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# Development of ta2o5 electrochemically reduced graphene oxide nanocomposite electochemical sensor for oxytetracycline detection in Milk

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# DEVELOPMENT OF Ta<sub>2</sub>O<sub>5</sub> ELECTROCHEMICALLY REDUCED GRAPHENE OXIDE NANOCOMPOSITE ELECTOCHEMICAL SENSOR FOR OXYTETRACYCLINE DETECTION IN MILK

Felista Sylivester Magesa

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

#### ABSTRACT

Various kinds of antimicrobials are being used in the veterinary sector for therapeutic and growth promotion purposes. Significant amounts of antimicrobial residues are released in the milk and exert harmful effects such as allergic reactions, carcinogenicity, mutagenicity and teratogenicity as well as disturbance of normal flora in the gastrointestinal tract. Thus, quantification of these residues is crucial. A number of conventional detection methods that are in application, which use chromatographic, immunological and spectroscopic techniques are sensitive, selective and used for confirmatory test. However, most of them are costly, time consuming, laborious and cannot provide onsite results. In addition, most of the rapid detection tests in the market are qualitative and non-confirmatory. Hence, for quick, portable, sensitive and cost effective detection of antimicrobials, novel detection tools are needed. In this study, novel tantalum pentoxide-electrochemically reduced graphene oxide nanocomposites modified glassy carbon electrode (Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE) was developed for the detection of oxytetracycline in milk. The composition, structure and morphology of GO, Ta<sub>2</sub>O<sub>5</sub>, and Ta<sub>2</sub>O<sub>5</sub>-ErGO were characterized by X-ray diffraction and scanning electron microscopy. Oxytetracycline electrochemical behavior on the bare GCE, GO/GCE, ErGO/GCE, and Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE was studied by cyclic voltammetry. The Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE showed 2 fold increased magnification of the oxytetracycline oxidation signal in comparison to GCE, GO/GCE, ErGO/GCE electrodes. Under the optimum conditions, the currents were proportional to the oxytetracycline concentration in the 0.2 to 10 µM range with 0.095 µM as low detection limit. The preparation of Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE in the current work provides an outlook for detecting ultra-trace oxytetracycline in milk.

#### DECLARATION

I, Felista Sylivester Magesa, declare that this dissertation is my own work. It is being submitted for the Master's degree in Life Sciences of the Nelson Mandela African Institution of Science and Technology. It has not been submitted for any degree or examination at any other University.

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#### CERTIFICATION

The undersigned certify that all supervisors have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology a dissertation entitled "Development of  $Ta_2O_5$  Electrochemically Reduced Graphene Oxide Nanocomposites Electrochemical Sensor for Oxytetracycline detection in Milk", in fulfillment of the requirements for the degree of Master of Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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# LIST OF ABBREVIATIONS AND SYMBOLS

CV	Cyclic Voltammograms
CVD	Chemical Vapor Deposition
DEOA	Diethanol amine
DRAMS	Dynamic Access Random Memory
ECL	Electrochemiluminescent
ECSs	Electrochemical Sensors
EL	Electroluminescent
ELISA	Enzyme-Linked Immunosorbent Assays
ErGO	Electrochemically reduced Graphene Oxide
FAO	Food and Agriculture Organization
GCE	Glass Carbon Electrode
GO	Graphene Oxide
$H_2O_2$	Hydrogen peroxide
$H_2SO_4$	Sulfuric acid
HPLC	High Performance Liquid Chromatography
HPLC	
JCPDS	Joint Committee Powder Diffraction Standards
JCPDs	Joint Committee Powder Diffraction Standards
$K_3Fe(CN)_6$	Potassium ferricyanide
KMnO <sub>4</sub>	Potassium permanganate
LC-MS	Liquid Chromatography-Tandem Mass Spectrometry
MRL	Maximum Residue Limit
MRL	Maximum Residue Limit
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	Disodium phosphate dodecahydrate
NaH <sub>2</sub> PO <sub>4</sub>	Sodium dihydrogen phosphate
NaOH	Sodium hydroxide
OTC	Oxytetracycline
PBS	Phosphate Buffer Saline
PEC	Photoelectrochemical
PL	Photoluminescent
RGO	Reduced Graphene Oxide
RSD	Relative Standard Deviation
SCE	Saturated Calomel Electrode

SCE	Saturated Calomel Electrode
SEM	Scanning Electron Microscopy
SEM	Scanning Electron Microscopy
SERS	Surface-Enhanced Raman Scattering
STM	Scanning Tunneling Microscope
STM	Scanning Electron Microscopy
$Ta_2O_5$	Tantalum Pentoxide
TaCl <sub>5</sub>	Tantalum chloride
TMONPs	Transition Metal Oxide Nanoparticles
UHT	Ultra Heat Treated
VICH	International Cooperation on Harmonisation of Technical
	Requirements for Registration of Veterinary Medicinal Products
VICH	International Cooperation on Harmonisation of Technical
	Requirements for Registration of Veterinary Medicinal Products
WHO	World Health Organization
XRD	X-ray Diffraction
$\alpha$ -Al <sub>2</sub> O <sub>3</sub>	Alpha aluminium oxide

#### **CHAPTER ONE**

#### Introduction

#### **1.1 Background of the Problem**

Antimicrobial residues are remnants or small amounts of antimicrobial drugs or their active metabolites which remain in milk after treating lactating cows. Each year antimicrobials are used in tons. In animal husbandry, antimicrobials especially antibiotics are used for treatment, prevention and growth promotion purpose (Sachi *et al.*, 2019). Globally, the use of antibiotics in livestocks is twice the use in humans and the application in food animal production is estimated to increase by 67% by 2030 worldwide (Delgado *et al.*, 1999; Van Boeckel *et al.*, 2015). A number of publications have reported that a significant proportion of the antibiotics used in animals or their residues find their way in food products such as milk, meats or eggs (Cháfer-Pericás *et al.*, 2010). The accumulation of antimicrobial residues in food products of animal origin have become a great global public health issue due to their contribution to hypersensitivity, disruption of normal flora in the intestine, antimicrobial drug resistance and teratogenic, carcinogenic as well as mutagenic effects (Priyanka *et al.*, 2017).

Oxytetracycline (OTC); an antibiotic in the tetracycline group has been commonly used to treat and prevent bacterial infections in livestock, as well as added in feeds as food additive for growth promotion (Ghodsi *et al.*, 2016). However, indiscriminate use of OTC in animals leads to accumulation and presence of the residues in milk and milk products (Navratilova *et al.*, 2009). Small doses of OTC above maximum residue limit (MRL) exposure to humans through the food chain poses health threats including allergic reactions such as hypersensitivity, antibiotic drug resistance and disturbance of gut microbiota in the gastrointestinal tract (Kang'ethe *et al.*, 2005). Furthermore presence of antibiotics or residues in milk interferes with start cultures in food product manufacturing in dairy industries such as yoghurt and cheese (Sachi *et al.*, 2019). Therefore, in order to protect the public from exposure to residues, early and precise detection of these residues in milk and milk food products is critical.

Different regulatory international organizations such as Food and Agriculture Organization (FAO), World Health Organization (WHO) and developed countries regulatory authorities including European Union, have established a maximum residue limit for OTC up to 0.2  $\mu$ M (Furusawa, 2003). In order to ensure consumption of safe milk and milk products, various tools/methods are used. The tools are designed to function based on techniques such as, chromatographic, immunological, microbiological, colorimetric, optical and spectroscopic

(Kebede *et al.*, 2014). These methods are sensitive and selective but laborious, time-consuming, extensive equipment requirements and cannot be applied easily in the field. Hence; novel, portable, rapid, sensitive, specific, and environmentally friendly analytical tools are highly desired.

In recent times, there has been a rise in the construction and use of electrochemical methods in the analysis of OTC concentration in different samples. Of these electrochemical tools, photoelectrochemical (PEC) (Sui *et al.*, 2018; Yan *et al.*, 2015) and fluorescence (Tan *et al.*, 2016; Zhao *et al.*, 2013) have shown great promise for use in sensing applications. Nanomaterials also, are potential candidates for designing and developing sensing platforms for different chemical substances (Joshi & Kim, 2020; Magesa *et al.*, 2019). The combination of electrochemical techniques and nanomaterials such as graphene and its derivatives (GO, rGO) and transition metals nanoparticles bring a bright opportunity for the creation of ultrasensitive tools of analysis (Krishna *et al.*, 2018). In this work, tantalum pentoxide nanoparticles were composited to reduced graphene oxide and fabricated successfully on a glass carbon electrode, as a result, a stable sensitive sensing platform for OTC in milk was formed.

# **1.2** Statement of the Problem

The conventionally used methods available for detection of antimicrobials in milk are laborious, time-consuming, need expensive equipments and cannot be applied easily in the field. Hence, novel, portable, rapid, sensitive, specific, and environmentally friendly analytical tools are highly needed to allow quick detection of antimicrobials on farm/field before milk is consumed or sold for processing into products. This will eliminate entry of antimicrobial-contaminated milk in the food chain.

# **1.3** Rationale of the Study

The wide range application of current emerging technologies such as nanotechnology and electrochemical techniques provides a new avenue in solving challenges associated with antimicrobial residues detection in milk. The nanostructured electrochemical sensing platforms have notably a number of usefulness such as free waste generators, miniaturized, highly sensitive, cost effective and very fast, thus provide a clean model for analytical application. Consequently, both intensive and extensive research and scientific understanding would fasten the commercial application nano-electrochemical sensors.

# 1.4 Research Objectives

## **1.4.1 General Objective**

The goal of this study was to develop an electrochemical sensor using  $Ta_2O_5$  nanoparticles composited with electrochemically reduced graphene oxide thus form nanocomposites for the detection of oxytetracycline in milk.

# 1.4.2 Specific Objectives

- (i) To synthesize and characterize  $Ta_2O_5$ -GO nanocomposites.
- (ii) To determine optimum parameters for the performance of the Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE sensor in oxytetracycline detection.

# **1.5** Research Questions

- (i) What are the synthesizing conditions and Characteristics of Ta<sub>2</sub>O<sub>5</sub>-GO nanocomposites which identify the material as good for sensing applications?
- (ii) What are the optimal parameters for the performance of Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE sensor in oxytetracycline determination?

#### **1.6** Significance of Study

Antimicrobial residues concentration level determination plays a substantial role in the management of the antimicrobial residues in milk and milk products. The fact that available methods for antimicrobial residues levels detection require long time, very costly, need trained personnale, require a lot of chemicals to run and cannot give onsite results. Thus, novel, rapid, cost effective, simple methods need to be created. The application of electrochemical sensing in conjunction to nanomaterials facilitates the development of timely and accurate sensing methods. This work contributes to the solution of developing the best electrochemical sensors for detecting OTC in milk.

# **1.7** Delineation of the Study

The current study goal was to develop a raw milk oxytetracycline sensing tool. The sensing platform design was focused mainly on application of electrochemistry techniques in conjunction with nanotechnology. The electrochemical sensor was developed by decorating a glass carbon electrode with Tantalum Pentoxide nanoparticles composited to electrochemically reduced graphene oxide which provides more adsorption sites thus increase surface area. The fabricated

electrode acts as a tool that is dipped in a solution containing pretreated raw milk then connected to an electrochemical machine for running a redox reaction which in the end provides the results. Thus, the developed tool could be literally called nano-electrochemical sensor.

#### **CHAPTER TWO**

#### LITERATURE REVIEW

#### 2.1 Sources of Antimicrobial Residues in Milk

Milk is a great source of macro and micro nutrients that are essential in the improvement of nutritional status of animals and human being. It is the first complete food of these animals' offspring (Pandey & Voskuil, 2011). Unlike animals humans can consume milk at any stage of their growth or life. A wide spread use of antimicrobial especially antibiotics in dairy industry has led to potential residues in milk (Beyene, 2016) and most of the antimicrobials are used for therapeutic purposes, though some are also used for prophylactic purposes and others are used to enhance animal growth. One of the most common reason for residues in milk is failure to adhere to the mandatory withdrawal periods (Miller, 1997). Withdrawal period is the waiting time after administration of last dose of the drug to the lactating cow (Nonga *et al.,* 2010). Withdrawal periods in milk are often given in the unit of hours taking into account the time the animal was injected or administered with the drugs, for instance if the animal received the last dose at the middle of the daytime, cannot be counted as a day.

Another reason is extra-label use of drugs and incorrect dosage levels (Weaver, 1992). This happens when antibiotics for humans are given to animals, or the dose bought for a certain animal e.g. a cow is given to a goat. Another way is using the drug to treat a condition which is not approved for or exceeding the dose for the given animal (Raymond *et al.*, 2006).

Prophylactic use of antibiotics and adulteration are other more sources of antibiotics residues in milk. For instance milk venders add some antibiotics to keep the milk from rotting so as to ensure their customers get fresh milk (Sawant *et al.*, 2005). In addition when the physiology of the animal changes due to diseases and nutrition, there could be interference of the antibiotic metabolism hence causing the drug to persist in the body longer than the established withdrawal period (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products [VICH], 2011).

#### 2.2 Methods Used to Detect Residues in Milk

A number of methods have been applied in the detection of OTC in milk. The conventional methods that have been mostly used include chromatographic, immunological, microbiological and spectrometric. Some of the conventional methods which work alone or coupled include liquid chromatography-tandem mass spectrometry (LC-MS) (Zhu *et al.*, 2016), high-performance liquid chromatography (HPLC) (Fritz & Zuo, 2007), enzyme-linked immunosorbent assays (ELISA)

(Aga *et al.*, 2003), surface-enhanced Raman scattering (SERS) (Meng *et al.*, 2017), capillary and many more. These methods are very sensitive, selective and used for confirmatory tests. However, the methods are time-consuming, require not only extensive and sophisticated equipment but also highly trained individuals in running and maintaining them, thus very expensive. Moreover, they require a lot of chemicals in doing the analysis as well as high magnitude of power supply. In addition, the sample need to undergo a lot of processing and preparation.

There has been some rapid detection kits developed and used in determining antimicrobial residues in animal food product including milk. Charm AIM screening test kit/Charm- Aim-96 test, Charm I and II, Charm Cowside test, Charm Farm test, Charm Blue Yellow Test, (Charm Sciences Inc. USA), Delvotest SP-NT Test Kit (Netherlands), Delvo-X-PRESS  $\beta$ L test (Gistbrocades BV The Netherlands), IDEXX SNAP test (Idexx Laboratories Inc., Westbrook, ME, USA), Beta-Star test (UCB Bioproducts, Belgium) and many other (NaVrátiloVá, 2008; Parthasarathy *et al.*, 2018), have been commercially available for use. These methods work on the principle of either microbiological, enzymatic, receptor binding or immunological. The test methods are simple, sensitive, cost-effective, enable detection of wide spectrum of antimicrobial agents and user friendly. Despite them being commercially available and already in application, they have been reported to have limitation such as, higher limit of detection, many false positive results, most provide qualitative results and they are not selective enough to distinguish subgroups of antibiotic (Parthasarathy *et al.*, 2018). Most of these rapid tests are applied for screening tests, however, further confirmation is done using conventional methods such as HPLC.

# 2.3 Nanotechnology and its Application in Designing Sensors

Nanotechnology is a field of applied sciences and technology which involves the manipulation of matter at atomic and molecular level with the size range of about one billionth of a meter. Fundamentally, nanotechnology encompasses design, synthesis, characterization, restructuring and application of materials at the size of 1 to 100 nm (Duncan, 2011). One would ask, why is it significant to work with matter at nanoscale? The early anticipation of the importance in dealing with nano-sized matter started since 1959 when a presentation entitled "There is Plenty of Room at the Bottom" was given by a Physist Richard Feynman (Gordon *et al.*, 2007). It has been about four decades now since nanotechnology took off and became practical due to advancement in instrumentation which brought to existence of specialized equipment such as SEM and STM that have enabled scientists and researchers to view matter at atomic level (Dowling, 2006).

Materials exhibit unique properties and behavior different from bulk or larger state when decreased to the dimensions of between 1to100 nanometer. These materials show significant change in physical and chemical properties due to increased surface area in comparison to volume as the size diminishes (Klabunde & Richards, 2009). Thus at this scale properties of materials are size and shape dependent. Some physical and chemical properties which are function of size include chemical properties such as reactivity and catalysis; thermal properties like melting point; Optical properties e.g. absorption and scattering of light; electrical properties; magnetic properties and mechanical properties for instance, adhesion forces, capillary forces, strain forces and many more (Hodes, 2007).

Scientists have been able to exploit the unique characteristics of materials at nanoscale (nanomaterials) and apply them in creating and producing devices and systems with novel features for use in a wide range of fields. Examples of potential areas where nanotechnology is used include, energy synthesis and storage, medicine and diagnostics, electronics, pharmaceuticals, construction, sensors, agriculture, security and many more as this megatrend technology provides new potential avenue in science and technology each day (Edwards *et al.*, 2017; Keyser, 2016; Korkin *et al.*, 2010; Kumar *et al.*, 2019; Manjunatha *et al.*, 2016; Mishra, 2019).

# 2.4 Graphene

Graphene is a new materials which has become prominent in scientific research due to its extraordinary properties. The material structure is a 2D monoatomic layer of  $sp^2$  bonded carbon atoms arranged in a flat hexagonal configuration (Kostya *et al.*, 2004), as illustrated in Fig. 1A. This structure is the reason contributing to the unique characteristics thus wide application of graphene.

Since its discovery in 2004 by Geim and Novoselov who received a Nobel Prize in Physics in 2010 for their graphene pioneering research, graphene has been surprisingly highly studied. Scientist have shown its remarkable properties such as large surface area, high strength mechanically, excellent thermal conductivity and stability, tunable band gap, chemically stable, high electrical conductivity and ability to act as molecular barrier. The 2D arrangement makes effective availability of the delocalized  $\pi$ -conjugate electrons on the surface which makes the electronic configuration chemically sensitive to the surrounding materials (Pumera. 2010); thus making graphene ideal for making sensing platforms. Due to the outstanding physicochemical properties, graphene has not only been applied in sensing applications but also extensively in energy storage, biomedical, electronic devices and catalysis fields (Magesa *et al.*, 2019).

Graphene characteristics can be manipulated to produce desired nanomaterials. Owing to its structure it can form multidimensional carbon nanomaterial such as zero dimension (fullerene), one dimension (carbon nanotubes), two dimension (graphene sheet), and three dimension (graphite) (Konstantin *et al.*, 2007). This control of graphene properties can be achieved through many ways. However the synthesis methods that produce nanomaterial suitable for electrochemical sensing include chemical vapor deposition (CVD), exfoliation, unzipping of carbon nanotubes, thermal decomposition, thermal reduction and chemical reduction just to mention a few. The methods allow the modification of graphene surface which endows more application of the material (Liu *et al.*, 2015; Magesa *et al.*, 2019).

Furthermore, graphene can be derived to form its oxidized form graphene oxide (GO). The graphene oxide can further be reduced to form reduced graphene oxide (RGO). The structure of GO is similar to that of graphene, refer to Fig. 1B. It's a hexagonal having oxygen containing functional groups, few lactone, carboxy, carbonyl, phenol and quinone are located at the edge of the sheet and filled with many hydroxyl and epoxy groups positioned at the basal plane (Chen *et al.*, 2012; Smith *et al.*, 2019). These oxygenated groups endows GO with extra characteristics in comparison to graphene. These unique properties include good hydrophilicity, dispersibility in aqueous conditions and easy functionalization of the surface which open wide avenue of the material to be used in nanocomposition (Roy *et al.*, 2011). Despite having the latter mentioned features, GO becomes less conductive due to presence of the functional groups and disrupted sp<sup>3</sup> carbon atoms (Huang *et al.*, 2013). Graphene oxide reduction leads to reduced GO, the reduction is done to minimize the oxygenated function groups and restore the conjugated structure. As the results, the reduction process makes rGO to have desired properties like excellent conductivity, solubility/dispersibility over GO.

The methods of GO synthesis depends on the target material of interest. Generally, methods involved in synthesis include chemical vapour deposition, epitaxial growth on silicon carbides wafers and some methods which produce GO which is good to be used as nanocomposites like Hoffman method, hummers' methods and modified hummer's method. In this work the GO used was commercial GO.



Figure 1: The schematic atomic structure of grapheme (A) and Graphene Oxide (B)

# 2.5 Tantalum Pentoxide Nanoparticles

Transition metal nanoparticles have attracted wide interest in various fields due to their physicochemical properties, particularly their oxides. Transition metal oxide nanoparticles (TMONps) exhibit potential features such as good photoelectric activity, good conductivity, superior thermal and chemical stability as well as tunable band gaps which can be manipulated to produce material for desired applications. These metal oxide nanoparticles have been extensively reported to be used in making devices and systems in fields of electronics, sensing, medical, catalysis and energy storage (Hernández *et al.*, 2002).

In this work, the focus is on sensing specifically electrochemical sensing, thus the examples discussed will base on sensing applications. Additional information on TMONps properties is on their small size and high affinity with working electrode surface. The TMONps can be fabricated easily on electrodes through various methods such as electrodeposition, electropolymerization, physical adsorption, and covalent bonding (Jiang *et al.*, 2012). There are many research works and reviews which have reported the use of TMONps in electrochemical sensing. One of the detailed work discussed in 2018, reports on the progress, design, synthesis of different metal nanoparticles and their application in electrochemical sensing (George *et al.*, 2018). The authors includes some of the TMONps such as ZnO, Cu<sub>2</sub>O, Fe<sub>2</sub>O<sub>3</sub>, MnO<sub>2</sub>, TiO<sub>2</sub> and Co, successive stories in sensing diverse range of bioanalytes and chemical analytes including glucose, uric acid, dopamine, food colorants and metals residues .

Tantalum Pentoxide (Ta<sub>2</sub>O<sub>5</sub>) (Fig. 2), being one of the transition metal oxide nanoparticle possess unique characteristics including high dielectric coefficient and refractive index, good photoelectric ability, excellent biological and electro-catalytic activity (Zhu *et al.*, 2016). It is a semiconductor material which has an approximated band gap of approximately 4 eV. Its exceptional properties enable its application in dynamic access random memory (DRAMS), electroluminescent (EL) display devices, antireflective coatings, wave guides, photocatalysis, sensors and corrosion coating in biomedical devices (Bonavita *et al.*, 2009).

The Ta<sub>2</sub>O<sub>5</sub> can be synthesized via many methods and manipulated to obtain the nanoparticle for a targeted use, however the most common ones are hydrothermal and sol gel methods (Anandan *et al.*, 2012; Sreethawong *et al.*, 2013). In this work, a hydrothermal method was used to produce Ta<sub>2</sub>O<sub>5</sub> nanoparticle for electrochemical sensing application, more is explained in Chapter 3. A number of studies have produced and applied Ta<sub>2</sub>O<sub>5</sub> NPs in electrochemical sensing. Zhou and colleagues in 2019 synthesized Ta<sub>2</sub>O<sub>5</sub>/rGO and fabricated a glass carbon electrode for the detection of tryptophan. The produced sensor exhibited a wider linear range and lower limit of detection (0.84  $\mu$ M). The sensor was applied practically in detection of tryptophan in human serum and the results were compelling. Tantalum pentoxide has also been applied in gas sensing. This provides an insight that there is potential application for the nanomaterial in sensing other materials (Zhou *et al.*, 2019).



#### Figure 2: Schematic atomic structure representation of Tantalum Pentoxide

# 2.6 Electrochemical Sensors

Electrochemical sensors are devices that process and produce analytically useful signal about the composition of the analyte, which is resulted from the chemical interaction between the

recognition element and transducer based on chemical energy transformation (Stetter *et al.*, 2003). For many years electrochemical sensors (ECSs) have been used in identification and quantification of substances owing to their excellent selectivity and sensitivity, cost effective as well as simple and user friendly (Faridbod *et al.*, 2011).

Simply an electrochemical sensor comprises of recognition element, transducer, and signal displaying device. The ECSs operate by involving the transducer element which is covered by a recognition element, this recognition element chemically interacts with the analyte and produces chemical signal. This chemical signal is transformed by the transducer into electrical signal. The electric signal produced is proportional to the concentration of the analyte and can be detected and displayed (Power & Morrin, 2013). The chemical interaction in sensing is a chemical reaction which occur at the interface between the electrode and target analyte suspended in electrolyte solution, the reaction is either oxidation or reduction. The oxidation process affects the electron flow to move from the sensing/working electrode to counter electrode through external circuit. On the other hand, reduction induces electrons flow to move from counter electrode to the sensing electrode. Either way electron flow direction results to current whose magnitude is relative to the concentration of the analyte (Brett & Oliveira-Brett, 2011; Kannan *et al.*, 2015).

Typically, an electrochemical sensor consists of a reference electrode, working electrode and counter electrode, the sensor structure scheme is depicted in Fig. 3. At the working electrode surface is where the reaction (sensing process) takes place, while the counter electrode acts as the second half reaction and thus completes the circuit of the electrochemical cell. On the other end, there is no current flowing to or away from the reference electrode. This electrode provides a constant and stable potential to the electrolyte (Kannan *et al.*, 2015). The electrochemical techniques used by electrochemical sensors include voltammetry, amperometry and potentiometry. In this study cyclic voltammetry (CV) was used given that, it is a very versatile technique that can derive information of the given mechanism of the electrochemical reactions. In CV current and voltage measurement are done between counter and working electrode and reference and working electrodes respectively. The resulted measurements are called voltammograms which is a plot of current versus potential.

Various electrochemical sensors have been discussed and reported by many researchers. These employ different techniques such as Photoelectrochemical (PEC), Electrochemiluminescent (ECL), Photoluminescent (PL), Flourescence, for the detection of a diverse chemical substances such as gases; O<sub>2</sub>, CO<sub>2</sub>, H<sub>2</sub>, Metals; Pb, Cu, Fe, Food colorants e.g amaranth and allura red,

antimicrobial residues and may more (Gaudin *et al.*, 2009; Magesa *et al.*, 2019; Santonico *et al.*, 2017).

Sun and coworkers developed a direct voltammetric sensor based on zinc cation-exchanged montmorillonite modified glassy carbon electrode (Zn-Mt/GCE) for the detection of OTC. The sensor exhibited a liner range of 0.80 to 40  $\mu$ M with the limit of detection being 0.12  $\mu$ M. The sensor was practically applied and gave promising results (Sun *et al.*, 2014). Other sensors for OTC determination are combined with biological materials such as enzymes, antibodies and aptamers to enhance sensitivity and selectivity. A number of these sensors which use techniques such as photoelectrochemical, electrochemiluminescence and fluorescent showed high sensitivity, specificity and reproducibility (Jalalian *et al.*, 2018; Li *et al.*, 2017; Zhao *et al.*, 2013). In antimicrobial residues detection many developed sensors require a biological material to achieve effectiveness which are mostly unstable. Due to this there is still a need for more knowledge in making electrochemical sensors which can directly and effectively analyze the material without requiring addition of biological materials which are difficult to handle.



Figure 3: Simple schematic representation of an electrochemical sensor (A), and Ta2O5/ERGO sensor for OTC (B)

## **CHAPTER THREE**

# **Materials and Methods**

# 3.1 Materials

# 3.1.1 Reagents

Graphite powder, tantalum chloride (TaCl<sub>5</sub>), potassium permanganate (KMnO<sub>4</sub>), diethanol amine (DEOA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), sodium hydroxide (NaOH), Trichloro acetic acid, potassium ferricyanide (K<sub>3</sub>Fe(CN)<sub>6</sub>);  $\alpha$ -aluminium oxide ( $\alpha$ -Al<sub>2</sub>O<sub>3</sub>, particle size: 1.0  $\mu$ m, 0.3  $\mu$ m and 0.05  $\mu$ m), and ethyl alcohol were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Disodium phosphate dodecahydrate (Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O); sodium dihydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>) and oxytetracycline (OTC) were supplied by Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). The milk used was obtained from the Renrenle supermarket (Zhuzhou, Hunan, China).

# 3.1.2 Equipment

Cyclic voltammetry (CV) was carried out on a CHI 660E electrochemical workstation of Shanghai Chenhua Instruments Company, China. A three-electrode system with GCE as working electrode, saturated calomel electrode (SCE) as a reference electrode, and a platinum electrode as a counter electrode was used in all electrochemical measurements. The pH value were determined using a pH-3c exact digital pH meter (Leichi Instrument Factory, Shanghai, China). Scanning electron microscopy (SEM) images were obtained using scanning electron microscope (EVO10, ZEISS, Jena, Germany).The crystal structure analysis was carried out using a powder X-ray difractometer (PANalytical, Amsterdam, Holland).

# 3.2 Methods

# 3.2.1 Specific Objective 1

# (i) Ta<sub>2</sub>O<sub>5</sub> Nanoparticle Synthesis

A hydrothermal method was applied in Ta<sub>2</sub>O<sub>5</sub> preparation as the procedure reported by Anandan and others (Anandan *et al.*, 2012). Briefly 100mm of 0.01 M NaOH was placed rapidly into 300 ml of 0.05 M TaCl<sub>5</sub>. Then 0.1ml of diethanolamine was added as a stabilizer. The solution was stirred for 1 h at room temperature and transferred into a hydrothermal autoclave then heated at 80 °C for 48 h for crystallization to occur. Upon cooling to room temperature, the powder was washed using distilled water. To remove impurities the powder was washed with ethanol three times and dried at room temperature using a vacuum drier. To obtain  $Ta_2O_5$  nanoparticles, the dried powder was calcinated at 700 °C for 3 h in a muffle furnace.

#### (ii) **Preparation of Ta<sub>2</sub>O<sub>5</sub>-GO Composition**

Commercial GO was used in preparing the dispersion. Twenty milligrams of GO powder was mixed with 20 ml of water to obtain GO solution with the concentration of 1mg/ml. Then 1 mg  $Ta_2O_5$  nanoparticles were mixed in 10 ml of the prepared GO solution. Subsequently, the mixed solution was ultrasonicated for 2 h to form  $Ta_2O_5NPs$ -GO dispersion.

## (iii) Characterization of the Synthesized Materials

Scanning electron microscopy (SEM) images were obtained at 2.0 KV of acceleration voltage using scanning electron microscope (EVO10, ZEISS, Jena, Germany). The crystal structure analysis of GO, Ta<sub>2</sub>O<sub>5</sub>NP, Ta<sub>2</sub>O<sub>5</sub>-GO nanocomposites was done using a powder X-ray difractometer (PANalytical, Amsterdam, Holland) with Cu K  $\alpha$  radiation (0.1542 nm). Electrochemical characterization was done using cyclic voltammetry (CV) technique performed on a CHI 660E electrochemical workstation. The conventional three-electrode system involving different working electrodes (Ta<sub>2</sub>O<sub>5</sub> NPs-ERGO/GCE, GO/GCE, ErGO/GCE, and Ta<sub>2</sub>O<sub>5</sub>-GO/GCE), a reference electrode (saturated calomel electrode, SCE), and a counter electrode (platinum electrode)) was used. The solution for electrochemical characterization contained 5mM K<sub>3</sub> [Fe(CN)<sub>6</sub>] and 0.1 M KCl, it was used to obtain cyclic voltammograms of bare GCE, GO/GCE, ErGO/GCE and Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE.

# (iv) Electrode Fabrication

A glassy carbon electrode (GCE) surface (3 mm in diameter) was polished with different fine size of alumina slurry ( $\alpha$ -aluminium oxide ( $\alpha$ -Al<sub>2</sub>O<sub>3</sub>)) starting with 1.0 um then 0.3 um and finalizing with 0.05 um. After that the polished electrode was ultrasonicated in distilled water, absolute ethanol then distilled water for 1 minute in each solution to clean them completely and left to dry under infrared lamp. After drying 5µL of Ta<sub>2</sub>O<sub>5</sub>-GO dispersion was drop casted on the surface of the clean GCE and left to dry at room temperature. The Ta<sub>2</sub>O<sub>5</sub>-ERGO/GCE was prepared by immersing the Ta<sub>2</sub>O<sub>5</sub>-GO/GCE in 0.1 M phosphate buffer solution (PBS, pH 6.5) and reduction process was run at a constant potential of -1.2 V for 120 s. The same method was used to prepare other modified electrodes such as a GO/GCE and ErGO/GCE for efficiency comparison.

# 3.2.2 Specific Objective 2

# (i) Optimization of pH and Deposition Parameters

# pН

In order to determine optimal pH of the solution, various 1M PBS solutions with different pH were prepared as follows; 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. Then Cyclic Voltammograms of  $10 \mu$ M oxytetracycline were recorded.

# **Deposition Potential and Time**

The effect of deposition potential for the current of the anodic oxytetracycline was investigated at the voltage range of  $-0.2 \sim 1.0$  V. Accumulation of OTC on the surface of Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE was done at numerous potentials for 90 s, then the  $i_{pa}$  were carried out by Cyclic Voltammetry. Furthermore, the influence of deposition time was studied at fixed deposition potential of 0 V as well.

#### (ii) Stability, Reproducibility and Selectivity Determination

# Stability

The developed sensing platform is usually designed to perform repeated measurements. Thus in testing its stability the modified electrode  $Ta_2O_5$ -ErGO/GCE was tested by running the Cyclic Voltammograms of 10  $\mu$ M oxytetracycline in 6.5 pH PBS, which was recorded approximately 25 times for 5 days.

# **Reproducibility**

For reproducibility determination, five  $Ta_2O_5$ -ErGO fabricated glass carbon electrodes were measured in 10  $\mu$ M oxytetracycline in 0.1 M PBS with pH of 6.5, potential window -0.2 -1.0 V, Deposition potential 0 V and deposition time 90 s. Then the Relative Standard Deviation for Oxytetracycline peak currents was calculated.

#### Selectivity

Selectivity test was done by taking 1 x  $10^{-3}$ M of Na+, K+, Zn<sup>2+</sup>, Al<sup>3+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup> and 2 x 10 <sup>-4</sup> M of amoxicillin, tetracycline and mix all in 10 x $10^{-5}$  M of oxytetracycline then the Cyclic Voltammograms of OTC with the interfering chemical substances was measured.

All electrochemical tests were carried out by CV technique using three electrode system (bare or modified GCEs as work electrodes, platinum wire electrode as counter electrode, and saturated calomel electrode (SCE) as reference electrode). Ten  $\mu$ M oxytetracycline was prepared in 1.0 M PBS (pH 6.5). With the deposition time (90 s) and potential (0 V), the cyclic voltammetry was recorded on the electrodes. Establishment of the calibration curve was done by plotting the relationship between the measured current signal and the concentration of analyte. The content of Oxytetracycline in milk sample solutions was determined by the standard addition method.

# (iv) Milk Sample Pretreatment

Ultra high temperature (UHT) treated whole milk samples were bought from local supermarkets at Zhuzhou town in China. For OTC analysis, the milk samples were pretreated as follows; 3 g of trichloroacetic acid was weighed and then dissolved in 100 ml of PBS solution with pH 6.5. Thereafter, 15ml of milk was put in a 50 ml centrifuge tube then, addition of 15 ml of the dissolved trichloroacetic acid followed by gently shaking. After that, the mixture was centrifuged at 4000 rpm for 30 minutes to separate between the clear milk fluid and the milk protein and fat. In order to obtain a very clear solution, the supernatant was filtered using the 0.22  $\mu$ m pore filter. In testing the fabricated sensor, milk samples 1, 2 and 3 were spiked with different concentration of OTC 0.5, 5 and 50  $\mu$ M respectively. The methodology discussed is summarized by the Fig. 4 below.



Figure 4: The preparation pathway of Ta<sub>2</sub>O<sub>5</sub>-rGO/GCE for the determination of Oxytetracycline

#### **CHAPTER FOUR**

#### **Results and Discussion**

#### 4.1 Results

# 4.1.1 Structures of the Synthesized Nanomaterials

The morphologies of the different synthesized materials were firstly explored by SEM. As Fig. 5A shows, the profusely plication and crumple-like surface structure are displayed, demonstrating the successful preparation of GO similar to studies reported by (Smith *et al.*, 2019). Figure 5B is the typical image of  $Ta_2O_5$  nanoparticles synthesized. Each homogeneous particle is clearly observed with estimated size of 750 nm, the large particles are formed by the small particles due to aggregation. Though these small particles are smaller than 100 nm, the exact size could not be determined with the low-resolution of the scanning electron microscope images. The morphology of  $Ta_2O_5$ -GO is represented in Fig. 5C, clearly showing majority of nanoparticles are uniformly dispersed on the surface of the 2D layer-structure GO, illustrating that the  $Ta_2O_5$  composited with GO nanosheets show the excellent disparity in contrast to the pure  $Ta_2O_5$ .



Figure 5: (A) Scanning electron microscopy images of the pure GO, (B) pure Ta<sub>2</sub>O<sub>5</sub> nanoparticles and (C) Ta<sub>2</sub>O<sub>5</sub>-GO composite nanoparticles

Crystalline structure of the GO, pure Ta<sub>2</sub>O<sub>5</sub> nanoparticles and Ta<sub>2</sub>O<sub>5</sub>-GO composite nanoparticles were further investigated by the X-ray diffraction (XRD). As the Fig. 6 presents, the (001) plane corresponding to blue column belongs to the typical XRD pattern of pure GO. Moreover, the obvious diffraction peaks of Ta<sub>2</sub>O<sub>5</sub> are observed at 2 $\theta$  =16.8°, 22.9°, 28.3°, 32.8°, 36.7°, 44.7°, 46.7°, 49.7°, 50.7°, 55.5°, 63.6° and 71.4°, corresponding to the green columns, which are related to (140), (001), (1110), (270), (1111), (340), (002), (0220), (2151), (1112), (2221) and (4160) reflections, respectively. The data is in agreement with the standard card (Joint Committee Powder Diffraction Standards, JCPDS, No. 25-0922) of  $Ta_2O_5$  (Zhou *et al.*, 2019), demonstrating that the synthetic  $Ta_2O_5$  nanoparticles was successfully prepared. Most importantly, not only the plane of GO, but also the ones of  $Ta_2O_5$  are observed in the XRD pattern of pure GO and  $Ta_2O_5$ -GO composites, indicating the successful synthesis of  $Ta_2O_5$ -GO composites.



Figure 6: X-ray diffraction patterns of pure Ta<sub>2</sub>O<sub>5</sub>, Ta<sub>2</sub>O<sub>5</sub>-GO composites and graphene oxide (GO) nanosheets

## 4.1.2 Electrochemical Features of the Modified Electrodes

A solution containing K<sub>3</sub>[Fe(CN)<sub>6</sub>] and 0.1 M KCl was used to obtain cyclic voltammograms of bare GCE, GO/GCE, ErGO/GCE and Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE and the results are indicated in Fig.7. Since ipa/ipc  $\approx$ 1, the redox peaks appeared in each electrode tested was a pair of the quasireversible ones. At the bare GCE, the potential separation ( $\Delta$ EP) of the redox peaks was 0.079 V with the weak current of 60.52 ( $i_{pa}$ )  $\mu$ A and 63.87 ( $i_{pc}$ )  $\mu$ A. While with the presence of the poor conductive material GO, the redox peaks currents of GO/GCE decrease precipitously with  $i_{pa}$ =23.96  $\mu$ A and  $i_{pc}$ =24.18  $\mu$ A. Moreover, after electrochemical reduction of GO to electrochemically reduced GO, the current on ErGO/GCE was higher than that of GO/GCE, implying a better conductivity of ErGO than that of GO. The intensity of the anodic and cathodic peak current of ErGO is 51.13 and 55.71  $\mu$ A, respectively. Above all, the largest redox peaks current ( $i_{pa} = 135.7 \ \mu$ A,  $i_{pc} = 134.5 \ \mu$ A) occurred to Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE, which increased by 2-fold approximately. The result shows Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE dramatically improved electrochemical performances owing to the large specific surface area. For further investigation, the effective electroactive areas of various electrodes could be figured out by the Randles–Sevcik equation (He *et al.*, 2019; Wan *et al.*, 2019):

$$i_{\rm pc} = 2.691 \times 10^5 \,{\rm n}^{3/2} \,{\rm D}^{1/2} \,{\rm v}^{1/2} \,{\rm AC}$$
 (1)

where  $i_{pc}$  indicates the reduction peak current of K<sub>3</sub>[Fe(CN)<sub>6</sub>]; **n** indicates the number of electron transferred during the redox process; **A** indicates the electrochemical active area (cm<sup>2</sup>); **D** (=7.6 ×10<sup>-6</sup> cm<sup>2</sup>s<sup>-1</sup>) indicates diffusion coefficient of K<sub>3</sub>[Fe(CN)<sub>6</sub>]; **C** indicates K<sub>3</sub>[Fe(CN)<sub>6</sub>] concentration in (mol·cm<sup>-3</sup>) of; **v** indicates the scanning rate (Vs<sup>-1</sup>). Thus, the electrochemical active areas of bare GCE, GO/GCE, ErGO/GCE and Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE can be worked out as 0.0545, 0.0206, 0.0475 and 0.1147 cm<sup>2</sup>, respectively. The Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE electrochemical active area increased by over 110% as compared to bare GCE, elucidating that the Ta<sub>2</sub>O<sub>5</sub>-ErGO composite modification enable the effective electroactive surface area to expand, promoting the conglutination of oxytetracycline on the electrode surface and proffer more catalytic active sites, and thus facilitate the redox process of oxytetracycline. As the results, Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE redox peak currents increased by more than two fold.



Figure 7: Cyclic Voltammetry (CV) obtained on bare GCE, GO/GCE ErGO/GCE and Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE in a 5.0 mM K<sub>3</sub>Fe(CN)<sub>6</sub> and 0.1 M KCL solution at the scan of 0.1V s<sup>-1</sup>

#### 4.1.4 Electrochemical Behavior of Oxytetracycline on Various Electrodes

Figure 8 displays the Cyclic Voltammograms responses of 10  $\mu$ M OTC in 0.1 M PBS obtained at various electrodes in a potential range of -0.2 to 1.0 V. Obviously, as shown in Fig. 8, there are no cathodic peaks at all electrodes, while the redox peak presented illustrates that the oxidation of OTC is an irreversible process. Relatively, there is a weak anodic peak observed clearly at bare GCE, which is similar to the one of GO/GCE. Most importantly, after the electrochemical reduction, the signal of the peak current of ErGO is magnified, but it is still less than that of Ta<sub>2</sub>O<sub>5</sub>-ErGO. The study shows Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE has an excellent detection performance for OTC when compared to the other electrodes. The results may be owing to the synergistic effect of ErGO and Ta<sub>2</sub>O<sub>5</sub> which enhance the current of the peak. Thus, in this work, Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE is the optimal choice for the determination of oxytetracycline.



# Figure 8: Oxytetracycline Electrochemical behaviours (10 μM) on bare GCE, GO/GCE ErGO/GCE and Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE (Cyclic voltammetry, scan rate: 0.1 V/s)

# 4.1.5 Oxytetracycline Electrochemical Kinetics on Ta<sub>2</sub>O<sub>5</sub>-ErGO Glass Carbon Electrode

For a further exploration of the reaction mechanism, the cyclic voltammograms of oxytetracycline  $(1.0 \times 10^{-5} \text{M})$  were plotted under different scan rate (30 ~300 mV/s) on Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE. The result is presented in Fig. 9A. Manifestly, with the enhancement of the scan rate, the  $i_{pa}$  of oxytetracycline aggrandize, while the background current increase as well. Probably, high scanning rates are able to enhance the charging current of double layer. Additionally, the linear relationship between the anodic peak currents of oxytetracycline ( $i_{pa}$ ) and the square root of the scan rate (v) is depicted in Fig. 9B. The linear equation of  $i_{pa(OTC)}(\mu A) = 52.315v^{1/2}$  (V/s)+2.150

with correlation coefficient ( $\mathbb{R}^2$ ) of 0.98, identifying the electrooxidation of oxytetracycline belongs to an adsorption-limited process on. Furthermore, for an adsorption-controlled and totally irreversible electrode process, the relationship between the peak potential and the scanning rate is based on Lavrion equation (Laviron, 1974):

$$E_{\rm p} = {\rm E}^0 + \left(\frac{RT}{\alpha nF}\right) \ln \left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{RT}{\alpha nF}\right) \ln \nu \tag{2}$$

where  $E_p$  is defined as the peak potential,  $E^0$  is defined as the formal potential (V), **T** is defined as temperature (298.15K),  $\alpha$  is defined as Electron transfer coefficient, **n** is defined as electron transfer number,  $k^0$  is defined as rate constant, **F** is defined as Faraday constant (F = 96485 C/mol), **R** = 8.314 J/(K·mol) and v is defined as scan rate (s). The linear relationship between the anodic peak potentials ( $E_p$ ) and Napierian logarithm of scanning rates ( $\ln v$ ) is shown in Fig. 9C. Consequently, the oxidation peaks are moved toward positive potential direct in different degrees at various scanning rate. The oxidation potentials ( $E_p$ ) are linearly proportional to Napierian logarithm of scan rate ( $\ln v$ ) with the linear equation:  $E_p = 0.040 \ln v + 0.822$  (R<sup>2</sup> = 0.9931). According to the equation (2) and the linear equation ( $dE_p/dlnv=RT/\alpha nF=0.040$ ),  $\alpha n$  was calculated as 0.64. As for an irreversible process, it is suggested  $\alpha$  to be 0.5, so **n** can be estimated to be 1. Thus, one electron (e<sup>-</sup>) participated in the oxidation of oxytetracycline on Ta<sub>2</sub>O<sub>5</sub>-ErGO-GCE.



Figure 9: (A) Oxytetracycline cyclic voltammograms on Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE at different scanning rates (B). Relationship between oxidation peak current (i<sub>pa</sub>) and scanning rate (v) (C). The oxidation potentials (Ep) are linearly proportional to the Napierian logarithm of scan rate (lnv) (the current values have been baseline-corrected)

## 4.1.6 **Optimal Determining Parameters**

#### (i) Effect of pH

For the investigation of the effect of solution pH, the CVs of 10  $\mu$ M oxytetracycline were recorded in different pH PBS, shown in Fig. 10A. With the enhancement of pH, the oxidation peaks shift directing towards negative potential, and the peak potentials ( $E_p$ ) decrease linearly with pH, as depicted in Fig. 10B. The linear equation of peak potential and pH is  $E_p$  = -0.049 pH +1.0313 (R<sup>2</sup> = 0.9818), illustrating protons (H<sup>+</sup>) participated in the electrochemical reaction of oxytetracycline. As Fig. 10C depicts, with the increase of pH, the  $i_{pa}$  increased constantly until pH is 6.5. Subsequently, the pH keeps aggrandizing, while the oxidation current of oxytetracycline is decreased. Thus, pH = 6.5 is the optimal pH for detecting the oxytetracycline in this study.



Figure 10: (A); Cyclic Voltammograms of oxytetracycline 10  $\mu$ M on Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE at different pH (B); the linear relationship of the oxidation peak potential of Oxytetracycline and the pH (C); the effect of pH on the oxytetracycline response peak current

# (ii) Effect of Deposition Parameters

The influence of deposition potential for the current of the anodic oxytetracycline was investigated at the voltage range of  $-0.2 \sim 1.0$  V. Oxytetracycline was accumulated on the surface of Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE at numerous potentials for 90 s, and then the  $i_{pa}$  of them were carried out by CV. As Fig. 11A shows, the peak current increases with the positive potential. When the deposition potential is over 0 V, the value of the peak current reduces, which is far less than that at 0 V. Hence, 0 V was suggested as the optimal deposition potential. Furthermore, the effect of deposition time was studied at fixed deposition potential of 0 Vas well. In Fig. 11B, it is evident to observe that the  $i_{pa}$  of oxytetracycline increases when the deposition time was prolonged from 0

to 180s. In the beginning, the  $i_{pa}$  is gradually increasing until 90 s. After 90 s, the currents are roughly the same as in the first 90 s. The result demonstrates the deposition time can effectively improve the sensitivity of detecting oxytetracycline, recommending 90 s for the optimal performance. Therefore, the deposition was performed at 0 V for 90 s in the following measurements.



Figure 11: The effect of deposition potential (A) and deposition time (B) on the oxidation peak currents of 10  $\mu$ M oxytetracycline (0.1 M PBS, pH=6.5) at Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE

#### 4.1.7 Selectivity and Reproducibility

At the optimum analytical conditions, the selectivity performance was studied by measuring the response peak currents in the presence of potential interfering substances containing some metal ions and other antibiotics. The experimental results showed that, 100-fold of Na+, K+,  $Zn^{2+}$ ,  $Al^{3+}, Ca^{2+}, Mg^{2+}, Cu^{2+}$  and 20-fold of amoxicillin, tetracycline have no apparent influences on the response peak current of 10  $\mu$ M oxytetracycline (signal change  $\leq \pm 5\%$ ). The experimental results illustrates that the proposed Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE has high selectivity for the OTC determination.

For the evaluation of reproducibility, 5 different electrodes modified with  $Ta_2O_5$ -ErGO were measured in 10  $\mu$ M oxytetracycline in 0.1 M PBS with pH of 6.5. The relative standard deviation (RSD) for oxytetracycline peak currents was 2.34 %, implying the good reproducibility.

## 4.1.8 Stability Test

This sensor is designed to perform repeated measurements, the intended operating mechanism is, a single electrode performs many test measurements repeatedly. As the results, a drop of 13% was observed on the current signal of oxytetracycline when the first and the last signal were compared as shown in Fig. 12. This shows that the  $Ta_2O_5$ -ErGO/GCE prepared has excellent stability.



Figure 12: The Cyclic Voltammograms obtained after running 25 cycles in 10 μM OTC in PBS the 5<sup>th</sup> day

# 4.1.9 Oxytetracycline Calibration Curve Characteristics

The calibration curve for oxytetracycline at Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE was characterized by cyclic voltammetry under the optimal experimental conditions. The typical Voltammograms are depicted in Fig. 13A. In the range of 0.2  $\mu$ M~100  $\mu$ M, the oxidation peak current is well linear to oxytetracycline concentration as Fig. 13B depicts. The linear regression equation can be described as:  $i_{pa}$  ( $\mu$ A) = 0.332c<sub>OTC</sub> ( $\mu$ M) +0.328 (R<sup>2</sup> = 0.9893). According to the equations LOD = 3S/N (S: blank solution standard deviation; N: the slope of calibration plots), the detection limit was identified as 0.095  $\mu$ M. It mainly ascribes the excellent sensing performance to the synergistic effect of Ta<sub>2</sub>O<sub>5</sub> and ErGO mentioned above. Table 1 shows the comparison of the current study limit of detection and a few selected from the literature.



Figure 13: (A) CVs obtained on Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE in 0.1 M PBS (pH 6.5) containing different concentrations of OTC (0.2, 0.4, 0.6, 0.8,1, 2, 4, 6, 8, 10, 30, 50, 70, 90 and 100  $\mu$ M). (B) The linear relationship between the anodic peak currents ( $i_{pa}$ ) of OTC and OTC, concentration (C<sub>OTC</sub>) in the range of 0.2-100  $\mu$ M

 Table 1: Comparison of various fabricated electrodes performance on the analysis of Oxytetracycline with the current study

Electrode/Fabrication	Techniques	Linear range(uM)	Detection Limit (uM)	References	
Montmorillonite/acetylene		Tange(µ11)			
black modified carbon paste	<sup>e</sup> DPV	0.5–50	0.087	Sun et al. (2015)	
microelectrode					
<sup>a</sup> Zn-Mt/GCE	DPV, <sup>f</sup> EIS	0.80-40	0.12	Sun et al. (2014)	
<sup>b</sup> Pd-HSiO <sub>1.5</sub> /Ni–Co	Electrodeposition	400-0.01	10	Du et al. (2018)	
°CS-MWCNTs/ AuNPs	CV, Amperometry	0.03-80	0.027	Lian et al. (2013)	
<sup>d</sup> Ni-Co Alloy electrode	CV	10-500	10	Xie et al. (2016)	
Ta <sub>2</sub> O <sub>5</sub> -ErGO/GCE	CV	0.2-100	0.095	Current study	

<sup>a</sup>zinc cation-exchanged montmorillonite modified glassy carbon electrode; <sup>b</sup>hierarchical nanoporous Pd-HSiO<sub>1.5</sub>/Ni–Co composite electrode; <sup>c</sup>chitosan-multiwalled carbon nanotube composite multilayer films and gold nanoparticles; <sup>d</sup>Nickel-cobalt alloy electrode; <sup>e</sup> differential pulse voltammetry; <sup>f</sup>Electrochemical impedance spectroscopy

#### 4.1.10 Detection of Oxytetracycline in Real Sample

The analysis of OTC was done in UHT milk bought from supermarket which was pretreated to get a clear solution. The technique employed for the quantitative analysis was Cyclic Voltammetry. The experimental results are listed in Table 2. In this specific milk sample there is no oxytetracycline detected. However the milk spiked with different OTC produced good recoveries (100.1-120.9 %) which indicate that the proposed Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE sensing platform can be functional to OTC detection in real samples.

Cyclic voltammetry (sample number = $3$ )							
Sample	Add (µM)	Total Found (µM)	RSD (%)	Recovery (%)			
NO. 1	0.50	0.599	3.50	119.8			
NO. 2	5.00	6.045	3.27	120.9			
NO. 3	50.00	50.04	2.76	100.1			

 Table 2: Determination of oxytetracycline in the spiked milk supernatant sample by Cyclic voltammetry (sample number = 3)

### 4.2 Discussion

The day to day demand for food products from animals has led to extensive use of antimicrobial agents. However owing to the indiscriminate use of these antimicrobial agents especially antibiotics, the global is facing serious health problems. Oxytetracycline is one of the broad spectrum tetracycline antibiotic which is commonly used for therapeutic, prophylactic as well as an agent for enhancing growth in animal husbandry. Despite useful application, abuse of OTC leads to residues above maximum residue limit in milk which could accumulate in human through food chain and in turn poses serious risks to human health. One of the better ways to monitor the levels of these residues is to have rapid and robust analytical test methods. In view of this fact, the current work is designed and has produced an electrochemical sensor for analysis of OTC in milk.

The SEM images and XRD pattern of  $Ta_2O_5$  in Fig. 5B and 6 respectively, exhibit a successfully synthesis at 700°C temperature. The micrographs show intelligible grain shape which indicate that a clear grain crystallization distribution occurred similar to data reported by Bonavita and colleagues (Bonavita *et al.*, 2009). Furthermore from the micrograph in Fig 5B, agglomerates can be seen. These large masses are formed by clustering of small particles due to adhesion. Figure 5C shows  $Ta_2O_5$  nanocomposites. The micrograph is different from Fig. 5A and 5B as it indicates the fusion of  $Ta_2O_5$  and GO. The crease part of GO layer is coated by the  $Ta_2O_5$  nanoparticles and the nanoparticles are well dispersed on the surface of the GO sheet. The XRD patterns in Fig. 6 confirms the  $Ta_2O_5$ -GO composite formation which agrees to similar results reported by preceding study (Zhou *et al.*, 2019).

The sensor developed in this study was fabricated using  $Ta_2O_5$ -ErGO nanocomposites. The results indicate that  $Ta_2O_5$ -ErGO/GCE has an excellent detection performance for OTC when compared to the bare GCE, rGO/GCE and  $Ta_2O_5$ -GO/GCE modified electrodes. The compelling performance is attributed to synergistic actions of ErGO and  $Ta_2O_5$ . ErGO increases the electrode surface area and enables good conductivity, thus enhancing the current of the peak. Consequently  $Ta_2O_5$ -ErGO/GCE in this study was observed to be the optimal choice for the determination of oxytetracycline.

The lowest quantity of Oxytetracycline that the modified Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE can determine was calculated to be 0.095  $\mu$ M. This amount means that the sensor can detect a very low amount of OTC residues in milk. This is below or in compliance with established maximum residue limit for Oxytetracycline in milk which is 0.2  $\mu$ M in reference to WHO, FAO and EU database (Furusawa, 2003). Furthermore, these results are better when further compared to other sensing platforms that have been reported although the list is not exhaustive in table 1. Sun and colleagues in 2014 produced an electrochemical sensor which could directly detect OTC in food samples (Sun *et al.*, 2014). The sensor produced had 10  $\mu$ M limit of detection which is even higher than the 0.095  $\mu$ M from the current study. This method of detection is highly promising for practical application.

Furthermore, this sensing platform exhibited compelling reproducibility because the relative standard deviation was 2.3% which is below the standard RSD 5% for this type of analysis (McFarren *et al.*, 1970). Moreover, the sensor showed outstanding selectivity towards ions, and other antibiotics that could be found in the milk while in stability test, a drop of 13% is observed between the first and the last peak. This could be due to impurities from the air which fouls the electrode and thus minimize the active sites for the redox reaction.

#### **CHAPTER FIVE**

#### **Conclusion and Recommendations**

## 5.1 Conclusion

In this study, the Ta<sub>2</sub>O<sub>5</sub> was successfully synthesized and then composited with GO to obtain Ta<sub>2</sub>O<sub>5</sub>-GO composition. Ta<sub>2</sub>O<sub>5</sub>-GO was dispersed in pure water and subsequent drop-coating on the surface of GCE. Subsequently, Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE was fabricated by electrochemical reduction method. In comparing the bare GCE, GO/GCE, ErGO and Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE, the oxidation signals of oxytetracycline are significantly enlarged on the nanocomposites modified GCE (Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE). In addition, this modified electrode has successfully been used to eliminate the interferences of oxytetracycline. The linear range for the determination of oxytetracycline was 0.2 ~100  $\mu$ M on Ta<sub>2</sub>O<sub>5</sub>-ErGO/GCE with the low detection limit of 0.095  $\mu$ M. The current developed method provides high sensitivity and selectivity, quick response and simple fabrication which give a basis for producing field ready portable commercial sensors for effective oxytetracycline detection in milk.

## 5.2 **Recommendations**

Antimicrobial residues availability in different substances still remains a global threat. Many methods for detections have been developed. However, none of them have been able to fulfil the effectiveness required in analyses of antimicrobial residues. As the demand for rapid, simple, ultrasensitive, selective and cost effective detection of analytes continues to grow, so does the research and development of electrochemical sensors needs to increase. In contrast to their chromatographic, immunological, microbiological and spectroscopic methods counterparts, electrochemical sensors have low cost, require low power and portable, thus, can be adapted for the identification of a wide range of analytes.

Despite the potential promise already demonstrated by electrochemical sensors, further studies are of high demand because technology improves when many persons work on it and not automatically. Electrochemical sensors work under redox principle thus the target analyte should be an electroactive species. Novel electrochemical sensors which can detect non electroactive species should also be developed. Furthermore, electrochemical sensors should be able to detect and quantify the lowest amount of analyte in a sample. Research on ultrasensitive sensors is very significant.

Moreover, many sensitive and selective detection methods are impractical in field analysis. In order to achieve onsite detection of antimicrobial residues, field-ready portable devices are to be

developed. The application of disposable electrodes in constructing field ready sensors could support real-time analysis of samples away from the laboratory. This would enable timely surveillance of antimicrobial residues especially in food substances such as milk. In the end, provide better models in managing residues in food.

Unlike their other chemical analytes e.g. gases counterparts, food substances analyses are very challenging. One major challenge is direct detection of target analytes in food or food products. Food matrix does not only interfere with the detection process but prevents the detection by covering the sensing surface. Therefore, for the detection of the target analyte, let us take the case of the current work, detection of OTC in milk. The milk has to undergo pretreatment to get a clear solution. This renders the electrochemical sensor unable to perform direct detection of target analyte. Therefore, more research should be focused on this aspect to solve this challenge.

The advancement of electrochemical sensing will rise sharply when the sensors will be developed while coupling nanomaterials. Due to their unique properties nanomaterials augment the sensing reactive surface area. Furthermore, the synergism with nanomaterials opens opportunity to miniaturize. Thus more research should be focused to this area to make the two fields work together to provide viable sensing platforms.

#### REFERENCES

- Aga, D. S., Goldfish, R., & Kulshrestha, P. (2003). Application of ELISA in determining the fate of tetracyclines in land-applied livestock wastes. *Analyst*, 128(6), 658-662.
- Anandan, S., Pugazhenthiran, N., Selvamani, T., Hsieh, S. H., Lee, G. J., & Wu, J. J. (2012). Investigation on photocatalytic potential of Au–Ta 2 O 5 semiconductor nanoparticle by degrading methyl orange in aqueous solution by illuminating with visible light. *Catalysis Science & Technology*, 2(12), 2502-2507.
- Beyene, T. (2016). Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. *Journal of Veterinary Science and Technology*, *7*(1), 1-7.
- Bonavita, A., Di Bartolomeo, E., Chevallier, L., D'Ottavi, C., Licoccia, S., & Traversa, E. (2009).
   Tailoring the Structural and Microstructural Properties of Nanosized Tantalum Oxide for
   High Temperature Electrochemical Gas Sensors. *Journal of Nanoscience and Nanotechnology*, 9(7), 4430-4436.
- Brett, C. M., & Oliveira-Brett, A. M. (2011). Electrochemical sensing in solution—origins, applications and future perspectives. *Journal of Solid State Electrochemistry*, 15(7-8), 1487-1494.
- Cháfer-Pericás, C., Maquieira, A., & Puchades, R. (2010). Fast screening methods to detect antibiotic residues in food samples. *TrAC Trends in Analytical Chemistry*, 29(9), 1038-1049.
- Chen, D., Feng, H., & Li, J. (2012). Graphene oxide: preparation, functionalization, and electrochemical applications. *Chemical Reviews*, *112*(11), 6027-6053.
- Delgado, C. L., Rosegrant, M. W., Steinfeld, H., Ehui, S. K., & Courbois, C. B. (1999). The coming livestock revolution. https://www.google.com
- Dowling, A. (2006). Nanoscience and nanotechnologies. Paper presented at the An international symposium on the nature, purposes, ethics and politics of evidence in a democracy. https://www.google.com
- Du, J., Song, Y., Xie, S., Feng, Y., Jiang, J., & Xu, L. (2018). Electrochemical Biosensor Based on Hierarchical Nanoporous Composite Electrode for Detection of Oxytetracycline. *Nanoscience and Nanotechnology Letters*, 10(8), 1095-1100.

- Duncan, T. V. (2011). Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. *Journal of Colloid and Interface Science*, 363(1), 1-24.
- Edwards, E., Brantley, C., & Ruffin, P. B. (2017). Overview of Nanotechnology in Military and Aerospace Applications. In O. T. Mensah, B. Wang, G. Bothun, J. Winter, & V. Davis(Eds), *Nanotechnology Commercialization: Manufacturing Processes and Products* (133-176). https://onlinelibrary.wiley.com
- Faridbod, F., Gupta, V. K., & Zamani, H. A. (2011). Electrochemical sensors and biosensors. International Journal of Electrochemistry, 2011, 1-3.
- Fritz, J. W., & Zuo, Y. (2007). Simultaneous determination of tetracycline, oxytetracycline, and 4-epitetracycline in milk by high-performance liquid chromatography. *Food Chemistry*, 105(3), 1297-1301.
- Furusawa, N. (2003). Isolation of tetracyclines in milk using a solid-phase extracting column and water eluent. *Talanta*, 59(1), 155-159.
- Gaudin, V. (2019). Receptor-based electrochemical biosensors for the detection of contaminants in food products *Electrochemical Biosensors* (pp. 307-365). Elsevier. https://www.google.com
- George, J. M., Antony, A., & Mathew, B. (2018). Metal oxide nanoparticles in electrochemical sensing and biosensing: a review. *Microchimica Acta*, 185(7), 1-26.
- Ghodsi, J., Rafati, A. A., & Shoja, Y. (2016). First report on electrocatalytic oxidation of oxytetracycline by horse radish peroxidase: application in developing a biosensor to oxytetracycline determination. *Sensors and Actuators B: Chemical*, 224, 692-699.
- Gordon, A. T., Lutz, G. E., Boninger, M. L., & Cooper, R. A. (2007). Introduction to nanotechnology: potential applications in physical medicine and rehabilitation. *American Journal of Physical Medicine & Rehabilitation*, 86(3), 225-241.
- He, Q., Liu, J., Liu, X., Li, G., Chen, D., Deng, P., & Liang, J. (2019). A promising sensing platform toward dopamine using MnO<sub>2</sub> nanowires/electro-reduced graphene oxide composites. *Electrochimica Acta*, 296, 683-692.

- Hernández-Santos, D., González-García, M. B., & García, A. C. (2002). Metal-nanoparticles based electroanalysis. *Electroanalysis: An International Journal Devoted to Fundamental* and Practical Aspects of Electroanalysis, 14(18), 1225-1235.
- Hodes, G. (2007). When small is different: some recent advances in concepts and applications of nanoscale phenomena. *Advanced Materials*, *19*(5), 639-655.
- Huang, X., Liu, F., Jiang, P., & Tanaka, T. (2013). Is graphene oxide an insulating material? Paper presented at the 2013 IEEE International Conference on Solid Dielectrics (ICSD). https://www.google.com
- Jalalian, S. H., Karimabadi, N., Ramezani, M., Abnous, K., & Taghdisi, S. M. (2018). Electrochemical and optical aptamer-based sensors for detection of tetracyclines. *Trends* in Food Science & Technology, 73, 45-57.
- Jiang, J., Li, Y., Liu, J., Huang, X., Yuan, C., & Lou, X. W. (2012). Recent advances in metal oxide-based electrode architecture design for electrochemical energy storage. *Advanced Materials*, 24(38), 5166-5180.
- Joshi, A., & Kim, K. H. (2020). Recent advances in nanomaterial-based electrochemical detection of antibiotics: Challenges and future perspectives. *Biosensors and Bioelectronics*, 153, 1-23.
- Kang'ethe, E. K., Aboge, G., Arimi, S., Kanja, L., Omore, A. O., & McDermott, J. J. (2005). Investigation of the risk of consuming marketed milk with antimicrobial residues in Kenya. *Food Control*, 16(4), 349-355.
- Kannan, P. K., Late, D. J., Morgan, H., & Rout, C. S. (2015). Recent developments in 2D layered inorganic nanomaterials for sensing. *Nanoscale*, 7(32), 13293-13312.
- Kebede, G., Zenebe, T., Disassa, H., & Tolosa, T. (2014). Review on detection of antimicrobial residues in raw bulk milk in dairy farms. *African Journal of Basic & Applied Sciences* 6(4), 87-97.
- Keyser, U. F. (2016). Enhancing nanopore sensing with DNA nanotechnology. *Nature Nanotechnology*, *11*(2), 106–108.
- Klabunde, K. J., & Richards, R. M. (2009). *Nanoscale materials in chemistry*: John Wiley & Sons. https://www.google.com

- Korkin, A., Krstić, P. S., & Wells, J. C. (2010). *Nanotechnology for electronics, photonics, and renewable energy*. https://www.google.com
- Korotcenkov, G., Han, S. D., & Stetter, J. R. (2009). Review of electrochemical hydrogen sensors. *Chemical Reviews*, 109(3), 1402-1433.
- Krishna, V. D., Wu, K., Su, D., Cheeran, M. C., Wang, J. P., & Perez, A. (2018). Nanotechnology: Review of concepts and potential application of sensing platforms in food safety. *Food Microbiology*, 75, 47-54.
- Kumar, A., Gupta, K., Dixit, S., Mishra, K., & Srivastava, S. (2019). A review on positive and negative impacts of nanotechnology in agriculture. *International Journal of Environmental Science and Technology*, 16(4), 2175-2184.
- Laviron, E. (1974). Adsorption, autoinhibition and autocatalysis in polarography and in linear potential sweep voltammetry. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 52(3), 355-393.
- Li, Y., Tian, J., Yuan, T., Wang, P., & Lu, J. (2017). A sensitive photoelectrochemical aptasensor for oxytetracycline based on a signal "switch off-on" strategy. *Sensors and Actuators B: Chemical*, 240, 785-792.
- Lian, W., Liu, S., Yu, J., Li, J., Cui, M., Xu, W., & Huang, J. (2013). Determination of oxytetracycline with a gold electrode modified by chitosan-multiwalled carbon nanotube multilayer films and gold nanoparticles. *Analytical Letters*, 46(7), 1117-1131.
- Liu, J., Liu, Z., Barrow, C. J., & Yang, W. (2015). Molecularly engineered graphene surfaces for sensing applications: A review. *Analytica Chimica Acta*, 859, 1-19.
- Magesa, F., Wu, Y., Tian, Y., Vianney, J. M., Buza, J., He, Q., & Tan, Y. (2019). Graphene and graphene like 2D graphitic carbon nitride: Electrochemical detection of food colorants and toxic substances in environment. *Trends in Environmental Analytical Chemistry*, 23, 1-12.
- Manjunatha, S., Biradar, D., & Aladakatti, Y. R. (2016). Nanotechnology and its applications in agriculture. *Journal of Farm Sciences* 29(1), 1-13.
- McFarren, E. F., Lishka, R. J., & Parker, J. H. (1970). Criterion for judging acceptability of analytical methods. *Analytical Chemistry*, 42(3), 358-365.

- Meng, F., Ma, X., Duan, N., Wu, S., Xia, Y., Wang, Z., & Xu, B. (2017). Ultrasensitive SERS aptasensor for the detection of oxytetracycline based on a gold-enhanced nano-assembly. *Talanta*, 165, 412-418.
- Miller, J. (1997). Problems associated with drug residues in beef from feeds and therapy. *Revue Scientifique et Technique (International Office of Epizootics), 16*(2), 694-708.
- Mishra, A. K. (2019). Application of Nanotechnology in Diagnosis, Drug Dissolution, Drug Discovery, and Drug Carrier. https://www.researchgate.net
- NaVrátiloVá, P. (2008). Screening methods used for the detection of veterinary drug residues in raw cow milk. *Czech Journal of Food Sciences*, 26(6), 393-401.
- Navratilova, P., Borkovcova, I., Dračkova, M., Janštova, B., & Vorlova, L. (2009). Occurrence of tetracycline, chlortetracycline, and oxytetracycline residues in raw cow's milk. *Czech Journal of Food Sciences*, 27(5), 379-385.
- Nonga, H., Simon, C., Karimuribo, E., & Mdegela, R. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro municipality, Tanzania. *Zoonoses and Public Health*, 57(5), 339-344.
- Novoselov, K. S., & Geim, A. (2007). The rise of graphene. Nature Materials, 6(3), 183-191.
- Novoselov, K. S., Geim, A. K., Morozov, S. V., Jiang, D., Zhang, Y., Dubonos, S. V., Grigorieva, I. V., &Firsov, A. A. (2004). Electric field effect in atomically thin carbon films. *Science*, *306*(5696), 666-669.
- Pandey, G., & Voskuil, G. (2011). Manual on milk safety, quality and hygiene. *Golden Valley agricultural Research Trust, Zambia*. https://www.google.com
- Parthasarathy, R., Monette, C. E., Bracero, S., & S. Saha, M. (2018). Methods for field measurement of antibiotic concentrations: limitations and outlook. *Microbiology Ecology*, 94(8), 1-10.
- Power, A. C., & Morrin, A. (2013). Electroanalytical sensor technology *Electrochemistry*. https://www.intechopen.com
- Priyanka, P. S., Sheoran, M. S., & Ganguly, S. (2017). Antibiotic residues in milk-a serious public health hazard. *Journal of Environment and Life Sciences*, 2(4), 99-102.

- Pumera, M., Ambrosi, A., Bonanni, A., Chng, E. L. K., & Poh, H. L. (2010). Graphene for electrochemical sensing and biosensing. *Trends in Analytical Chemistry*, 29(9), 954-965.
- Raymond, M., Wohrle, R., & Call, D. (2006). Assessment and promotion of judicious antibiotic use on dairy farms in Washington State. *Journal of Dairy Science*, 89(8), 3228-3240.
- Roy, S., Soin, N., Bajpai, R., Misra, D., McLaughlin, J. A., & Roy, S. S. (2011). Graphene oxide for electrochemical sensing applications. *Journal of Materials Chemistry*, 21(38), 14725-14731.
- Sachi, S., Ferdous, J., Sikder, M. H., & Hussani, S. A. K. (2019). Antibiotic residues in milk: Past, present, and future. *Journal of Advanced Veterinary and Animal Research*, 6(3), 315-332.
- Santonico, M., Pennazza, G., Parente, F. R., Grasso, S., Zompanti, A., Stornelli, V., Ferri, G., Bizzarri, M., & D'Amico, A. (2017). A gas sensor device for oxygen and carbon dioxide detection. Paper presented at the Multidisciplinary Digital Publishing Institute Proceedings. https://www.google.com
- Sawant, A., Sordillo, L., & Jayarao, B. M. (2005). A survey on antibiotic usage in dairy herds in Pennsylvania. *Journal of Dairy Science*, 88(8), 2991-2999.
- Smith, A. T., LaChance, A. M., Zeng, S., Liu, B., & Sun, L. (2019). Synthesis, properties, and applications of graphene oxide/reduced graphene oxide and their nanocomposites. *Nano Materials Science*, 1(1), 31-47.
- Sreethawong, T., Ngamsinlapasathian, S., & Yoshikawa, S. (2013). Facile surfactant-aided sol-gel synthesis of mesoporous-assembled Ta<sub>2</sub>O<sub>5</sub> nanoparticles with enhanced photocatalytic H2 production. *Journal of Molecular Catalysis A: Chemical*, 374, 94-101.
- Stetter, J. R., Penrose, W. R., & Yao, S. (2003). Sensors, chemical sensors, electrochemical sensors, and ECS. *Journal of The Electrochemical Society*, 150(2), S11, 1-7.
- Sui, C., Zhou, Y., Wang, M., Yin, H., Wang, P., & Ai, S. (2018). Aptamer-based photoelectrochemical biosensor for antibiotic detection using ferrocene modified DNA as both aptamer and electron donor. *Sensors and Actuators B: Chemical*, 266, 514-521.

- Sun, J., Gan, T., Meng, W., Shi, Z., Zhang, Z., & Liu, Y. (2015). Determination of oxytetracycline in food using a disposable montmorillonite and acetylene black modified microelectrode. *Analytical Letters*, 48(1), 100-115.
- Sun, J., Gan, T., Zhu, H., Shi, Z., & Liu, Y. (2014). Direct electrochemical sensing for oxytetracycline in food using a zinc cation-exchanged montmorillonite. *Applied Clay Science*, 101, 598-603.
- Tan, B., Zhao, H., Du, L., Gan, X., & Quan, X. (2016). A versatile fluorescent biosensor based on target-responsive graphene oxide hydrogel for antibiotic detection. *Biosensors and Bioelectronics*, 83, 267-273.
- Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, 112(18), 5649-5654.
- VICH, G. (2011). International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products. https://www.google.com
- Wan, X., Yang, S., Cai, Z., He, Q., Ye, Y., Xia, Y., Li, G., & Liu, J. (2019). Facile synthesis of MnO<sub>2</sub> nanoflowers/N-doped reduced graphene oxide composite and its application for simultaneous determination of dopamine and uric acid. *Nanomaterials*, 9(6), 847, 1-16.
- Weaver, L. (1992). Antibiotic residues in milk and meat: Perceptions and realities. Veterinary Medicine (USA). https://www.google.com
- Xie, S., Xu, J., Du, J., Li, N., & Xu, L. (2016). Preparation of Ni–Co Alloy Electrodes by Pulsed Electrodeposition and Its Application in Detection of Oxytetracycline. *Nanoscience and Nanotechnology Letters*, 8(6), 527-531.
- Yalkowsky, H., & Press, Y. H. C. (2003). Handbook of Aqueous Solubility Data. https://www.google.com
- Yan, K., Liu, Y., Yang, Y., & Zhang, J. (2015). A cathodic "signal-off" photoelectrochemical aptasensor for ultrasensitive and selective detection of oxytetracycline. *Analytical Chemistry*, 87(24), 12215-12220.

- Zhao, H., Gao, S., Liu, M., Chang, Y., Fan, X., & Quan, X. (2013). Fluorescent assay for oxytetracycline based on a long-chain aptamer assembled onto reduced graphene oxide. *Microchimica Acta*, 180(9-10), 829-835.
- Zhou, S., Deng, Z., Wu, Z., Xie, M., Tian, Y., Wu, Y., Liu, J., Li. G., & He, Q. (2019). Ta2O5/rGO nanocomposite modified electrodes for detection of tryptophan through electrochemical route. *Nanomaterials*, 9(6), 1-13.
- Zhu, G., Lin, T., Cui, H., Zhao, W., Zhang, H., & Huang, F. (2016). Gray Ta2O5 nanowires with greatly enhanced photocatalytic performance. *Applied Materials & Interfaces*, 8(1), 122-127.
- Zhu, W. X., Yang, J. Z., Wang, Z. X., Wang, C. J., Liu, Y. F., & Zhang, L. (2016). Rapid determination of 88 veterinary drug residues in milk using automated TurborFlow online clean-up mode coupled to liquid chromatography-tandem mass spectrometry. *Talanta*, 148, 401-411.

# **RESEARCH OUTPUTS**

# (i) Publications

- Magesa. F., Wu, Y., Dong, S., Tian, Y., Li, G., Vianney, J. M., Buza, J., Liu, J., & He, Q. (2019). Electrochemical sensing fabricated with Ta<sub>2</sub>O<sub>5</sub>/rGO Nanoparticle-Electrochemically Reduces Graphene Oxide Nanocomposites for the detection of Oxytetracycline. *Biomolecule*, 9, 1-13
- Magesa. F., Wu, Y., Dong, S., Tian, Y., Vianney, J. M., Buza, J., He, Q. & Tan, Y. (2019). Graphene and graphene like 2D graphitic carbon nitride: Electrochemical detection of food colorants and toxic substances in environment. *Trends in Environmental Analytical Chemistry*, 23 (2019), 1-11.

# (ii) Poster Presentations