl solution containing 1.0 mM  $Fe[(CN)_6]^{3-/4-}$  (1:1). The frequency range was scanned from 0.01 to  $1 \times 10^5$  Hz. right inset: the  $R_s(CPE[R_{ct}W])$  equivalent circuit model.

#### 4.6.7 Amperometric detection of glucose on ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles

The amperometric response of the  $ZnFe_2O_4/PPy$  core-shell-modified GCE towards the effect of glucose concentration from 0.0 to 8.0 mM in 0.1 M NaOH is depicted in Figure 4.19. A typical calibration curve of the sensor can be prepared from the amperometric response data (left inset). A quick increase in the current after each glucose addition was detected and the amperometric signal exhibited linear correlation to glucose concentration in the range from 0.1 mM to 8.0 mM. The sensor electrode also
shows a fast response time within 6 seconds right after every injection of glucose into the stirred electrolyte. The proposed sensor displays a linear response ( $R^2 = 0.9943$ ) to glucose with a sensitivity of 145.36  $\mu$ A mM<sup>-1</sup> and detection limit of 0.09 mM, at room temperature. Comparison of the present enzyme-free glucose sensor with other reported glucose sensors based on Zn materials is shown in Table 4.5.



**Figure 4.19:** The typical current–time dynamic response of the ZnFe<sub>2</sub>O<sub>4</sub>/PPy (4.0 ml of PPy) modified GCE towards various concentrations of glucose; left inset: the calibration curve for glucose detection.

| with other greese sensors based on 21 materials. |           |           |                    |              |               |
|--|-----------|-----------|--------------------|--------------|---------------|
| Electrode  | Detection | Detection | Sensitivity        | Linear range | Ref.          |
|  | potential | limit     | $(\mu A m M^{-1})$ |              |               |
|  | (vs. SCE) |           |                    |              |               |
| ZnO nanotubes                                    | - 0.8 V   | 1.0 mM    | 21.7               | 0.05-12      | (Kong, T. et  |
|  |           |           |                    | mM           | al., 2009)    |
| ZnO nanonails                                    | -         | 5.0 µM    | 24.6               | 0.1-7.1 mM   | (Umar et al., |
|  |           |           |                    |              | 2008)         |
| ZnO hollow                                       | 0.8 V     | 1.0 µM    | 65.82              | 0.005-13.15  | (Fang et al., |
| nanospheres                                      |           |           |                    | mM           | 2011)         |
| ZnFe <sub>2</sub> O <sub>4</sub> /PPy/co         | 0.85 V    | 0.09 µM   | 145.36             | 0.1-8.0 mM   | This work     |
| re-shell   |           | -         |                    |              |               |

**Table 4.5:** Comparison of the present ZnFe<sub>2</sub>O<sub>4</sub>/PPy nanoparticles enzyme-free glucose sensor with other glucose sensors based on Zn materials.

### 4.6.8 Interference study

To study the anti-interference ability of the fabricated glucose sensor, the amperometric response of the sensor recorded by successive injection of 0.1 mM glucose and blood interfering species (0.1 mM uric acid, UA and 0.1 mM ascorbic acid, AA) into solution containing 0.1 M NaOH (Figure 4.20). The performance of the sensor to differentiate glucose from the other sugars like sucrose and fructose was also demonstrated. It can be seen from Figure 4.20 that although the addition of 0.1 mM AA and UA increased the current of ZnFe<sub>2</sub>O<sub>4</sub>/PPy, the intensity increase is much smaller than the addition of 0.1 mM glucose, which is around 120  $\mu$ A. As a result small amount of AA, UA, fructose and sucrose can be neglected as ZnFe<sub>2</sub>O<sub>4</sub>/PPy modified electrode exhibits high selectivity for glucose sensing.



Figure 4.20: Interference test of the sensor in 0.1 M NaOH with 0.1 mM glucose and other interference as indicated.

#### 4.6.9 Reproducibility, stability of the sensor and detection of real samples

The reproducibility and stability of the sensor were evaluated. Four electrodes were prepared under the same conditions and relative standard deviation (RSD) of the current response towards 0.1 mM glucose was found to be 4.4%, confirming that the results can be reproducible. In addition, the stability of the  $ZnFe_2O_4/PPy$  core-shell nanoparticles electrode was tested by measuring responses for 17 days (Figure 4.21). The performance of the modified electrode was investigated every two days (electrode was placed in ambient condition). The current response did not show a big change during the first 5 days, and only 8% of the sensor activity was lost after one week time. It can be seen from Figure 4.20 that the sensor retains around 88.2% of its initial response after two weeks ( $I_0$  and I are the response current in the first and later days). Evaluation of the applicability of the proposed sensor to the determination of glucose in real samples was tested and the results are shown in Table 4.6.



**Figure 4.21:** Long-term stability of ZnFe<sub>2</sub>O<sub>4</sub>/PPy core-shell nanoparticles measured in more than two weeks.

| Blood serum | Glucose concentration  | Glucose            | Relative standard  |  |  |  |
|-------------|------------------------|--------------------|--------------------|--|--|--|
| samples     | measured by University | concentration      | deviation          |  |  |  |
|             | Malaya Medical Centre  | measured by sensor | measured by sensor |  |  |  |
|             | (mM)                   | (mM)               | (%)                |  |  |  |
| 1           | 4.56                   | 4.72               | 4.68               |  |  |  |
| 2           | 8.38                   | 8.96               | 3.94               |  |  |  |
| 3           | 6.62                   | 6.18               | 3.72               |  |  |  |
| 4           | 7.44                   | 6.98               | 4.26               |  |  |  |

**Table 4.6:** Determination of glucose in real sample of blood serum.

#### 4.7 Electro-oxidation mechanism of glucose on ZnFe<sub>2</sub>O<sub>4</sub>/PPy electrode

Glucose oxidation in alkaline solution is easier as the oxidation of glucose is the highest in alkaline media due to the formation of an intermediate known as enediol which makes glucose oxidation easier in these media. As it can be seen in Scheme 4.2, glucose loses one of its protons to form the enediol structure in alkaline solution then the enediol structure will be oxidized by  $ZnFe_2O_4$  nanoparticles. The mechanism of glucose electro-oxidation on  $ZnFe_2O_4/GCE$  and  $ZnFe_2O_4/PPy/GCE$  is similar to each other as  $ZnFe_2O_4$  nanoparticles only serve as the nucleation sites for the polymerization of pyrrole without any chemical interaction between the metal oxides and polymer in the composites, but the peak currents are different. A broad oxidation peak at about +0.85 V *vs.* SCE with a peak current of 500 µA is observed for  $ZnFe_2O_4/PPy$  modified electrode. All these results indicated that the electro-catalytic activity toward glucose oxidation on  $ZnFe_2O_4$  is highly improved. This enhanced electrochemical behavior is attributed to the special property of PPy which can provide better catalytic activity as well as facilitate the electron transfer rate compared to  $ZnFe_2O_4$  (Qian *et al.*, 2013).



Scheme 4.2: Electro-oxidation mechanism of glucose on ZnFe<sub>2</sub>O<sub>4</sub>/PPy/GCE.

# 4.8 Conclusion

In this work, a novel enzyme-free glucose sensor based on chemical oxidative polymerization of pyrrole on  $ZnFe_2O_4$  nanoparticles surface is presented. The sensor showed good activity towards the determination of glucose with the linear concentration range of 0.1-8.0 mM. The current response for 0.1 mM glucose in the presence of normal physiological concentrations of interferes (ascorbic acid and uric acid) and sugars like fructose and sucrose did not have a significant change. The modified electrode is stable enough in electrochemical measurements which are possibly due to supporting matrix of PPy. In application of electrochemistry, it showed a very high electrochemical active surface area and high electro-catalytic activity for the glucose oxidation, a lower detection limit of 0.09  $\mu$ A and high sensitivity of 145.36  $\mu$ A mM<sup>-1</sup>. All these advantageous features hold the promise for the development of a practicable application in the future.

Part 3: Reduced graphene oxide-supported copper ferrite hybrid for glucose sensing

#### 4.9 Introduction

A stable enzyme-free glucose sensor was fabricated via a facile in situ hydrothermal route by the formation of  $CuFe_2O_4$  nanoparticles into the graphene oxide sheets. The contents of graphene oxide in composites were varied from 10 to 40 wt%. The morphology of formed  $CuFe_2O_4/rGO$  nanocomposite was found by TEM analysis. The electro-catalytic activity of different  $CuFe_2O_4/rGO$  samples towards glucose oxidation was studied by employing cyclic voltammetry and chronoamperometry techniques.

# 4.10 Characterization of CuFe<sub>2</sub>O<sub>4</sub> and CuFe<sub>2</sub>O<sub>4</sub>/rGO magnetic nanocomposite 4.10.1 Fourier transform infrared spectroscopy (FTIR)

Figure 4.22 shows FTIR spectra of GO,  $CuFe_2O_4/rGO$  magnetic nanocomposite ( $CuFe_2O_4/rGO(30 \text{ wt\%})$  and pure  $CuFe_2O_4$ . The appearance of peak at 3410 and 1743 cm<sup>-1</sup> are attributed to the stretching vibrations of O–H and C=O, respectively. Additionally, two strong bands at 1620 and 1278 cm<sup>-1</sup> appear in the spectrum of GO which is assigned to the vibration of carboxyl groups (Fu *et al.*, 2012). A strong band at 570 cm<sup>-1</sup> appears in the spectrum of CuFe<sub>2</sub>O<sub>4</sub> which is assigned to the Fe–O stretching vibration mode (Ramankutty & Sugunan, 2001). Compared to the spectrum of CuFe<sub>2</sub>O<sub>4</sub> nanoparticles, the CuFe<sub>2</sub>O<sub>4</sub>/rGO composite showed weaker peaks which can be due to the nucleation and growth of CuFe<sub>2</sub>O<sub>4</sub> nanoparticles into the layered GO sheets. After the hydrothermal reaction, the GO peaks became weak or disappear in CuFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite. The similar peaks were observed for CuFe<sub>2</sub>O<sub>4</sub>/rGO composite with different rGO contents (10, 20 and 40 wt%).



Figure 4.22: FTIR spectra of (a) GO, (b)  $CuFe_2O_4/rGO$  nanocomposite and (c)  $CuFe_2O_4$  nanoparticles.

#### 4.10.2 X-ray diffraction (XRD)

Figure 4.23 shows the XRD patterns of the starting materials and nanocomposite CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%). As displayed in curve c, there is a peak at around  $2\theta \approx 10.68^{\circ}$  corresponding to the (001) reflection but this peak in rGO had disappeared and there is a new diffraction peak assigned to graphene at 26.78, with consequences the indices of (002) that confirmed the reduction of graphene oxide. There are two stronger peaks corresponding to (211) and (103) reflections at  $2\theta \approx 34.7^{\circ}$  and  $35.9^{\circ}$  which merged into a single broad peak, indistinguishable from the strongest reflection of the cubic CuFe<sub>2</sub>O<sub>4</sub> phase ( $2\theta \approx 35.9^{\circ}$ ). The Bragg plane corresponds to tetrahedral structure of CuFe<sub>2</sub>O<sub>4</sub> nanoparticles at  $2\theta \approx 54^{\circ}$  which appears in all the XRD patterns (Paul Joseph *et al.*, 2011). The XRD results indicated that the GO reduced to rGO in hydrothermal reduction process, while the CuFe<sub>2</sub>O<sub>4</sub> were also formed along this process. CuFe<sub>2</sub>O<sub>4</sub>/rGO magnetic nanocomposite with different rGO contents (10, 20 and 40 wt%) showed the similar XRD patterns.



Figure 4.23: XRD patterns of (a) CuFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite, (b) rGO and (c) GO.

### 4.10.3 Surface morphology study

Transmission electron microscopy (TEM) was carried out to observe morphologies of samples. These images showed spherical shape of  $CuFe_2O_4$ nanoparticles with diameters ranging from 20 to 90 nm. An image of the transparent sheets of reduced graphene oxide was illustrated in Figure 4.24. As the sheet structure properties of graphene are dependent on its morphology, the preservation of that has important effect on the performance of graphene. The TEM results indicated that the reduced graphene oxide was flake-like with wrinkles and reduction process has kept the morphology of graphene without any damages. The morphology of  $CuFe_2O_4/rGO$ nanocomposite showed that the  $CuFe_2O_4$  nanoparticles were homogeneously distributed on the graphene sheets without obvious aggregation. In hydrothermal reduction through electrostatic attraction, the oxygen-containing functional groups on the graphene oxide sheets can adsorb the positive copper and iron ions and this good dispersion and larger surface area enhanced electro-catalytic activity of CuFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite towards glucose.



**Figure 4.24:** The SEM images of (a) CuFe<sub>2</sub>O<sub>4</sub> and TEM images of (b) CuFe<sub>2</sub>O<sub>4</sub>, (c) reduced graphene oxide (d) and (e) CuFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite.

# 4.10.4 Cyclic voltammetry studies

The cyclic voltammograms of the bare GCE and the modified GCEs in presence of 2.0 mM glucose in 0.1 M phosphate buffer solution with a scan rate of 10 mV s<sup>-1</sup> are shown in Figure 4.25. The bare GCE and rGO/GCE hardly responded for this

concentration of glucose. After the GCE was modified with CuFe<sub>2</sub>O<sub>4</sub> nanoparticle, the peak currents significantly enhanced to confirm great glucose catalytic ability of these magnetic nanocomposites. The electro-catalytic activity of the different CuFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposites modified GCE were investigated as well. As can be seen, the obtained redox peak of CuFe<sub>2</sub>O<sub>4</sub>/rGO(10 wt%)/GCE increases compared to those of CuFe<sub>2</sub>O<sub>4</sub>/GCE and the redox peak current rising continued with increasing graphene oxide content from the range of 10 wt% to 40 wt%. Unique transport properties of graphene due to zero bandgap and two-dimensional  $\pi$ -conjugation structure lead to these results. It can be concluded that the existence of graphene nanosheets as a carbon support with excellent electronic conduction features is responsible for improve the electro-catalytic behaviour at CuFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite modified electrode. This high electro-catalytic activity can be attributed to the good synergistic coupling effects between the CuFe<sub>2</sub>O<sub>4</sub> nanoparticles and rGO nanosheets. The cyclic voltammograms of the CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%) which has the best performance among other modified electrodes, recorded in 0.1 M phosphate buffer solution (pH 7.4) at different scan rates (Figure 4.26). It is found that in the range of 10-150 mV s<sup>-1</sup>, both anodic and cathodic peak currents increase clearly with the applied scan rate, implying that the electrochemical kinetics is a typical surface-controlled electrochemical process.



**Figure 4.25:** Cyclic voltammograms of (a) bare GCE; (b) rGO/GCE; (c) rGO/GCE; (d) CuFe<sub>2</sub>O<sub>4</sub>; (e) CuFe<sub>2</sub>O<sub>4</sub>/rGO(10 wt%)/GCE; (f) CuFe<sub>2</sub>O<sub>4</sub>/rGO(20 wt%)/GCE; (g) CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE and (h) CuFe<sub>2</sub>O<sub>4</sub>/rGO(40 wt%)/GCE in presence of 2.0 mM glucose in 0.1 M phosphate buffer solution (pH 7.4) at the scan rate of 10 mV s<sup>-1</sup>.



Figure 4.26: Cyclic voltammograms of CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE in 0.1 mM PBS solution (pH 7.4) at different scan rates of 10, 20, 50, 100, 120 and 150 mV s<sup>-1</sup>.

#### 4.10.5 Electrochemical impedance spectroscopy (EIS) studies

EIS was used to analyze these modified electrodes with a frequency ranging from 0.1 to  $1 \times 10^5$  Hz in 0.1 M KCl solution containing 1.0 mM Fe[(CN)<sub>6</sub>]<sup>3-/4-</sup>(1:1). The Nyquist plots of the bare GCE, rGO/GCE, CuFe<sub>2</sub>O<sub>4</sub>/GCE and CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE in the presence of redox probe are shown in Figure 4.27. The diameter of semicircle portion is equal to the electron transfer resistance (R<sub>ct</sub>) which reflects conductivity. It is obvious that the bare GCE reveals maximum R<sub>ct</sub>. However, the EIS of rGO/GCE exhibits less R<sub>ct</sub> than those of bare GCE due to conductivity of graphene oxide, significant difference in the impedance spectra was observed after modifying GCE with CuFe<sub>2</sub>O<sub>4</sub> magnetic nanoparticles. The electron transfer ability of GCE has been significantly improved by depositing CuFe<sub>2</sub>O<sub>4</sub> nanoparticles on the surface of GCE. The modification of GCE with CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%) gives rise to a considerable reduction in R<sub>ct</sub>, indicating that the electron transfer speed of the  $CuFe_2O_4/rGO(30 \text{ wt\%})$  is faster than that of the  $CuFe_2O_4$  nanoparticles. This can be attributed to the coating of CuFe<sub>2</sub>O<sub>4</sub> nanoparticles by graphene oxide which enhances the electrical conductivity of the magnetic nanoparticles. For the CuFe<sub>2</sub>O<sub>4</sub>/rGO(40 wt%)/GCE, the charge transfer resistance increased and indicated that nanocomposite with 30 wt% reduced graphene oxide gave the best activity with better glucose detection ability which in great agreement with cyclic voltammetry results.



**Figure 4.27:** EIS of (a) bare GCE; (b) rGO/GCE; (c)  $CuFe_2O_4/GCE$ ; (d)  $CuFe_2O_4/rGO(30 wt\%)/GCE$  and (e)  $CuFe_2O_4/rGO(40 wt\%)/GCE$  in 0.1 M KCl solution containing 1.0 mM  $Fe[(CN)_6]^{3^{-/4^{-}}}(1:1)$ . The frequency range was from 0.1 to  $1 \times 10^5$  Hz. right inset: the  $R_s(CPE[R_{ct}W])$  equivalent circuit model.

# 4.10.6 Amperometric detection of glucose on CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE

Amperometry is the most common electrochemical technique based on the measured reduction or oxidation current at a given specific potential over a fixed period of time. Among all the studied nanocomposites with different graphene content,  $CuFe_2O_4/rGO(30 \text{ wt\%})/GCE$  showed the highest electro-catalytic activity towards glucose in previous measurements, therefore this nanocomposite is chosen for the amperometric measurement of glucose. The dependence of the amperometric response on the applied potential of the  $CuFe_2O_4/rGO(30 \text{ wt\%})$  coated GCE under the batch conditions was calculated over the range of -1.0 V to +1.0 V (*vs.* SCE) to obtain the best applied potential for optimizing the glucose detection. The suitable working potential was achieved at -0.2 V *vs.* SCE and selected as the optimized applied potential. Figure 4.28 shows the amperometric response of the  $CuFe_2O_4/rGO(30 \text{ wt\%})/GCE$  as an enzyme-free sensor to the successive additions of glucose at different concentrations under optimized detection potential at -0.2 V. As indicated,  $CuFe_2O_4/rGO(30 \text{ wt\%})/GCE$ 

wt%)/GCE displayed increment of current rapidly after each injection of glucose solution to the stirred supporting electrolyte. The sensor displayed a linear response with the glucose concentration from 0.1 mM to 7.5 mM glucose (correlation coefficient was 0.9947) with a specific sensitivity value of 1824.22  $\mu$ A mM<sup>-1</sup> cm<sup>-2</sup> at a signal/noise ratio of 3. The obtained high sensitivity and low detection limit should be attributed to the synergistic electro-catalytic activity by combining CuFe<sub>2</sub>O<sub>4</sub> nanoparticles with graphene. The proposed sensor showed detection limit of 1.0  $\mu$ M. The sensitivity and detection limit at this modified electrode are comparable and better than those obtained by using other modified electrodes based on metals or metal oxide nanoparticles (Table 4.7).



**Figure 4.28:** The typical current–time dynamic response of the  $CuFe_2O_4/rGO(30 \text{ wt\%})/GCE$  towards various concentrations of glucose; left inset: the calibration curve for glucose detection.

| grucose sensor with other grucose sensors. |             |           |           |             |         |         |
|--|-------------|-----------|-----------|-------------|---------|---------|
| Electrode                                  | Electrolyte | Detection | Detection | Sensitivity | Linear  | Ref.    |
|  |             | potential | limit     | (μΑ         | range   |         |
|  |             | (vs. SCE) |           | $mM^{-1}$ ) |         |         |
| GOx/Pt-Au/TiO <sub>2</sub> NT              | pH= 7.3     | - 0.189 V | 0.1 mM    | 0.08366     | 0-1.8   | (Kang,  |
|  | PBS         |           |           |             | mM      | Q. et   |
|  |             |           |           |             |         | al.,    |
|  |             |           |           |             |         | 2008)   |
| TiO <sub>2</sub> /CNT/Pt/GOx               | pH= 7.2     | 0.38 V    | 5.7 µM    | 0.24        | 0.006-  | (Pang   |
|  | PBS         |           |           |             | 1.5     | et al., |
|  |             |           |           |             | mM      | 2009)   |
| GE/CuO/GOx/Nafion                          | pH = 7.4    | 0.54 V    | 1.37 μM   | 47.19       | 0.01-   | (Umar   |
|  | PBS         |           |           |             | 10 mM   | et al., |
|  |             |           |           |             |         | 2009)   |
| $CuFe_2O_4/rGO(30)$                        | pH = 7.4    | -0.2 V    | 0.1 µM    | 164.18      | 0.6-5.6 | This    |
| wt%)                                       | PBS         |           |           |             | mM      | work    |

**Table 4.7:** Comparison of the present CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%) nanocomposite enzyme-free glucose sensor with other glucose sensors.

# 4.10.7 Interference study

Discrimination ability is one of the most significant factors for an enzyme-free glucose sensor, therefore the CuFe<sub>2</sub>O<sub>4</sub>/rGO/GCE response attributed to the presence of ascorbic acid and uric acid is determined by applying chronoamperometry and the data shows no obvious current response with the addition of 0.1 mM AA and 0.1 mM UA (Fig 4.29). Furthermore, the sensor performance to differentiate glucose from the other sugars like sucrose and fructose was demonstrated. These sugars solution caused negligible interference to the remarkable current responses of glucose at the CuFe<sub>2</sub>O<sub>4</sub>/rGO/GCE.



**Figure 4.29:** Interference test of the sensor in 0.1 M phosphate buffer solution (pH 7.4) with 0.1 mM glucose and other interferes as indicated.

#### 4.10.8 Reproducibility, stability and sample analysis studies

The redox peak current of the sensor was measured every two days to evaluate its stability. The current signal decreased 12% after two weeks, retained around 88% from its initial response. In addition, the reproducibility of the sensor was examined by preparing four electrodes under the same conditions and relative standard deviation (RSD) of the current response towards 0.1 mM glucose was found to be 4%. All these results are in close agreement with excellent electronic conduction features of graphene oxide for improving electro-catalytic behaviour of  $CuFe_2O_4/rGO(30 \text{ wt\%})/GCE$ . In order to further determine the performance of the proposed sensor, its applicability was evaluated by the analysis of real samples. The obtained results were given in Table 4.8. A good agreement between the values obtained by the proposed glucose sensor and those data obtained from University Malaya Hospital lab analysis. These results indicated that the presented modified electrode can be used as electrochemical sensor.



Figure 4.30: Stability of a CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE measured in more than two weeks.

| Blood serum | Glucose concentration  | Glucose concentration | Relative standard  |
|-------------|------------------------|-----------------------|--------------------|
| samples     | measured by University | measured by sensor    | deviation          |
|             | Malaya Medical Centre  | (mM)                  | measured by sensor |
|             | (mM)                   |                       | (%)                |
|             |                        |                       |                    |
| 1           | 4.5                    | 4.2                   | 2.2                |
| 2           | 5.5                    | 5.4                   | 3.1                |
| 3           | 5.8                    | 5.88                  | 3.4                |
| 4           | 6.3                    | 6.4                   | 2.9                |

**Table 4.8:** Determination of glucose in real sample of blood serum.

# 4.11 Conclusion

In summary, an enzyme-free glucose sensor based on glassy carbon electrode modified by CuFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%) nanocomposite had been developed. This low cost, easily fabricated and stable sensor exhibited great electro-catalytic activity towards the determination of glucose with linear concentration range of 0.1-7.5 mM. There was no significant change in current response for 1.0 mM glucose in the presence of normal physiological interferents such as ascorbic acid and uric acid and also for selected sugars. The modified electrode showed great stability and excellent sensitivity value of 1824.22  $\mu$ A mM<sup>-1</sup> cm<sup>-2</sup> with a detection limit of 0.1  $\mu$ M and can hold the promise for the development of an accurate enzyme-free glucose sensor.

## Part 4: Electrochemical sensing of glucose by reduced graphene oxide-zinc ferrite

#### 4.12 Introduction

 $ZnFe_2O_4$  magnetic nanoparticles/reduced graphene oxide nanosheets modified glassy carbon ( $ZnFe_2O_4/rGO$ ) as a novel system for the electrochemical glucose sensing is reported here. Using a facile in situ hydrothermal route, the reduction of GO and the formation of  $ZnFe_2O_4$  nanoparticles happened at the same time to decorate the graphene sheets by  $ZnFe_2O_4$  nanoparticles. Characterization of nanocomposite by X-ray diffraction (XRD) and transmission electron microscopy (TEM) clearly demonstrate the successful attachment of  $ZnFe_2O_4$  nanoparticles to graphene sheets.

#### 4.13 Characterization of ZnFe<sub>2</sub>O<sub>4</sub> and ZnFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite

# 4.13.1 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy is a powerful technique for the characterization of graphene based materials. GO,  $ZnFe_2O_4$  nanoparticles and  $ZnFe_2O_4/rGO$  nanocomposite were examined by FTIR. The peaks at 3410 cm<sup>-1</sup> and 1743 cm<sup>-1</sup> correspond to the stretching vibrations of O–H and C=O respectively, while the vibration of carboxyl groups are found at 1620 cm<sup>-1</sup> and 1278 cm<sup>-1</sup> (Shen *et al.*, 2011). The obtained  $ZnFe_2O_4/rGO$  nanocomposite was also explored with FTIR. The adsorption peak around 1570 cm<sup>-1</sup> may be assigned to the stretching vibrations of the unoxidized carbon backbone. Moreover the presence of absorption peak at 550 cm<sup>-1</sup> can be assigned to the stretching vibrations of the Fe–O bonds in tetrahedral positions (Ramankutty & Sugunan, 2001). After the hydrothermal reaction, the most characteristic peaks of GO disappeares which confirmed the presence of rGO in the ZnFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite. Magnetic nanocomposites with different rGO contents (10, 20 and 40 wt%) have also showed the similar peaks.



Figure 4.31: FTIR spectra of (c) ZnFe<sub>2</sub>O<sub>4</sub>/rGO; (b) ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles and (a) GO.

#### 4.13.2 X-ray diffraction (XRD)

The structures of GO, rGO and zinc ferrite/graphene composites were characterized using the XRD analysis (Figure 4.32). The diffraction pattern of GO showed a strong peak at around  $2\theta = 10.2^{\circ}$ , originated from its (001) reflection which is consistent with the lamellar structure of GO. This peak disappeared in rGO, indicating the oxygen groups have been removed and GO has been reduced to rGO nanosheets. It has been observed that the two stronger peaks corresponding to (220) and (311) reflections at  $2\theta \approx 30^{\circ}$  and  $35.5^{\circ}$ . These peaks merged into a peak (400) which is the reflection of the cubic ZnFe<sub>2</sub>O<sub>4</sub> phase  $2\theta \approx 38.18^{\circ}$  (Yao *et al.*, 2014). The Bragg planes of (422) and (440) were corresponded to the tetrahedral structure of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles ( $2\theta \approx 51^{\circ}$ -  $60^{\circ}$ ). Moreover there is no visible sign of (001) in diffraction peak of ZnFe<sub>2</sub>O<sub>4</sub>/rGO which due to growth of magnetic nanoparticles within GO interlayers and exfoliation of graphene oxide (Fu *et al.*, 2012).



**Figure 4.32:** XRD patterns of (a) ZnFe<sub>2</sub>O<sub>4</sub>/rGO nanocomposite; (b) ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles; (c) rGO; (d) GO.

# 4.13.3 Surface morphology study

The morphology of the pure  $ZnFe_2O_4$  nanoparticles, reduced graphene oxide and the resulting  $ZnFe_2O_4/rGO(30 \text{ wt\%})$  nanocomposite (Figure 4.33) were characterized by TEM. The images related to the pure  $ZnFe_2O_4$  nanoparticles with diameters ranging from 20 to 90 nm and reduced graphene oxide with curled and corrugated structure are shown in Figure 4.33a and b. Graphene oxide as a supporting substrate can minimize the metallic nanoparticles agglomeration. Moreover, reduced graphene oxide has the unique 2D structure which enables it to be a great electron-transporting material. Densely distribution of the  $ZnFe_2O_4$  nanoparticles on the graphene sheets can be seen in Figure 4.33f which illustrated the electrostatic adsorption of the positive zinc and iron ions on the graphene oxide sheets by the oxygen-containing functional groups.



**Figure 4.33:** The SEM images of (a)  $ZnFe_2O_4$  and TEM images of (b)  $ZnFe_2O_4$ ; (c and d) reduced graphene oxide at different resolutions; (e and f)  $ZnFe_2O_4/rGO$  nanocomposite at different resolutions.

# 4.13.4 Cyclic voltammetry studies

The electro-catalytic activities of the modified GCEs were investigated to detect their responses to the oxidation of glucose in 0.1 M PBS solution in the presence of 2.0 mM glucose with scanning rate of 10 mV s<sup>-1</sup> (Fig 4.34). There are no obvious redox peaks in given CV responses of the bare GCE and rGO/GCE, indicating poor redox activity of the selected electrodes. A strong peak appeared after modification of the GCE with ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles confirms great glucose catalytical ability of this nanomagnetic. As can be seen with further modification of the GCE by the different  $ZnFe_2O_4/rGO$  nanocomposites, the obtained redox peaks improved significantly compared to those of  $ZnFe_2O_4$  modified electrode and the redox peak current rising continuously with increasing graphene oxide content from the range of 10 wt% to 40 wt%. All these results are in close agreement with excellent electronic conduction features of graphene oxide for improving electro-catalytic behaviour of  $ZnFe_2O_4/rGO/GCE$ .

In the present work, a pair of Zn(I)/(II) redox couple were observed that acts as a catalyst for the oxidation of glucose. When glucose diffuses to the electrode surface, the Zn(II) oxidizes it rapidly to glucolactone on the electrode. The electro-catalytic oxidation mechanism of glucose at the working electrode surface may be simply described by:

 $Zn(I) \rightarrow Zn(II) + e^{-1}$ 

 $Zn(II) + glucose \rightarrow Zn(I) + glucolactone$ 

The relationship of scan rate and the redox peak current of the  $ZnFe_2O_4/rGO(30 wt%)/GCE$  which had the best performance among other modified electrodes, is also investigated by CV in PBS solution with 2.0 mM glucose at different scan rates in the range of 10-120 mV s<sup>-1</sup> (Figure 4.35). It is found that both anodic and cathodic peak currents increased with the applied scan rate, suggesting that the electrochemical kinetics is a typical surface-controlled electrochemical process.







**Figure 4.35:** Cyclic voltammograms of ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE in 0.1 M PBS solution (pH 7.4) at different scan rates.

#### 4.13.5 Electrochemical impedance spectroscopy (EIS) studies

Figure 4.36 presents the representative impedance spectrum for modified electrodes in 0.1 M KCl solution containing 1 mM Fe[(CN)<sub>6</sub>]<sup>3-/4-</sup>(1:1). The semicircle diameter of impedance equals the electron transfer resistance ( $R_{ct}$ ), which controls the electron transfer kinetics of the redox probe at the electrode interface. The Nyquist semicircle of the rGO/GCE decreased compared with the bare GCE, which indicates better conductivity of graphene oxide. After modifying GCE with ZnFe<sub>2</sub>O<sub>4</sub>, the semicircle significantly decreased; such decreased impedance may be ascribed to the great electron transfer ability of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles. Furthermore, ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%) composite was cast on the GCE surface, the resistance decreased most presumably due to the synergic excellent electric conductivity of graphene oxide and high electron transfer ability of ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles.



**Figure 4.36:** EIS of (a) bare GCE; (b) rGO; (c)  $\text{ZnFe}_2O_4$  nanoparticles; (d)  $\text{ZnFe}_2O_4/\text{rGO}(30 \text{ wt\%})/\text{GCE}$  and (e)  $\text{ZnFe}_2O_4/\text{rGO}(40 \text{ wt\%})/\text{GCE}$  in 0.1 M KCl solution containing 1.0 mM  $\text{Fe}[(\text{CN})_6]^{3^{-/4^-}}(1:1)$ . The frequency range was from 0.1 to  $1 \times 10^5$  Hz. right inset: the  $R_s(\text{CPE}[R_{ct}W])$  equivalent circuit model.

# 4.13.6 Amperometric detection of glucose at ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE

Amperometry is a widely used electroanalytical technique which involves the application of a constant reducing or oxidizing potential to a fixed period of time. The best way to find out the proper potential is by measuring the relationship between applied potential in chronoamperometry and oxidation current of glucose. Therefore the applied potential of the ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE was evaluated over the range of -0.1 to +0.1 V. The modified electrode showed the highest current response at -0.2 V and this potential was selected as the optimized condition. Since ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE indicated the highest electro-catalytic activity among all the synthesized nanocomposites with different graphene oxide, it is chosen for the amperometric measurement of glucose. Figure 4.37 revealed the typical current-time dynamic responses of the glucose sensor based on ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE which were measured under different concentrations of glucose solution. The corresponding electrochemical response was recorded while the successive increment of glucose concentration from 0.1 mM to 7.5 mM to the buffer solution was performed at applied potentials of -0.2 V. An immediate increase in the current after each addition of glucose was noticed. The calibration curve of the proposed sensor displays a linear response of  $R^2=0.9951$  to glucose with a sensitivity of 110.92 µA mM<sup>-1</sup> and limit of detection (LOD) of 1.2 µM.



**Figure 4.37:** The typical current–time dynamic response of the ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE towards various concentrations of glucose; left inset: the calibration curve for glucose detection.

# 4.13.7 Interference study

Presence of some interfering species coexist with glucose in human serum is a big challenge in glucose detection as they can be simultaneously oxidized along with glucose at the electrode surface. Therefore, selectivity is one of the vital characteristics of high-performance enzyme-free glucose sensors. The amperometric response of ZnFe<sub>2</sub>O<sub>4</sub>/rGO (30 wt%)/GCE sensor towards the addition of 0.1 mM glucose and 0.1 mM blood interfering species is indicated in Figure 4.38. The corresponding changes in the oxidation current upon the addition of 0.1 mM glucose is much greater than those of other interfering species and this confirmed that the addition of ascorbic acid and uric acid has little impact on the detection of glucose. Besides, the effect of the fructose and sucrose which added to the mixed solution of phosphate buffer and 0.1 mM glucose was

also tested and their current peak is found to remain almost unchanged. The sensitivity and detection limit at this modified electrode are comparable and better than those obtained by using other modified electrodes based on metals or metal oxide nanoparticles (Table 4.9).



Figure 4.38: Interference test of the sensor in 0.1 M phosphate buffer solution (pH 7.4) with 0.1 mM glucose and other interferes as indicated.

| Greesse sensor with other 24 based greesse sensors. |           |           |                    |          |               |
|---|-----------|-----------|--------------------|----------|---------------|
| Electrode   | Detection | Detection | Sensitivity        | Linear   | Ref.          |
|   | potential | limit     | $(\mu A m M^{-1})$ | range    |               |
|   | (vs. SCE) |           |                    |          |               |
| ZnO nanotubes                                       | - 0.8 V   | 1.0 mM    | 21.7               | 0.05-12  | (Kong, T. et  |
|   |           |           |                    | mM       | al., 2009)    |
| ZnO nanonails                                       | -         | 5.0 µM    | 24.6               | 0.1-7.1  | (Umar et al., |
|   |           |           |                    | mM       | 2008)         |
| ZnO hollow  | 0.8 V     | 1.0 µM    | 65.82              | 0.005-   | (Fang et al., |
| nanospheres   |           |           |                    | 13.15 mM | 2011)         |
| ZnFe <sub>2</sub> O <sub>4</sub> /rGO(30            | -0.2 V    | 1.2 µM    | 110.92             | 0.1-7.5  | This work     |
| wt%)  |           |           |                    | mM       |               |

**Table 4.9:** Comparison of the present ZnFe<sub>2</sub>O<sub>4</sub>/rGO (30 wt%) nanocomposite enzyme-free glucose sensor with other Zn based glucose sensors.

#### 4.13.8 Reproducibility, stability and real sample analysis studies

Reproducibility and stability of the electrode are important parameters to evaluate the performance of an electrochemical sensor. To study the reproducibility of the sensor, four electrodes were prepared under the same conditions and relative standard deviation (RSD) of the current response towards 0.1 mM glucose was found to be 4.1%. Moreover the stability of  $ZnFe_2O_4/rGO(30 \text{ wt\%})$  nanocomposite was investigated by periodically recording its current response to 2.0 mM glucose (Figure 4.39). The performance of modified electrode was investigated every two days and the sensor retains around 88% of initial response after two weeks ( $I_0$  and I are the current response of fresh sensor and the current response after storage respectively.). The good stability could be attributed to the great composites compatibility. The overall performance showed that this sensor displayed a good superiority in terms of sensitivity, selectivity and linear calibration. In order to verify the reliability of the proposed sensor,  $ZnFe_2O_4/rGO(30 \text{ wt\%})$  nanocomposite was applied to the determination of glucose in real sample of blood serum. 0.2 ml of the serum sample was added to 10 ml of 0.1 mM phosphate buffer solution (pH=7.4) as testing solution for amperometric measurement. The analytical results were shown in Table 4.10, implied that this electrode has the sensing ability to be used to test on real human serum samples.



Figure 4.39: Stability of ZnFe<sub>2</sub>O<sub>4</sub>/rGO(30 wt%)/GCE measured in more than two weeks.

| Blood serum samples | Glucose concentration | Glucose concentration | Relative standard  |
|---------------------|-----------------------|-----------------------|--------------------|
|                     | measured by           | measured by sensor    | deviation          |
|                     | University Malaya     | (mM)                  | measured by sensor |
|                     | Medical Centre        |                       | (%)                |
|                     | (mM)                  |                       |                    |
| 1                   | 5.56                  | 5.82                  | 4.32               |
| 2                   | 8.01                  | 8.24                  | 4.22               |
| 3                   | 7.53                  | 7.85                  | 4.81               |
| 4                   | 9.60                  | 10.22                 | 5.12               |

**Table 4.10:** Determination of glucose in real sample of blood serum.

# 4.14 Conclusion

In this study, the glucose sensor was fabricated by hydrothermally formation of  $ZnFe_2O_4$  nanoparticles into graphene sheets. GO prevented the aggregation of  $ZnFe_2O_4$  nanoparticles without changing its electrical properties and also improved the electron transfer. The synthesized  $ZnFe_2O_4/rGO$  nanocomposite was characterized by X-ray diffraction and also characterized by transmission electron microscopy and their results are consistent to one another. The fabricated electrode  $ZnFe_2O_4/rGO(30 \text{ wt\%})$  displayed excellent catalytic property to glucose in the range of the glucose concentrations from  $1.0 \times 10^{-2}$  to  $7.5 \times 10^{-2}$  M with sensitivity of  $110.92 \mu \text{A mM}^{-1}$ . Moreover, the enzyme-free glucose sensor showed good repeatability, reproducibility, selectivity and stability.

#### **CHAPTER 5 : SUMMARY & FUTURE WORK**

The goal for this work was to design high sensitive and selective enzyme-free glucose sensors having the potential for glucose level detection. To address this, four glucose sensors were proposed based on combination of magnetic nanoparticles, polypyrrole and graphene oxide. Although many efforts have been tried for the development of enzyme-free glucose sensors using various nanostructures of metal, metal alloys, metal oxides and carbon nanotubes which displayed fast response, high sensitivity, lower detection limit, better stability and lower cost, these sensors need to improve in selectivity and using novel nanoparticles can realize this objective. Irreversible aggregation of the magnetic nanoparticles which can affect the magnetic properties was minimized by steric stabilization. This property made MNPs suitable to be used as the sensing material. Polypyrrole and graphene oxide were candidates for magnetic nanoparticles coating in this thesis and these two materials had improved the surface area and also enhanced the stability via ionic interactions.

In this work, glucose level was determined by four proposed modified electrodes.  $CuFe_2O_4$  and  $ZnFe_2O_4$  nanoparticles as selected magnetic nanoparticles showed good detection ability towards glucose concentrations. Role of graphene oxide in improving the sensing performance of selected nanoparticles was tested. The enhanced electrocatalytic ability of  $CuFe_2O_4/rGO$  and  $ZnFe_2O_4/rGO$  modified electrode compared to those of only magnetic nanoparticles modified electrode is considered to be the result of a large surface area and high conductivity as well as fast electron transfer provided by graphene sheets, confirming the important role of graphene sheets. The electro-activity of  $CuFe_2O_4$  and  $ZnFe_2O_4$  nanoparticles modified by PPy were investigated and the finding showed the considerable improvement in glucose sensing after modification. These modified electrode were stable enough in electrochemical measurements which

possibly due to supporting matrix of PPy. All four prepared enzyme-free glucose sensors showed good repeatability, reproducibility and selectivity towards glucose detection. There were no significant changes of current response during the detection of 1.0 mM glucose in the presence of normal physiological interferents such as ascorbic acid and uric acid and other selected sugars. All the fabricated sensors were further evaluated their performance in the determination of glucose in real samples where the results indicated the low RSD values which confirmed high possibility to use the presented magnetic nanocomposites in future for clinical diagnostics. Compared to graphene sheets, PPy revealed better influence electrical conductivity.  $CuFe_2O_4/PPy$ nanoparticles displayed the highest glucose sensing performance among the other fabricated sensors with a low detection limit of 0.1  $\mu$ M and excellent sensitivity value of 164.18  $\mu$ A mM<sup>-1</sup> with linear concentration range of 0.1-7.5 mM.

Based on this work, future development should concentrate on further applications of these magnetic nanoparticles in other diagnostic purposes. The conjugation of magnetic nanoparticles with electrochemical sensing systems promises large evolution in actual electro-analysis method.

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## LIST OF PUBLICATIONS AND PRESENTATIONS

1. <u>Shahnavaz, Z</u>., Lorestani, F., Woi, P. M., Alias, Y. (2014). Polypyrrole-ZnFe<sub>2</sub>O<sub>4</sub> magnetic nanocomposite with core-shell structure for glucose sensing. *Applied Surface Science*, 317(30): 622-629.

2. <u>Shahnavaz, Z</u>., Lorestani, F., Woi, P. M., Alias, Y. (2015). Core-shell– CuFe<sub>2</sub>O<sub>4</sub>/PPy nanocomposite enzyme-free sensor for detection of glucose. *Journal of Solid State Electrochemistry*, 19(4): 1223-1233.

3. <u>Shahnavaz, Z</u>., Woi, P. M., Alias, Y. (2015). A hydrothermally prepared reduced graphene oxide-supported copper ferrite hybrid for glucose sensing. *Ceramics International*, 41(10): 12710-12716.

## Manuscript submitted

4. <u>Shahnavaz, Z</u>., Woi, P. M., Alias, Y. (2015). Electrochemical sensing of glucose by reduced graphene oxide-zinc ferrospinels. *Applied Surface Science*.

## Conference presentations

 <u>Shahnavaz, Z</u>., Woi, P. M., Alias, Y. A nonenzymatic glucose biosensor based on zinc ferrite nanoparticles. 6<sup>th</sup> International Conterence on Sensors, 27-29<sup>th</sup> August 2013, Malacca, Malaysia.–Poster presentation.

2. <u>Shahnavaz, Z</u>., Woi, P. M., Alias, Y. Core-Shell—CuFe<sub>2</sub>O<sub>4</sub>/PPy hybrid nanocomposite enzyme free sensor for detection of glucose. International Conference

on Ionic Liquids (ICIL13), 11-13<sup>th</sup> December 2013, Langkawi Island, Malaysia.–Poster presentation.

3. <u>Shahnavaz, Z.</u>, Woi, P. M., Alias, Y. Zinc ferrite-reduced graphene oxide nanocomposite for non-enzymatic amperometric glucose detection. 3<sup>rd</sup> International Conterence on Sensors on Advances in Engineering & Technology (ICAET), 26-27<sup>th</sup> December 2014, Kuala Lumpur, Malaysia.–Oral presentation.

4. <u>Shahnavaz, Z.</u>, Woi, P. M., Alias, Y. Hydrothermal synthesis of magnetic reduced graphene oxide nanocomposite for electrochemical glucose sensor application. The 2nd International Conference on Engineering and Natural Science (ICENS), 22-24<sup>th</sup> July 2015, Tokyo,Japan.–Poster presentation.