TULEEN SHEGEM

SYNTHESIS OF SULFAMETHOXAZOLE SURFACE IMPRINTED CORE-SHELL MAGNETIC NANOPARTICLES FOR SELECTIVE DETECTION OF SULFAMETHOXAZOLE IN POULTRY BY AN ELECTROCHEMICAL SENSOR

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NEAR EAST UNIVERSITY INSTITUTE OF GRADUATE STUDIES
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Approval

We certify that we have read the thesis submitted by Tuleen Shegem titled "**SYNTHESIS OF SULFAMETHOXAZOLE SURFACE IMPRINTED CORE-SHELL MAGNETIC NANOPARTICLES FOR SELECTIVE DETECTION OF SULFAMETHOXAZOLE IN POULTRY BY AN ELECTROCHEMICAL SENSOR"** and that in our combined opinion, it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

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Head of the Institute

Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Tuleen Shegem

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Tuleen Shegem

Abstract

Synthesis Of Sulfamethoxazole Surface Imprinted Core-Shell Magnetic Nanoparticles For Selective Detection Of Sulfamethoxazole In Poultry By An Electrochemical Sensor

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Foodborne illness poses a threat to both human and animal health. In recent years, *Campylobacter* and *Salmonella spp* have been found to be the most frequent causes of human foodborne bacterial diseases connected to poultry. To ensure the long-lasting availability of poultry products, Sulfamethoxazole (SMX) is administered into the diets of poultry products as a preventive-treatment technique for future illnesses. Unfortunately, they have been abusively used, and due to the extensive abusive use of antibiotics over many years, dissemination of antibiotic-resistant strains of different bacterial organisms, such as *Carbapenem-Resistant Acinetobacter*, have been introduced to the environment. Sulfamethoxazole was determined using a unique sensitive voltammetric sensor that is presented in this study. As a recognition element, the developed sensor uses a carbon paste electrode sensor modified with Sulfamethoxazole surface imprinted core-shell magnetic nanoparticles. For the electrosensitive determination of SMX, a carbon paste electrode with a non-imprinted polymer (NIP) modification was also created. Electrochemical techniques such as CV, DPV, and EIS were used to evaluate the electrochemical behavior of Sulfamethoxazole at a modified carbon paste electrode. The integration of Fe_3O_4 nanoparticles into the CPE sensor resulted in a sensor with exceptional sensitivity, repeatability, selectivity, reproducibility, stability, and low preparation cost. The Limit of detection LOD and the limit of quantification LOQ are

6.45788 x10^{-8} M and 2.1311 x10^{-8} M, respectively. The concentration of SMX in actual tap water samples was determined using the modified electrochemical sensor.

Keywords*: s*ulfamethoxazole, resistant bacteria, molecularly imprinted polymer, nanotechnology, carbon paste electrode

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List of Abbreviations

AMX:	Amoxicillin
APS:	Ammonium Persulfate
BDD:	Boron Doped Diamond
BDD:	Boron-Doped Diamond
CDL:	Double-Layer Capacitor
CE:	Counter Electrode
CEPH:	Cephalexin
CNPE:	Carbon Nanopowder Paste Electrode
CO:	Cobalt
CV:	Cyclic Voltammetry
DC:	Consistent Potential Detection
DPV:	Differential Pulse Voltammetry
EGDMA:	Ethylene Glycol Dimethacrylate
EIS:	Electrochemical Impedance Spectroscopy
FAO:	Food and Agriculture Organization
FE:	Iron
GCE:	Glassy Carbon Electrode
GPL:	Graphite Pencil Leads
HEMA:	2-Hydroxyethyl Methacrylate
LC-MS:	Liquid Chromatography–Mass Spectrometry
LOD:	Low Detection Limit
M-IONPs:	Magnetic Iron Oxide Nanoparticles
MIP:	Molecularly Imprinted Polymer

MRLS:	Maximum Residue Limits
MWCNT:	Multi-Walled Carbon Nanotube Paste Electrodes
NAT:	Rylamine N-Acetyltransferase
NI:	Nickel
NIP:	Non-Imprinted Polymer
NMR:	Nuclear Magnetic Resonance
PABA:	Para-Aminobenzoic Acid
PBNC:	Prussian Blue Nanocube
PGE:	Pencil Graphite Electrode
PV:	Peroxide Value
PVA:	Poly Vinyl Alcohol
PY:	Pyrrole
RCT:	Transfer Resistance
RE:	Reference Electrode
RP:	Polarization Resistance
RS:	Solution Resistance
SAs:	Sulfonamides
SbNPs:	Antimony Nanoparticles
SDS:	Sodium Dodecyl Sulfate
SEM:	Scanning Electron Microscope
SMX:	Sulfamethoxazole
SPCE:	Screen Printed Carbon Electrode
SPP:	Plural For Species
SWV:	Squarewave Voltammetry
WE:	Working Electrode

CHAPTER I

Introduction

Poultry-derived products are the most widely consumed in the meat industry; more than 337.3 million tons of chicken meat was consumed in 2020 due to its availability and low price, as the food and agriculture organization (FAO) declared. Despite the significant improvements in hygienic practices and technology at all poultry production stages in developed countries, accompanied by advanced public sanitation improvement, foodborne diseases remain a persistent threat to human and animal health. Thus, foodborne diseases are still a significant concern in those countries. In developing countries, the need to produce sufficient food to meet population increases, accompanied by bad economic situations, often overshadows the need to ensure safe food products (Hafez & El-Adawy, 2019).

Campylobacter and *Salmonella spp* are the most frequent causes of human foodborne bacterial infections associated to poultry, according to numerous reports, therefore, to ensure long-lasting availability for poultry products, more than 60% of all different drug types such as antibiotics (i.e., Sulfonamides and Fluoroquinolones) such as (Sulfamethoxazole (SMX), Amoxicillin (AMX), Cephalexin (CEPH)), antiparasitic, antifungals, hormones, and growth promoters as mentioned by the food and agriculture organization (FAO, 2017) are administered into the diets of chickens and other varieties of poultry products as a preventive-treatment technique for future diseases, although those drugs have many positive outcomes, they have been abusively used since the early 1940s by farmers, as this method proved and still proves to be highly profitable since this reduces the mortality rate amongst farm animals (Hafez & El-Adawy, 2019).

However, due to the extensive, abusive use of antibiotics over many years, dissemination of antibiotic-resistant strains of different bacterial organisms, such as *Carbapenem-Resistant Acinetobacter*, *Pseudomonas Aeruginosa*, *Staphylococcus aureus*, and *Vancomycin-Resistant Enterococcus species*, have been introduced to the environment. In addition, because they were capable of surviving and even grow in

the presence of medicines, rare antibiotics used to combat resistant bacteria are expensive.

Patients with resistant infections stay in hospitals on average twice as long as those who are not, also incurring further loss in terms of lost workdays. Subsequently, more extended follow-up periods are required to treat patients, and they are possible to remain out of work for some time, impacting familial finances. Multi-resistant organisms (MRO) are organisms that are resistant to a variety of antibiotics. However, the most serious concern about antibiotic resistance is that some microorganisms have developed resistance to practically all antibiotics now available. These bacteria can cause serious sickness and can be spread from person to person in society, and it is getting more frequent, making it a major public health issue.

Because of their low cost and broad spectrum antibacterial activity, sulfonamides, often referred as sulfa medications, are widely used in veterinary and human medicine to treat infections caused by bacteria. They are given to food-producing animals as feed and water additives or intrauterine infusions. Due to the relevance of these medications, there has been a gradual increase in interest in investigating these substances and determining their concentrations in commercial formulations using analytical methods (Meshki, et al., 2014).

Sulfonamides such as Sulfamethoxazole (SMX) and other antibiotics such as Amoxicillin (AMX) and Cephalexin (CEPH) are used to treat many different diseases; SMX treats uncomplicated, acute urinary tract infections. The action of AMX against penicillin-sensitive Gram-positive pathogens, as well as some Gram-negative bacteria, is used to treat some bacterial illnesses in poultry. Furthermore, *alpha- and beta-hemolytic streptococci*, several *Staphylococci species*, and *Clostridia* organisms are all part of the gram-positive spectrum of action. Amoxicillin is also efficient against *E. coli*, a variety of *Salmonella species*, and *Pasteurella multocida*.. However, for the CEPH, it is a first-generation Cephalosporin antibiotic. It is given mainly for abstruse skin infections, such as bumble-foot. It is effective against acute bacterial infections, primarily those that are associated with Gram-positive organisms.

Sulfonamides treat opportunistic infections in transplantation and for AIDS-related complications of hematological abnormalities such as Thrombocytopenia, Megaloblastosis, Agranulocytosis, Sulfhemoglobinemia, and Eosinophilia uncomplicated, acute urinary tract infections; They are well absorbed and eliminated in the urine when administered orally (Cliquet, et al., 2003). They get attached significantly to plasma proteins. Sulfonamides are widely disseminated throughout bodily tissues and fluids after they are ingested, as well as the central nervous system (Cliquet, et al., 2003).Sulfonamides are metabolized in a variety of ways, the most common of which being acetylation of the para-amino moiety. The acetylated metabolite is less toxic and soluble than the parent medication, yet it has antibacterial activity. Both the parent compound and metabolites are excreted largely through the kidney, with renal function having a significant impact on excretion. Sulfonamides are expelled in small quantities in stools, bile, breast milk, and other bodily fluids. (Enna & Bylund, 2007). The majority of sulfonamides, particularly the older agents, are insoluble in the acid urine, precipitating in the urinary tract. However, sulfonamides consist of many types, such as Sulfamethoxazole(SMX), Sulfasalazine, Sulfisoxazole, and many more. They have a p-amino benzoyl ring moiety with an aromatic amino group at the N4 position, but the N1 position is substituted differently. (Zhang & Wang, 2009).

Sulfonamides(SAs) are among the most popular antimicrobial agents due to their broad spectrum against Gram-negative and Gram-positive microorganisms, and they get effective against P*rotozoa*. However, while they were initially broad-spectrum agents, a significant amount of resistance has developed to them during the past 60 years of use. Furthermore, correct withdrawal times are not observed before slaughtering or milking treated animals; hence, food products generated from these animals, such as milk and meat, may be tainted with leftover sulfonamides (Franco, et al., 1990). As evidenced by the fact that nearly 5% of patients who received sulfonamides therapy undergone undesirable side effects such as hypersensitivity reactions and alterations of the hematopoietic system, kidney damage due to sulfonamides drug crystals accumulation in the kidney, and renal damage due to sulfonamides drug crystals deposition in the kidney, these traces may cause negative effects in some humans (Enna & Bylund, 2007).

Cliquet et al. (2003) At least nine sulfonamides are allowed in veterinary medicine in Europe, the United States, Canada, and the Netherlands, according to the report. Maximum residual limits (MRLs) have been set by law in many countries to protect users from the hazards associated with drug residues. The MRL for the total amount of sulfonamides in consumable tissues is 100 gkg^{1} according to EU rules (1999) and the Canadian Food and Drug Law (1991), but the MRL in Japan is 20 gkg^{1} (Haasnoot, et al., 2000).

Numerous analytical methods have been reported to determine SMX in biological or pharmaceutical formulations samples during past experiments. These include the Spectrophotometric technique, Bratton–Marshall technique, Titrimetric assay technique, low injection Spectrophotometric method, are all examples of chromatographic methods. However, the majority of the published procedures have numerous drawbacks, such as being time-consuming, costly, and necessitating sophisticated sample preparation (Lahcen, et al., 2016). In comparison to other analytical methods, electroanalytical methods offer numerous advantages for the detection of sulfonamides, including ease of use, the lack of sample preparation, low-cost apparatus, low detection limits, high dynamic range, sensitivity, and strong selectivity (Issac, et al., 2009).

The modified electrochemical sensors with molecularly imprinted polymer-decorated magnetite nanoparticles have gained much attention. They are one of the sensor types that have distinct properties as analytical tools when compared to other electrodes. They also have a number of benefits, including the ability to identify and attach specific target molecules through noncovalent strong interactions between the host material and the guest molecule (Galvez, et al., 2016), their ease of preparation, chemical stability, cost-effectiveness, renewability, resilience, low ohmic resistance, steady response, suitable for diverse sensing, and large detecting applications (Lahcen, et al., 2016).

Nanotechnology, which only started a few decades ago, has become a very active research area due to its impressive physicochemical characteristics and properties, making it possible to develop highly sensitive biosensors. Therefore,

when the nanoparticles' size decreases, the increasing volume percentage of surface atoms within the total particle affects the surface. In addition, due to the imperfect coordination sphere, the regularity of the chemical surroundings of magnetic metal cations near the surface is diminished. As a result, the magnetic structure of the upper layers differs greatly from that of the nanoparticle body, and magnetic interactions in the surface layer may have a major impact on the magnetic characteristics of nanoparticles. (Vestal, et al., 2003). For example, magnetite iron oxide nanoparticles possess exceptional electrical and catalytic characteristics, many active adsorption sites, and a large surface-to-volume ratio, making them mainly appropriate for analytical determinations (Pena-Pereira, et al., 2012). Furthermore, the magnetite iron oxide nanoparticles are promising supercapacitor cathode tools due to their high abundance, inexpensive, easy synthesis, phenomenal electrochemical execution, and biocompatibility (Mao, et al., 2014).

Statement of the Problem

Antibiotics, such as Sulfamethoxazole(SMX), Amoxicillin(AMX), Cephalexin(CEPH), are being widely used in the poultry industry. This method has become economically favorable amongst farmers and factories, as it decreases the chicken mortality rate. Consequently, bacteria in the poultry-derived products have been developed and become resistant among the bacterial strains, Antibiotic-resistant medicine is ineffective as a result of this (Yu, et al., 2011). Furthermore, overuses for the antimicrobial agents in veterinary medicine or feed additives might cause potentially severe food safety effects and affect veterinary and human health (Piddock, et al., 2000). For example, Sulfonamide residues would be left inside slaughtered animals if the animals were treated incorrectly with sulfonamides. (Lu, et al., 2007), and the residual amounts of sulfonamide in specific concentrations may cause cancer, diabolism, dermatitis, severe allergic reactions, and disorders in the central nervous system (Nesterenko, et al., 2009). Therefore, biosensor use has become crucial to stop the abusive use of antibiotics in the poultry industry to stop the spread of resistant bacteria.

Purpose of the Study

The focus of this research was to see if the modified CPE could be used as an electrochemical sensor to detect Sulfamethoxazole (SMX) in water.

Research Question/Hypothesis

Could the CPE with imprinted core-shell magnetic nanoparticles be used as a practical electrochemical sensor for voltammetric detection of Sulfamethoxazole in water?

Significance of the Study

Various methods for determining SMX in complicated materials have been proposed, including Chromatography with various detectors, Spectrophotometry, Potentiometric, Photometric, and Titrimetric assay. However, the previously mentioned strategies have several difficulties: knowing how the instrument operates, the high instrumental cost, and the time consumed while preparing the experiment. Among these methods, the study's significance lies in applying the electrochemical sensor because it is a simple, quick, economical, highly sensitive, and environmentally friendly analytical approach. Furthermore, due to their strong catalytic efficiency, several functional materials such as graphene, carbon nanotubes, MIPs, and iron oxide can boost the sensor's sensitivity.

Limitations

Electrochemical biosensors are commonly used due to their rapid response, high sensitivity, and selectivity, but limitations are found in every experiment. In this experiment, the limitations may include that internal temperature compensation is used in biosensors.

As a result, it's preferable to keep the sample temperature as constant as feasible. Biosensors could also have a short or limited shelf life. For example, depending on the material to be identified and the conditions in which it is used, an electrochemical sensor has a life span of 6 months to one year.

Moreover, Sulfamethoxazole and Trimethoprim always come in pairs; this study examines only Sulfamethoxazole and because most articles address the determination of both, there is not enough research that talks about Sulfamethoxazole alone.

Definition of Terms

Biosensor: It is a device that detects chemical compounds using specialized biochemical reactions mediated by tissues, isolated enzymes, immunosystems, organelles, or whole cells, usually via electrical, thermal, or optical signals.

Carbon Paste Electrode: A particular electrochemical sensor used mainly for voltammetric measurements; it is simple to make and offers an easily renewable electron exchange surface. A mixture makes the electrode of conducting carbon powder and a binder.

Cyclic Voltammetry: It's a type of electrochemical potentiodynamic analysis. The electrochemical features of a molecule adsorbed onto to the electrode or of an analyte in a solution are studied using cyclic voltammetry.

Deferential Pulse Voltammetry: With a sequence of regular voltage pulses superimposed on the potential linear sweep or stairsteps, this voltammetry method is used to make electrochemical measurements and is a derivative of linear sweep voltammetry or staircase voltammetry.

Electrochemical Impedance Spectroscopy: A technique used to study kinetic behavior of a battery system near the electrode's surface.

Electrochemical: The study of chemical processes that cause electrons to flow is known as chemistry. This flow of electrons is known as electricity, and it is created when electrons travel from one element to another in an oxidation-reduction reaction.

Fe_3O_4 Nanoparticles: At room temperature, they are colloidal iron oxide (Fe_3O_4) compounds with superparamagnetic characteristics. Magnetic nanotechnology and

nanotoxicology research and development employ this material. The small, non-toxicity, and superparamagnetic properties of magnetite nanoparticles makes them appealing for use in a variety of domains, such biosensors, catalysis, Magnetic fluids, magnetic isolation, and MRI contrast agents.

Molecularly Imprinted Polymer: Using imprinting technology, a may replicate natural molecular receptors (e.g., antibodies) and specifically detect targets.

Nanotechnology: The technology part deals with dimensions of less than 100 nanometers, especially manipulating individual atoms and molecules.

Resistant Bacteria: When microorganisms (such as bacteria, fungi, viruses, and parasites) are subjected to antimicrobial medications, they alter and become less efficient for (antibiotics, antifungals, antivirals, antimalarials, and anthelmintics).

Sulfamethoxazole: Is an antibacterial sulfonamide that stops bacteria from producing dihydrofolic acid, a chemical that they need to survive.

CHAPTER II

Theoretical Framework

Sulfonamides

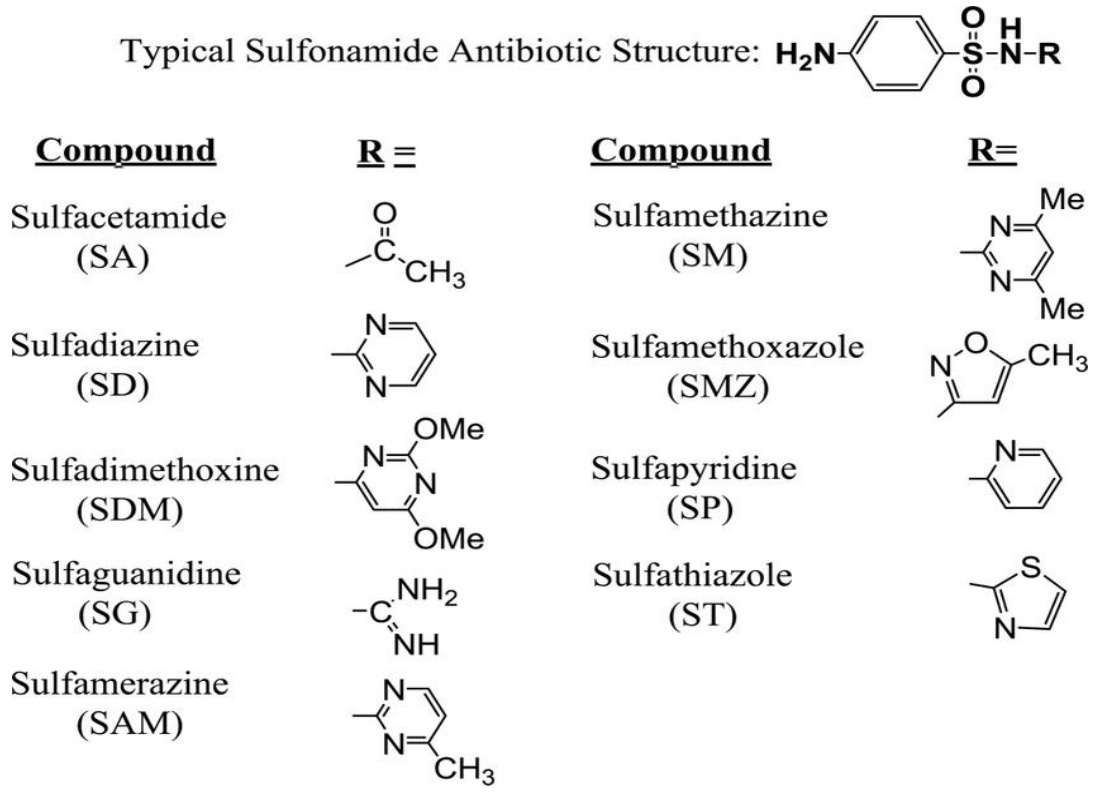
Synthetic antimicrobial compounds that are structural analogs of para-aminobenzoic acid are known as sulfonamides (PABA). They are used as a preventative agent in treatment for a variety of disorders, as indicated in Figure 1. They are well-absorbed orally and eliminated primarily in the urine. Sulfa medicines include Sulfisoxazole, Trimethoprim and Sulfamethoxazole, Sulfadiazine and Sulfasalazine; their functional group is in clinical use, such as antifungal, antibacterial, antiprotozoal, and anti-inflammatory due to the presence of the SO_2NH^- group (Rehman, et al., 2017). More recent use of sulfonamides is an antiviral HIV, anticancer agent, and Alzheimer's disease. (Rehman, et al., 2017) In addition, they are used effectively to treat ulcerative colitis, urinary and intestinal infections. They have a promising future in veterinary and agricultural sciences, in addition to their critical function in human medicine. Tetrahydrofolate is required for the synthesis of bacterial DNA and RNA, which is hindered by sulfonamides, hence new DNA and RNA creation decreased due to a lack of tetrahydrofolate, causing bacteria to decay (Rehman, et al., 2017). Despite the fact that they were originally broad-spectrum drugs, they have developed serious resistance during the last 60 years of use (Rehman, et al., 2017). As a result, sulfonamides are now routinely used as monotherapy in conjunction with other antimicrobial medicines.

However, sulfonamides have been linked to a variety of side effects, including renal damage from drug crystals forming in the kidney, allergic responses, and changes to the hematological system. Furthermore, newer sulfonamides and their variants have attracted a lot of attention in the pharmaceutical industry as a way to combat life-threatening challenges produced by drug-resistant bacteria, such as Methicillin resistance, because they have a remarkable ability to acclimate to antibiotic stress (Rehman, et al., 2017). When disease-causing organisms are treated therapeutically with common antibiotic medication molecules, other species emerge as a result of permutation, coupling, transmission, or conversion. As a result, researchers are paying more attention to the synthesis of novel sulfonamides and

their variants for use in health research and pharmaceutical applications (Rehman, et al., 2017).

Figure 1

The Molecular Structure of Sulfonamides (Balakrishnan, et al., 2014).



Sulfamethoxazole

Sulfamethoxazole (SMX) is a kind of sulfanilamide that is N1-(5 methyl 3 isoxazolyl) sulfanilamide with the chemical formula $C_{10}H_{11}N_3O_3S$. It has a white-yellowish hue, is tasteless, and odorless. In the previous few decades, the chemical structure of it is seen in Figure 2 with the structure of Amoxicillin and Cephalexin. (SMX, AMX, and CEPH), respectively. Furthermore, throughout an electrochemical oxidation and reduction, the various ionization forms of SMX are seen in Figure 2, 4 (Hai, et al., 2017). Sulfonamides, on the other hand, have been gradually categorized based on their symptoms and duration of action in the body (short ,intermediate, or long-acting). Water versus lipid solubility or impact duration is the most well-known categorization (Boothe, 2015). It is, nevertheless, a bacteriostatic antibiotic that is

widely used in human medicine to treat bronchitis and urinary bladder infections, as well as AIDS-related problems, and in veterinary medicine for treating, prevent infections and stimulate growth in food-producing animals (Arvand & Alirezanejad, 2012).

Furthermore, because of its structural similarities to an endogenous PABA substrate, It inhibits di-hydropteroate-synthase, an essential enzyme for bacterial conversion from dihydrofolic acid to PABA (Zhou, et al., 2009). As a result, most bacteria synthesize folic acid from para-aminobenzoic acid (PABA) rather than Animalia, which requires foreign folic acid supplies. Inhibition of this process also hinders the production of tetrahydrofolate, which in turn prevents the creation of bacterial purines and DNA (Yun, et al., 2012).

Furthermore, it is frequently used with Trimethoprim, which inhibits bacterial folic acid production in a sequential manner (Bushby & Bushby, 1976). As a result, these medicines function together to prevent two processes in the manufacture of nucleic acids and proteins, both of which are required for bacterial growth and division, and their use together helps to inhibit the growth of bacterial resistance. SMX is used in this combination to treat bacterial infections of the urinary, respiratory, and gastrointestinal systems (Yun, et al., 2012). Its metabolism is predominantly handled by arylamine N-acetyltransferase (NAT) enzymes, which are essential for SMX acetylation at the N4 site (Khalil, et al., 2003).

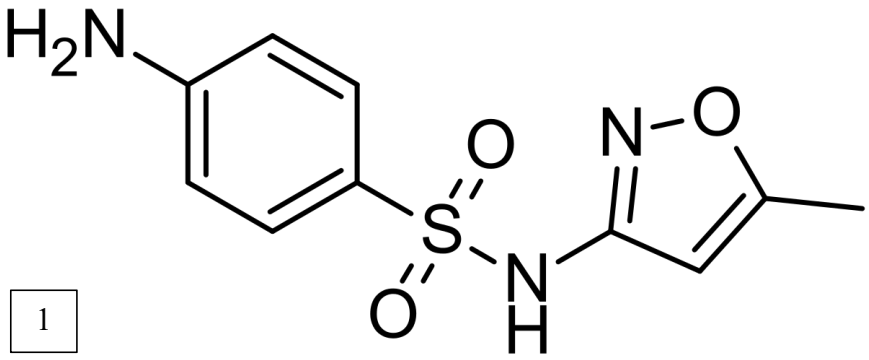
SMX is eliminated in human milk after spreading through most bodily tissues including sputum, vaginal secretions, inner ear fluid, and placental barrier. Furthermore, about 70% of the medication is linked to plasma proteins, maximum blood levels for individual components occur from 1 to 4 hrs after oral administration, and SMX has a mean serum half-life of 10 hrs (Khalil, et al., 2003). The kidneys' glomerular filtration and tubular secretion are the primary routes of excretion; urine concentrations are often greater than serum concentration. Within 72 h, over 85% of a single oral dosage of SMX is recovered in the urine, with 30% being loose SMX and the rest being the N4-acetylated metabolite (Zhou, et al., 2009). Constipation, nausea, puking, starvation, headaches, drowsiness, sleepiness, and unconsciousness are indications or indications of sulfonamide overdose or allergic responses.

Treatment may increase the chance of folate deficit, thus it should be taken with caution in individuals who are at a higher risk of acquiring one (Khalil, et al., 2003). In addition, hemolysis has been seen in individuals using Sulfamethoxazole/Trimethoprim who had glucose-6-phosphate dehydrogenase impairment.

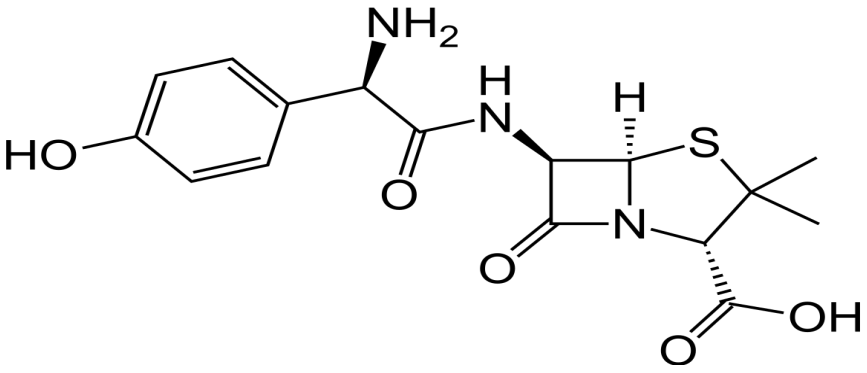
Furthermore, in overdose situations, therapy should be symptomatic and supportive, and may involve forced emesis or stomach lavage if appropriate (Yun, et al., 2012). Additionally, the patient should be monitored through test work for signs of electrolyte imbalances or blood dyscrasias. Bacterial resistance develops more slowly with the combination of the two medications than with either Sulfamethoxazole or Trimethoprim alone, according to studies, but they limit successive stages in the bacterial folate production pathway when used jointly (Bushby & Bushby, 1976).

Figure 2

Chemical Structure of 1)Sulfamethoxazole, 2)Amoxicillin, 3)Cephalexin 4)SMX in various ionization forms (Hai, et al., 2020).



1



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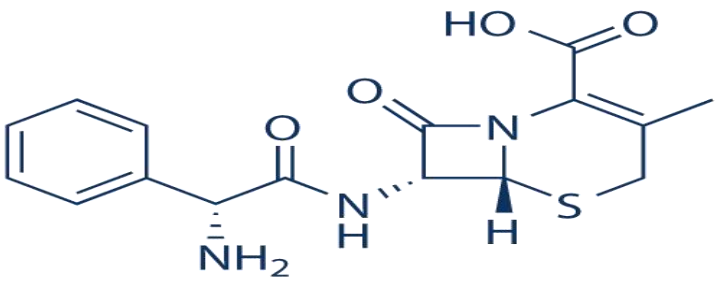

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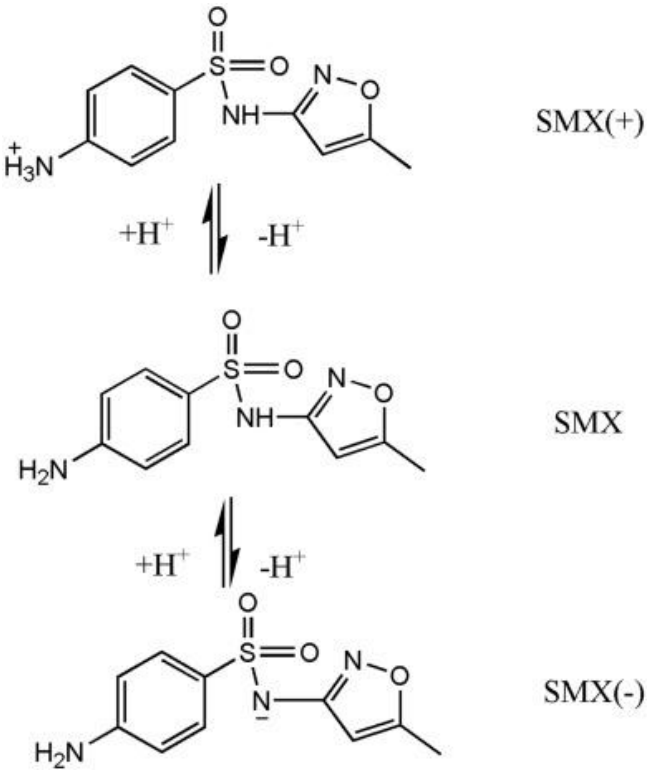


4

Electrochemical Measurement

In recent years, a lot of work has gone into inventing simple, quick, and cost-effective approaches. With their great simplicity, sensitivity, low cost, and compactness, electrochemical methods are on the rise. To detect particular and label-free sulfonamide, Galvez et al. (2016) employed a material built on (MIP) modified nanoparticles of magnetite. Cesarino et al. (2013) also created an antimony nanoparticles (SbNPs)-modified multi-walled carbon nanotubes (MWCNT) to detect SMX. Fifield emission gun SEM and cyclic voltammetry were applied to the MWCNT/SbNPs nano composite morphology of as well as the the composite electrode's electrochemical performance. Arvand et al. (2010) were the first to use MWCNT paste electrodes to explain electrooxidation and SMX measurement. Classic cyclic voltammograms were produced using a three-electrode cell with a functionally adjusted electrode carbon paste, with a saturated reference electrode (Ag/AgCl), and a counter electrode (platinum wire). Although SMX produced an anodic current for both electrodes, there was no discernible reduction output on reverse scan, indicating that the drug has been permanently oxidized at the electrodes.

Cyclic Voltammetry

The potential ramps linearly with time in cyclic voltammetry, it is also known as a scan-rate. It is a powerful and effective approach for studying the reduction and oxidation reactions of molecular species; it is also very simple and inexpensive.

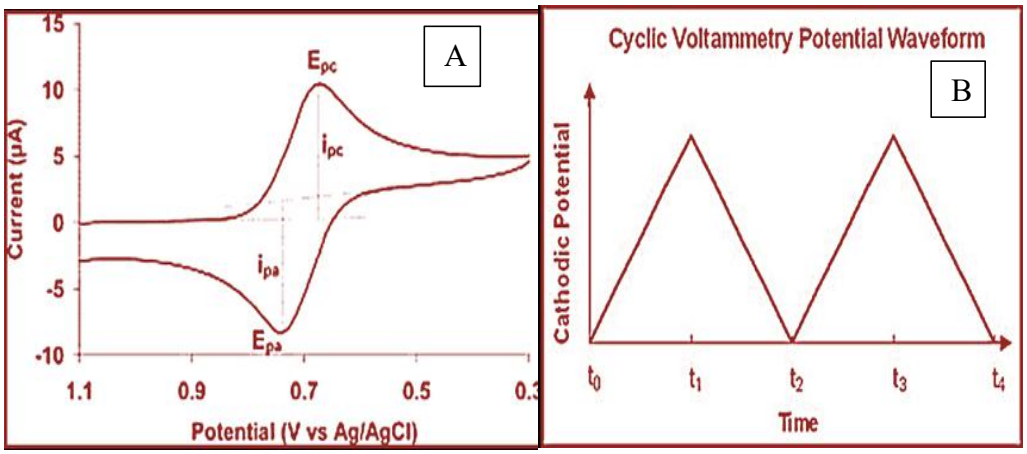
Moreover, it is Also useful for researching electron transfer-initiated chemical processes, as well as catalysts for obtaining qualitative data on electrochemical reactions (Elgrishi, et al., 2018). The findings of CV can quickly offer critical info about the redox thermodynamics reactions, the kinetics of electron transport events, as well as the chemical changes or adsorption processes that are associated with them (Elgrishi, et al., 2018). It involves screening the potential of a stationary working electrode linearly using a triangle potential waveform; single or several cycles can be utilized depending on the information being explored. The potentiostat monitors the current generated by the applied potential during the potential sweep. A CV is the result of plotting current vs. potential. Electrochemical properties is frequently the first experiment carried out in an electrochemical investigation since it allows for a

quick determination of the electroactive species' redox potentials and a quick assessment of the influence of media on the redox process.

The working electrodes' potential ramp is inverted when the potential reaches a preset value. During a single experiment, this inversion might occur multiple times. The inserted potential is triangular in form, with two parts: anodic and cathodic, or, more accurately, forward scan and inversion. The likelihood of re-oxidation or re-reduction of the product generated by the forward scan is a critical aspect in CV. The usual voltammogram is seen in Figure 3.

Figure 3

A)Typical Cyclic Voltammogram, B)The Cyclic Voltammetry Potential Waveform. (Eliska, 2014).



Note. (Ip a,c - Anodic and Cathodic Peaks, Reversible Reaction)

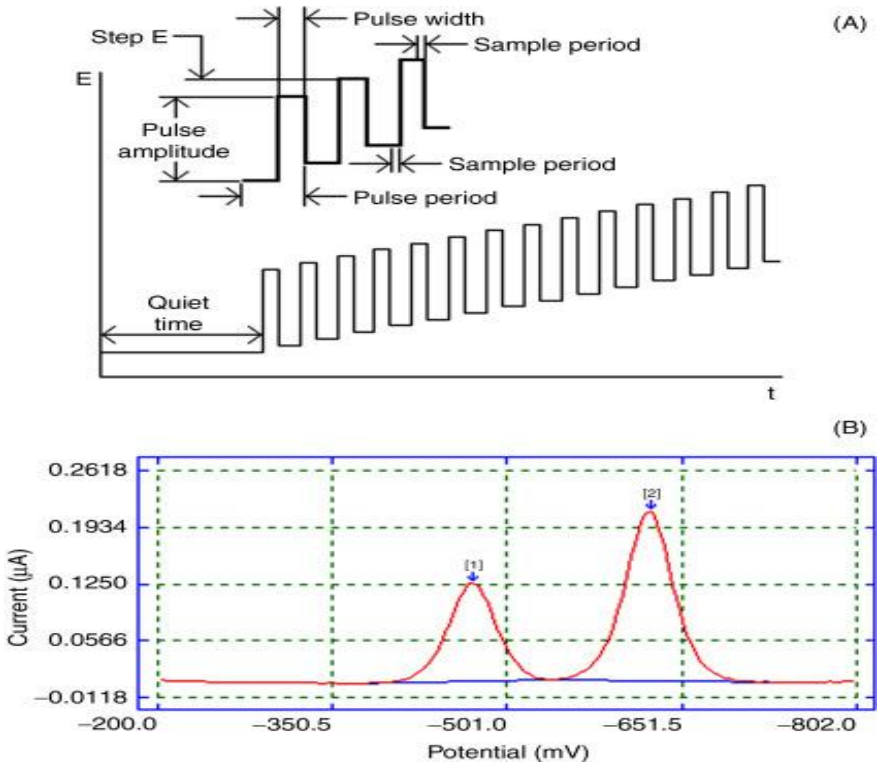
Differential Pulse Voltammetry

With regular voltage pulses superimposed on the potential linear stair-steps or sweep, the voltammetry method yields electrochemical measures like staircase voltammetry or derivative of linear sweep voltammetry.The DPV technique is extremely effective for determining trace quantities of organic and inorganic compounds (Kurbanoglu, et al., 2017). A DPV is one the best voltammetric methods in terms of sensitivity, owing to the charging current being strongly discriminated against concerning the faradaic current, and the percentage of faradaic to charging current is high. The current is sampled twice after they are applied to the working

electrode. One is just before the pulse is delivered, and the other is later in the pulsing life, after the charging current has decreased. Using an instrument, the first current is subtracted from the second current. The applied potential is displayed against the current differential [i=i(t2)–i(t1)]. The present peaks on the DPV appear to be proportionate to the concentrations of the analytes involved. Small pulses with constant amplitude in the scope (10-100 mV) are overlaid on a changing base potential in DPV (Kurbanoglu, et al., 2017). Figure 4 shows that the current estimates of two focuses for each of the pulses, one is when the potential is at the start and the other at the conclusion of the applied pulse.

Figure 4

A)Differential Pulse Voltammetry Potential Wave Form B)A Differential Pulse Voltammogram of a Typical Differential Pulse Voltammogram.(Kurbanoglu, et al., 2017).



Electrochemical Impedance Spectroscopy(EIS)

The EIS is an electrochemical method that analyzes the impedance of a system's independence from the frequency of the AC potentials. It's employed in a variety of industries, including electrocatalysis, energy, and medicine. This technique is a valuable tool for analyzing surface working conductors' interfacial characteristics, which gives critical data concerning the impedance change in the electrode's working surface. In addition EIS is very surface sensitive, which makes many changes visible that other techniques do not see.

However, electrochemical impedance spectroscopy uses the frequency scan of alternating current and voltage modulated over direct current bias to elicitation the equivalent circuit. When a potential is performed, the response to this potentially generates an alternating current, it may be thought of the sum of sinusoidal functions. Setting the input voltage and monitoring the induced current can be used to determine impedance. (Zhang, et al., 2017).

Similar resistance is formed by a single kinetically regulated electrochemical reaction. We don't have a mixed potential in the case below, but rather a single response at balance.

Take the case of a metal in interaction with an electrolyte. The metal can decompose electrolytically in the electrolyte, and this technique always includes a non-Faradaic component, as shown in equation (1) below:

$$\mathbf{Red \Leftrightarrow Ox + ne^-} \qquad \mathbf{(1)}$$

The oxidant is O, the number of electrons transferred is n, and the reduce portion is R. There are portions of the charge motion that are Faradaic and sections that are not Faradaic (Chang & Park, 2010). Electrons enter the metal in the fourth reaction of the first equation, and metal ions spread into the electrolyte, transferring a charge. This charge transfer reaction moves at a set rate. Temperature, concentration in the reaction products, kind of reaction, and voltage all influence reaction speed.

The charge-transfer resistance statement becomes equation when the overpotential, η, is exceedingly small and the electrochemical system is in equilibrium (2):

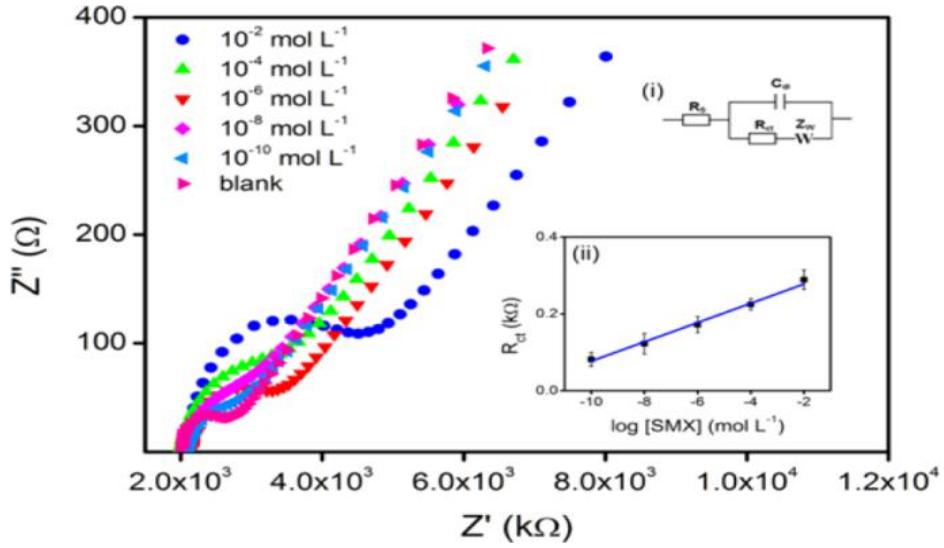
$$\mathbf{R\,c_t} = \frac{RT}{nFi} \qquad \textbf{(2)}$$

When R_{ct} is known, the exchange current density may be determined using equation (2).

In summary, there are two methods for measuring EIS: Non-Faradaic and Faradaic approach. Faradaic EIS are often performed with a reversible redox probe that allows electrons to travel via a reaction over the interface that overcomes a suitable activation obstacle, such as polarization resistance (R_p) and uncompensated solution resistance (R_s) (Chang & Park, 2010). Non-Faradaic impedance measurements, on the other hand, are performed without the use of a redox probe. The results begin with the charging of the double-layer capacitor (CDL). The mass transit of products and reactants is critical in measuring the speed of electrons moving at the interface, which is dependent on the generation of reducing agents and the consumption of oxidants near to the electrode region (Galvez, et al., 2016). The recognition events involved in MIP have been studied using both Faradaic and non-Faradaic impedance spectroscopy. In particular, enhanced sensitivity for Faradaic readings has been recorded (Galvez, et al., 2016). The Nyquist plot shows conventional impedance data, with semicircular portion at high frequency, representing the interfacial charge-transfer process and a linear section at low frequency indicating the rate of diffusion. The charge transfer resistance (R_{ct}), which reflects the transfer of the redox probe's electron kinetics across the surface of the electrode, corresponds to the semicircle width in the Nyquist plot, as shown in Figure5.

Figure 5

In the presence of Various SMX Concentrations, Nyquist Plots EIS of Fe_3O_4 MNP-SPCE MIP- Modified Electrode.(GaLvez, et al., 2016).



*Note. (*i) The Impedance Information Randles Equivalent Circuit Model. (ii) R_{ct} data were plotted against the log for SMX concentrations to determine curve calibration..

Molecularly Imprinted Polymer

"A polymer that has been processed with molecular imprinting technologies, which involves co-polymerizing cross-linkers and active monomers in the presence of a template molecule." According to Arvand and Alirezanejad (2011). MIPs were utilized as selective sorbent materials in sample pretreatment techniques for sulfonamide analysis, such as the analysis of (SMX).When biorecognition components that target tiny molecules, for example, are technically challenging to make. In terms of structure, size, and functionalities, MIPs are complementary to the target analyte, which is used as a template molecule, as shown in Figure 6. They may selectively detect and bind particular target molecules as a consequence of noncovalent interactions between the host material and the guest molecules, such as h - bonding, electrostatic properties, Van der Waals forces, hydrophobic phenomena, and metal-ion coordination. (Galvez, et al., 2016). MIPs are made in a three-step process: initially, monomers are blended with the target analyte, then polymerization begins, and eventually, analytes are eliminated from polymeric matrices using

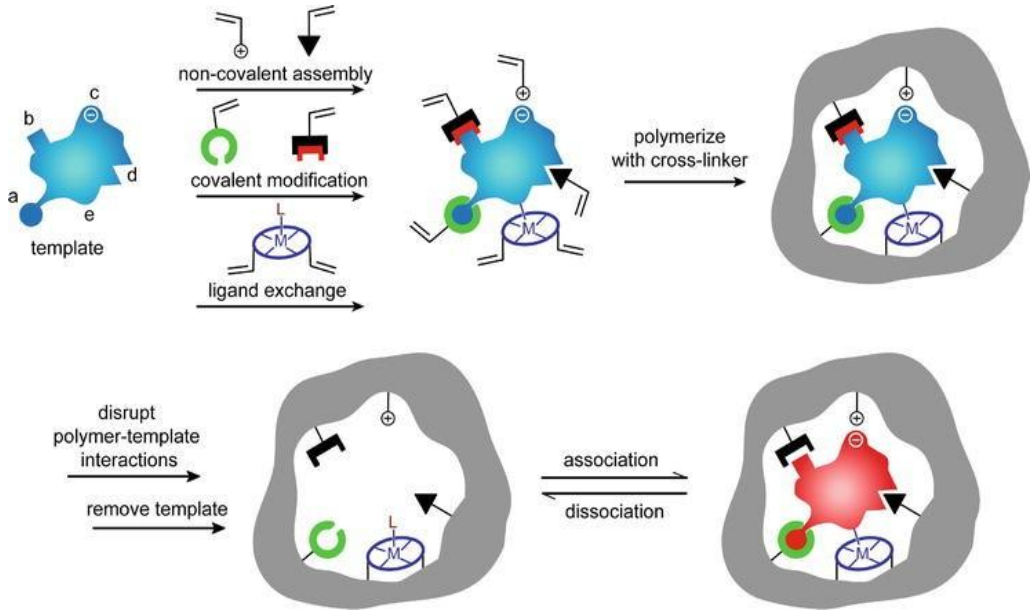
appropriate washing procedures, leaving complementary fillings inside the polymer. The remaining cavities, which are the same size, form, and matrix as the template, allow selective rebinding of the template molecule (Turco, et al., 2017).

MIPs are a viable alternative to natural antibodies, and they are discovering applications in all techniques in which specific interactions are required, such as solid-phase extraction and/or sensor, due to the many advantages of their easy preparation, low cost, adaptability, and stability high endurance levels that MIPs offer. MIPs act as recognition elements in sensors and must be integrated on transducer surfaces to achieve satisfactory sensor performance. They have obtained such widespread acceptance in the scientific community that they have begun to be used in a variety of fields. Biosensing innovation is one of the sectors that is utilizing MIP, for example, the biomimetic detection element.(Arvand & Alirezanejad, 2011). The researchers present a composite material made up of MIP and Fe_3O_4 nanoparticles that are superparamagnetic and can detect (SMX) with extreme sensitivity.

The analyte is preconcentrated, segregated, and manipulated using the composite's superparamagnetic properties, which are gathered selectively onto the composite's surfaces by the MIP. Detachable screens printed electrodes, on the other hand, have been used to track the overall resistance of materials, which has been determined by the amount of analyte collected utilizing electrochemical biosensor analysis (Galvez, et al., 2016).

Figure 6

Synthesizing of Molecularly Imprinted Polymer.(Piletsky, et al., 2013).



Fe_3O_4 Superparamagnetic Nanoparticles (MNPs)

Nanotechnology has gotten a lot of press because of its potential medicinal applications. Nanotechnology is defined by Jeevanandam et al. (2018) "Understanding, fabrication, and control of matter at the nanometer dimensions (nanoparticles) in order to produce technologies, novel nanomaterials, and structures" and Nanoparticles are identified by Jeevanandam et al. (2018) as "particles comprised of three types of layers: the surface layer, the shell layer, and the core." With three dimensions of less than 100 nm for each particle." Metals, metal oxide, carbon - containing nanomaterials, nanowires, nanorods, and magnetic nanoparticles are some of the nanomaterials found in nanoparticles. Furthermore, those nanomaterials may be classified depending on their nanostructure dimensions; for example, nanofibers, nanowires, nanorods, and nanotubes are all 1D (second dimension) nanomaterials. Owing to its better nature above sheer-sized particles, bigger surface-to-volume ratio, strong magnetic features, high activity, and innovative optical qualities, nanotechnology, which just began a few decades ago,

has become a very active study topic for highly sensitive biosensors (Ganapathe, et al., 2020).

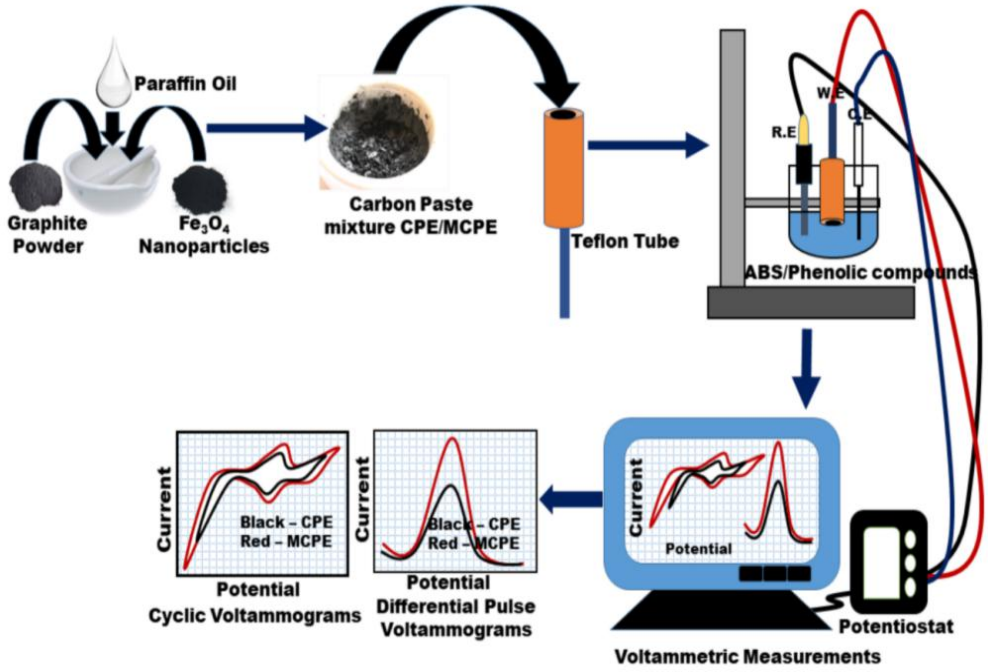
According to Wu et al. (2016), nanotechnology has also been utilized to detect antibiotic residues in chicken products. (Fe_3O_4), (-Fe_2O_3), and (-Fe_2O_3) are the three most common magnetic iron oxide nanoparticles (M-IONPs), and their names are derived from the term 'Magnesia,' which is a region in Asia Minor where massive quantities of magnetite were discovered, and the two most common M-IONPs are magnetite, and maghemite (Ganapathe, et al., 2020). As a result, iron oxide nanoparticles have shown promise in a variety of biological applications. Magnetite (Fe_3O_4) nanoparticles, in particular, are frequently used because of their biocompatibility, strong magnetic susceptibility, chemical stability, innocuity, strong saturation magnetization, and low cost. Furthermore, when its size falls in the single-domain area to roughly 20 nm, magnetite (Fe_3O_4) shows superparamagnetism, an important trait for biological applications (Ganapathe, et al., 2020).

Carbon Paste Electrode

Carbon paste has become one of the most prominent electrode materials utilized in the laboratory manufacture of different electrodes, sensors, and detectors in recent decades, according to the literature. This position is unquestionably the outcome of the carbonaceous substrate's optimal combination of physicochemical and electrochemical features. As a result, each carbon paste is unique, with chemical, physical, and electrochemical characteristics that differ from one preparation to the next; as a result, each probe must be calibrated and surface polished before each test to assure measurement repeatability. Traditional electrodes can be replaced by a throwaway and reusable electrode, such as the CPE. Similarly, the behavior of voltammetry of the analyte is influenced by; type of the functioning electrode's active surface, and hencel; morphologies of the voltammograms may change dramatically. CPEs are composites made up of three parts: graphite powder, pasting solutions, and the electrode, which can be a polyethylene syringe, a glass tube, or a Teflon rod linked by a conducting wire, as illustrated in Figure 7. the behavior of voltammetry of an analyte can be effected by the CPE type, resulting in the maximum electrochemical signal (Vytas, et al., 2009).

Figure 7

Electrochemical Sensor Preparation Using a Carbon Paste Electrode .(Pwavodi et al. 2021)



Related Research

SMX electrochemical detection has been the subject of several studies. The following are some of the studies that will be summarized:

Alkhawaldeh (2020) The approach used in this work was based on the voltammetric measurement of SMX utilizing a molybdenum oxide-modified glassy carbon electrode, which was effectively applied by DPV and shown great selectivity for identifying these compounds. The two components were oxidized at the modified electrode surface, with current development linearly proportional to their concentrations of 7.04 $x10^{-7}$-1 $x10^{-3}$ M for SMX. The molybdenum coating makes the carbon electrode less susceptible to adsorption and impurity effects. The current peak and the concentration of SMX were found to be linear, with LOD of 1.44 $x10^{-4}$ M for SMX. The two components' oxidation reactions were pH-dependent. Britton-Robinson buffer with a pH of 7.00 was utilized. The regression factors for SMX and TMP were 0.9790 and 0.9812, respectively, based on the calibration curves.
The calibration curves yielded regression determinants of 0.9790 for SMX and 0.9812 for TMP. The technique of analysis was confirmed, and the (LOD) and (LOQ) of SMX were determined to be 1.44 $x10^{-4}$M and 4.36 $x10^{-4}$ M, respectively. The technique of analysis was verified, and the percentage recovery of both components was determined to be 81% for SMX.

Xu et al. (2017) To extract sulfonamides (SAs) from ambient water, this study employed a selective and easy process based on magnetic separation technology, which was followed by HPLC-tandem mass spectrometry. The super-paramagnetic surface that is magnetic, MIP (Fe_3O_4@SiO_2@MIPs) were created as magnetic adsorbents and were extremely selective for SAs. The enrichment and extraction were carried out simultaneously by simply mixing the water samples with the adsorbent mixture. The use of magnetic adsorbents gave the suggested approach a number of benefits, including quicker adsorbent regeneration and preparation, a simpler sample preparation procedure, greater extraction recovery, and lower solvent consumption. SEM, vibrating sample magnetometry and Fourier-transform infrared spectroscopy, were used to identify the Fe_3O_4@SiO_2@MIPs. Furthermore, as

compared to typical magnetic MIPs, Fe_3O_4@SiO_2@MIPs had a substantially larger area of specific surface and a quicker analyte desorption/adsorption frequency. In concentration range of 10–1000 ngL^1, the calibration curves derived from matrix-matched references show a strong linear connection. The R equals 0.9994–0.9999, and LOD were in the spectrum of 1.4–2.8 ngL^1. This method is utilized to identify SAs in six environmental water samples, with SAs being found in four of them at varying concentrations from 10.5 to 120.2 ngL^1. As a result, this approach is gaining popularity and may be a suitable alternative trace analysis methods for a wide range of complex materials.

Lahcen et al. (2016) The purpose of this research was to see which of the various carbonaceous material-based electrochemical sensors was the most effective in detecting sulfonamides in pharmaceutical preparations and (enriched) water samples. CPE with carbon nanopowder have a high electrooxidative power, graphite, multi-walled carbonnanotubes, acetylene black, and glassy carbon powder at pH 6.0 is examined using square wave voltammetry with SMX as the model analyte. Carbon paste electrodes made with carbon nanopowder or graphite had the lowest detection limit and the maximum sensitivity, according to the findings. The experiment was conducted at a voltage of 0.930 vs. Ag/AgCl. The LOD are 0.4 to 1.2 M, the voltammetric response follows a linear pattern., the concentration range is 1 to 75 M, and the sensitivities are between 10 and 38 nAM1. CNPE has the lowest LOD (0.12 M) for SMX as a consequence.

Galvez et al. (2016) Sulfamethoxazole might be screened in a complex mixture (seawater) using a hybrid material made of (MIP) enhanced magnetite nanoparticles. EIS was applied to evaluate (composite). Analyte is preconcentrated, separated, and manipulated using the composite's magnetic characteristics, which the MIP selectively collects onto the composite's surface. The EIS levels of the whole material were measured in response to the amount of analyte collected using screen-printed electrodes.The sensitivity of this composite-based sensing device (LOD about 1102 mol L^1 2.8104 ppb) is comparable to sulfonamide monitoring utilizing HPLC mass spectrometry.

Zhao et al. (2015) This research presented a creative electrochemical sensor (MIP) customized Boron-doped diamond (BDD) electrode for selective,quantitative, and sensitive electrochemical sensing of (SMX) in groundwater, which increased at varying concentrations levels and conducted well with a recovery of 96.0–106.2 percent. In situ electro polymerization of pyrrole (Py) on the BDD electrode in the presence of SMX produced the MIP/BDD electrode. The sensor showed a linear reaction for SMX from 0.1 to 100 mM, with a LOD of 24.1 nM, a good repeatability (2.32%), and a low LOD (24.1 nM). The high-selectivity finding is also due to the MIP fifilm's imprinted sites, which gain from an appropriate homologous functional groups and appropriate cavity size for specific identification of SMX molecules. As a consequence, the custom-built sensor provided a simple, selective, sensitive, and ecologically friendly method for determining SMX

Sgobbi et al. (2015) Using a new electrochemical sensor modified with multi-walled carbon nanotubes ornamented with Prussian blue nanocubes (MWCNT/PBnc), this study employed DPV to detect SMX and TMP in urine. The MWCNT/PBnc composite combines the MWCNT platform with the increased incidence of active catalytic sites in PBnc edges, resulting in better PBnc stability at neutral pH. As a result, the MWCNT/PBnc film was extremely sensitive to SMX and TMP while being highly selective. The MWCNT/PBnc/SPE was estimated for SMX, with a linear range of 1.0-10.0 mmol L^1 and a LOD of 38 nmol L^1 and a linear range of 1.0-10.0 mmol L^1 and a LOD of 38 nmol L^1. TMP has a linear spectrum of 0.1-10.0 mmol L^1 and a LOD of 60 nmol L^1.

Motaharian and Sadeghi (2013) A novel sensitive voltammetric sensor for detecting sulfadiazine was disclosed in their work. As a recognition element, the proposed sensor used a CPE enhanced with sulfadiazine (MIP). (NIP/CPE) was created for comparing. Employing CV and differential pulse voltammetry, binding event and electrochemical behavior of sulfadiazine at modified CPE were investigated. Due to sulfadiazine oxidation, DPV at 0.92 V vs. Ag/AgCl was used to quantify sulfadiazine after it was extracted onto the electrode surface. Under ideal operational conditions, the highest current generated by the MIP modified carbon paste electrode was equivalent to the sulfadiazine concentration ranging 2.0107–1.0104 mol L^1, with LOD and sensitivity of 1.4107 mol L^1 and 4.2105A mol L^1,

correspondingly. The designed sensor exhibits a repeatability of 2.6 percent in terms of relative SD. The sensor proved successful in detecting sulfadiazine in contaminated cow milk and human blood samples, with recovery rates ranging from 96.7 to 100.9 percent.

Alirezanejad, Arvand (2012) The potentiometric approach utilizing MIP doped in a CPE presented a very interesting option for the SMX evaluation, and the sensor was successfully deployed to the analysis of biological specimens and food, according to the resulting results of this study. The host cavity was sculpted on a polymeric surface that was radical polymerized using the methacrylic acid monomers. Graphite powder, ionic site, and paraffin oil were combined with (MIP). The Taguchi approach was used to investigate the best conditions for producing carbon paste electrodes. Graphite, MIP, paraffin oil, and the ionic site were all employed as controlled elements in this investigation. Each controllable factor's % contribution was calculated. In a wide range of concentrations of 6.09 $x10^{-8}$ to 3.19 $x10^{-3}$ mol L^{-1}, the MIP modified electrode showed a Nernstian response (57.2mVdecade-1) with a lower LOD of 3.59 $x10^{-9}$mol L^{-1}. As a result, when compared to their earlier work, this sensor had benefits such as ease of design, rapid reaction, wide linear range, consistent, reproducible data, higher predictive throughput, good accuracy, low limit of detection, and strong selectivity. Because of its simplicity, accuracy, precision, and cheap cost in terms of reagent use and equipment, the proposed approach was suited for routine SMX screening.

Arvand et al. (2011)A MWCNT paste electrode was examined and found to have good electrocatalytic activity for Sulfamethoxazole oxidation. The increase in strength of SMX oxidation was significantly reduced by the designed MWCNT paste electrode compared to a typical CPE, with markedly enhanced signal-to-noise features without the need for any modifier, as well as improved sensitivity to those reported at a conventional CPE, which was measured at around 110 mV. For the oxidation of Sulfamethoxazole, the modified electrode has high stability, catalytic activity, and repeatability. The catalytic peak currents achieved with DPV were linearly related on the Sulfamethoxazole concentrations due to the broad linear dynamic range. Because of the enhanced reactivity of the MWCNT paste electrode for the identification of SMX, the overpotential for oxidation of the sulfamide

activity has been considerably lowered. The calibrated graphs for the oxidation process under ideal conditions are linear in the range of concentrations of 0.35–30 gmL^1, with a slope of 0.0955A/gmL^1, LOD 0.1 gmL^1, and LOQ 0.33 gmL^1, showing that the pH of the supporting electrolyte is important. The technique for assessing the medication in suspension or tablet form was carried out without the use of excipients, and the variation from the target concentrations was acceptable.

Arvand and Alirezanejad (2011) used a potentiometric transduction approach to create a novel biomimetic sensor material for Sulfamethoxazole, which was used in the examination of food and biological materials. It is made from ethylene glycol dimethacrylate as a crosslinker, acetonitrile as a porogenic,As a functional monomer, methacrylic acid is used as a solvent., 2,2 azobisisobutyronitrile like a radical initiator for radical polymerization. The Sulfamethoxazole sensor, which had 10.90 percent particles that are imprinted, with a detection limit (6.3108mol L^1) and slope (58.3 mV/decade) responses. This electrode also showed high selectivity for sulfonamide antibiotics, calcium, glucose, nickel, ammonium, sodium, and zinc, among other things. Furthermore, pH 2.2 and pH>6 had no effect on the sensor.

CHAPTER III

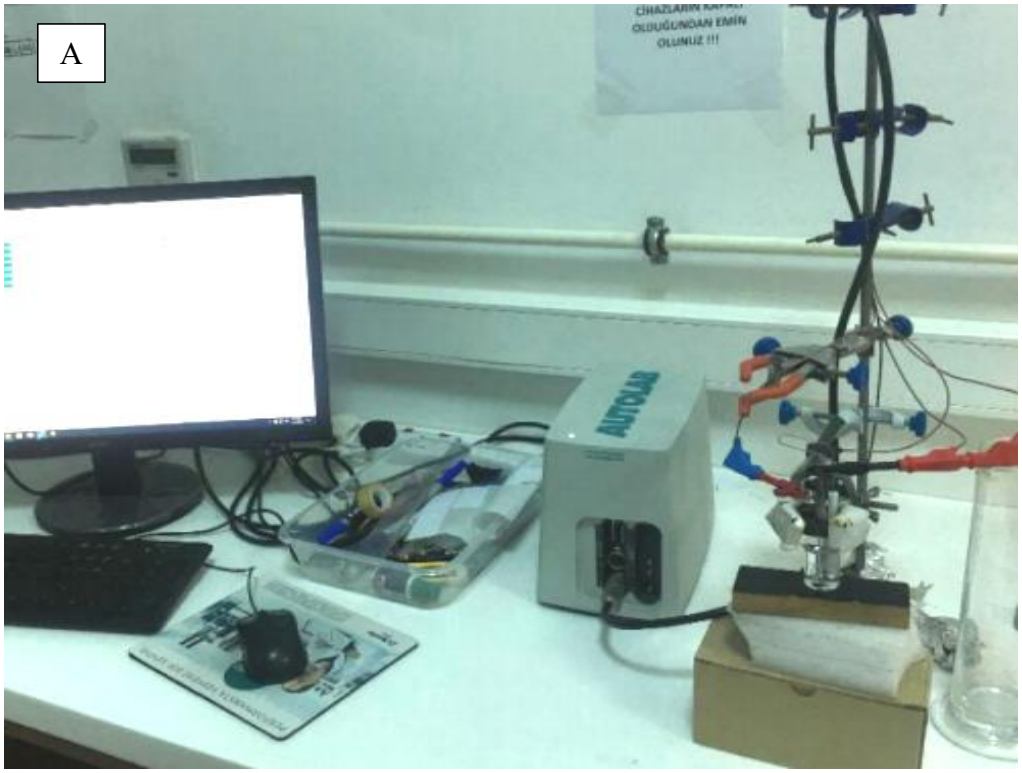
Methodology

Instrumentation and Methods

The potentiostat–galvanostat (AUTO LAB-PGSTAT204, Metrohm, Utrecht, The Netherlands) and Nova 2.1.4 software were used to perform electrochemical cyclic voltammetric, differential pulse voltammetric, and Electrochemical Impedance Spectroscopy (EIS) experiments. The potentiostat–galvanostat was linked to the three-electrode system cell, a carbon paste electrode, and a modified carbon paste electrode as working electrodes. In a 10 ml cell containing 0.5M ABS pH 7.4 as a supporting electrolyte, an Ag/AgCl/3M KCl reference electrode was employed, and a platinum wire was used as an auxiliary electrode.As indicated in Figure 8, A, all pH measurements were taken with an edge H12002 pH meter (Hanna Instruments, Woonsocket, RI, USA). All of the studies were conducted at a constant temperature of 25 degrees Celsius. The nanoparticles were centrifuged using a Beckman Coulter centrifuge (Optimum TM L-look Ultracentrifuge, USA). The nanoparticles were examined using a scanning electron microscope (SEM, Joel JEM1200 EX, Tokyo, Japan) and a spectrophotometer (UV mini-1240, SHIMADZU, Tokyo, Japan) in a 1-cm optical path quartz cuvette in the 1 to 9 nm spectral region. As illustrated in Figure 8, B, the microbeads were made in a batch reactor system with a motor stirrer at 600 rpm and 45°C (Heidolph RZR 2021, AC, DC input 220 V AC, Germany).

Figure 8

A)Auto Lab Pgstat 204 Potentiostat with Nova 2.12 - B)Batch Reactor System.



Chemicals and Reagents

Fulka A.G. supplied ethylene glycol dimethacrylate (EGDMA) (Buchs, Switzerland). Merck A.G. contributed the remaining chemicals (Darmstadt, Germany). Aldrich provided the magnetic nanoparticle Iron (II, III) oxide, 50-100 nm. As demonstrated in Figure 9, all analyses were carried out using distilled water. The powdered chemicals Sulfamethoxazole molecularly imprinted polymer and Sulfamethoxazole non-imprinted polymer were made using the SMX MIP/NIP synthesis process outlined below. Standard HEMA (2-hydroxyethyl methacrylate), PVA (polyvinyl alcohol), SDS (sodium dodecyl sulfate), APS (ammonium persulfate), methacrylic acid, sodium bicarbonate, sodium bisulfite, Sulfamethoxazole, cephalexin, paraffin oil, carbon powder, and amoxicillin, on the other hand, were all commercially available from Sigma All of the reagents were analytical standards, and they were used exactly as they were. The pH rate of the acetate buffer solutions (ABS) utilized in this investigation is 0.5M, with an ABS pH of 7.4. Standard antibiotic stock solutions were made using ultra-pure water at a concentration of 1000 ppm (3.95103 M for SMX, 2.9103 M for CEPH, and 2.8103 M for AMX). The stock solution was then lowered to 200 ppm standard concentrations (0.79103 M for SMX, 0.58103 M for CEPH, and 0.55103 M for AMX), which were then utilized as working solutions. The laboratory provided the water samples utilized in the real sample analysis.

Figure 9

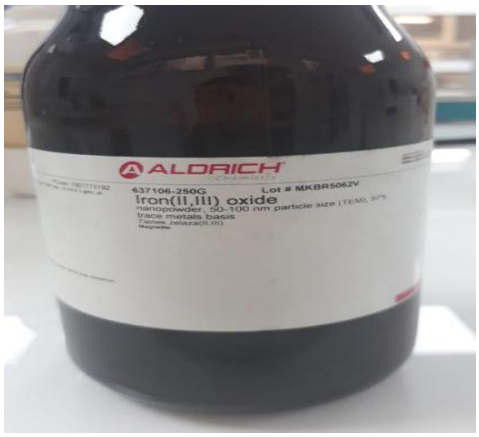
Materials Used in the Experiment (Fe_3O_4, NIP Nanoparticles, Sulfamethoxazole etc).

Synthesis of Magnetic Nanoparticles

Magnetic nanoparticles were synthesized by the polymerization method of mini-emulsion two-phase. First, two aqueous phases and one organic phase were prepared: for the primary aqueous phase, 28 mg of SDS, 187 mg of PVA, and 23.4 mg of sodium bicarbonate were dissolved in 10 ml deionized water; for the second aqueous phase, 100 mg SDS and 100 mg PVA, was dissolved in 150 ml of deionized water, and for the organic phase 4 ml of HEMA was dissolved in 2.1 ml of EGDMA. Finally, the mixture of the organic phase and first aqueous phase was homogenized at 25000 rpm by a homogenizer. After homogenization, 100 mg of the Iron (II, III) oxide nanopowder, as shown in Figure 10, was added to the organic phase mixture and the first aqueous phase. Next, the second aqueous phase was added to the mixture of the first aqueous and the organic phase; then, this combination was moved to the polymerization system. Next, the heat of the polymerization system was adjusted to 45 C, and the reactor was stirred at 600 rpm. Next, adding sodium bisulfate (115 mg) and ammonium persulfate (125 mg) as initiator and stabilizer, polymerization it was proceeded for 24 h. The nanoparticles were then rinsed with water and an alcohol/water combination to remove any unreacted monomers, surfactants, or initiators. In addition, the washing procedure was performed in several steps; each washing step was performed by centrifuging nanoparticles in water, and the deionized water containing 0.5% sodium azide was applied for storing the nanoparticles at 4°C.

Figure 10

Iron (II, III) Oxide Nanopowder.



Synthesis of Sulfamethoxazole Surface Imprinted Core-Shell Magnetic Nanoparticles

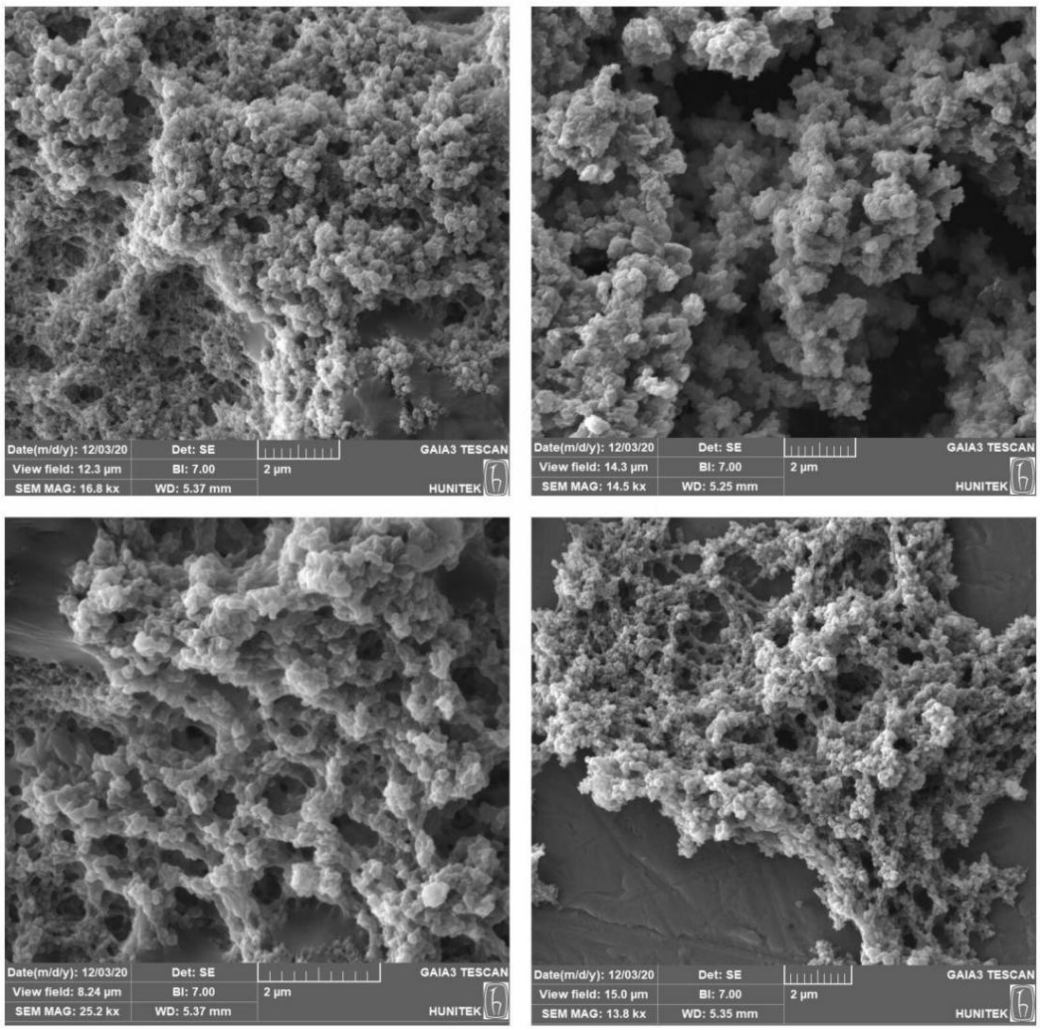
The Mini-emulsion polymerization method was used to obtain a magnetic nanoparticle core coated with a Sulfamethoxazole imprinted polymer shell. The very first phase was made using 78 mg PVA, 29 mg SDS, and 27 mg sodium bicarbonate dissolved in 10 ml deionized water, while the second phase was made with 100 mg PVA and 100 mg SDS dissolved in 200 ml deionized water. Then, 100 mg magnetic nanoparticles, Sulfamethoxazole (0.1 mmol, 25.3 mg) as a template, and methacrylic acid monomer (0.2 mmol, 16.2 ml) were dissolved in 10 ml HEMA and 2 ml EGDMA to form the monomer phase. The monomer phase was then added to the first phase, and the mixture was homogenized for 15 minutes at 25000 rpm. The resultant combination was then added to the second liquid phase one drop at a time. The resultant mixture was transferred to the polymerization system, and sodium bisulfite (120 mg) and ammonium persulfate (130 mg) were added to start the polymerization, which was agitated at 600 rpm for 24 hours at 45 degrees Celsius.

The magnetic core covered with Sulfamethoxazole imprinted polymer shell nanoparticles was prepared by centrifugation of the solution, and the precipitate was then washed with deionized water and ethanol to remove unreacted monomers, surfactants, and initiator molecules. The template molecule was removed from the imprinted nanoparticle by washing it with phosphate buffer solution (7.4). A UV-vis spectrophotometer set to 271 nm was used to examine the nanoparticle. The nanoparticles were then manufactured three times more using the same procedure but varying concentrations of Sulfamethoxazole (0.2, 0.5 mmol), as shown in Figure 11. The nanoparticles were further examined using a scanning electron microscope (SEM).

Adsorption experiments will be conducted with these Sulfamethoxazole imprinted magnetic nanoparticles and will be checked to see which ratio of monomer and template molecule gives a high amount of absorbance. Then the best ratio of monomer and template molecule will be used for the sensor experiment. Without the inclusion of Sulfamethoxazole, the same experimental approach was used to make non-imprinted nanoparticles.

Figure 11

Sulfamethoxazole Surface Imprinted Core-Shell Magnetic Nanoparticles Via SEM.



The surface morphology of SMX MIP Fe_3O_4 NPs was investigated with a scanning electron microscope, as shown in Figure 11, after the modification process; polymerization aiming to obtain a molecularly imprinted polymer layer on a magnetic core; and polymerization aiming to obtain a molecularly imprinted polymer layer on a magnetic core. As a result, a Fe_3O_4 layer may be seen. Furthermore, a huge zone of dark region may be seen, which is related with a greater particle dispersion and a more homogenous distribution. Meanwhile, once the template molecule was removed, MMIPs had a more evident porous structure with voids.

Data Collection Procedures

Preparation of 0.5M, pH 7.4 ABS Buffer Solution

500 ml of ABS was a set-up from adding 14.45 ml of glacial acetic acid and was dissolved in 250 ml of ultra-pure water; its pH was changed to 7.4 by putting 1N NaOH (20g/500 ml). After that 0.84 g, NaCl was added to the pH-adjusted acetic acid solution and made up the volume to 500 ml. All aqueous solutions were made with ultra-pure water.

Preparation of Stock Solutions for (SMX, AMX, CEPH)

SMX, AMX, CEPH solution of (10 mg/L) was prepared directly from ultra-pure water. To prepare the solution, 10 mg of each SMX, AMX, CEPH powder separately was weighed on a weighing scale and then made into solution in a 10 ml beaker using ultra-pure water.

Preparation of Working Solutions for (SMX, AMX, CEPH)

SMX, AMX, CEPH working solution was prepared by taking 200 ppm of each antibiotic from the stock solution, which is 2 ml separately, and then inserted into a 10 ml beaker by topping it up with ABS 0.5M, pH 7.4 buffer solution.

Preparation of Bare MIP/CPE and NIP/CPE

The 60:20:20 and 60:30:10 (wt/wt/wt percent) MIP to carbon powder to paraffin oil (binder) ratios were evaluated for optimum results, and the 60:30:10 ratio was chosen as the optimal percentage for the study. MIP/CPE was made by hand, using 60 mg of MIP, 30 mg of carbon powder, and 10 mg of paraffin oil to make a homogenous combination of 60, 30, and 10 mg. For NIP/CPE combined with paraffin oil, the same technique was followed. The homogeneous pastes were made to fill two distinct Teflon tubes with 4 mm diameter cavities, one for MIP/CPE and the other for NIP/CPE. The electrodes' ends were joined by a copper wire for conductivity (Teflon tubes). To create a smooth and crack-free surface, the electrodes' surfaces were cleaned by smoothing them with smooth paper. Following any examination, new electrode surfaces were created by putting the paste into

Teflon tubes and polishing their surfaces. Before each new measurement, this technique was repeated throughout the studies.

Preparation and Detection Procedure of Real Sample

The SMX chemical concentration in contaminated water samples was determined using the DPV method. The voltammograms were created by adding successive dilutions of known volumes and concentrations of the SMX chemical using a standard addition technique. A volume of 1 ml of the water sample was placed into a 10 ml volumetric flask, followed by a volume of 10 ml of ABS (0.5 M, pH 7.4). Aliquots of the standard SMX compound (0.210-3–0.6103 M) were then added to the 10 ml volumetric flasks 5 ml of water samples and 10 ml of ABS as a supporting electrolyte. After that, a magnetic stirrer was used to agitate them for two minutes. Each beaker containing aliquots of the standard SMX compounds had its DPV analytical measurements recorded.

Electrochemical Measurements

CV, EIS, and DPV methods were used to determine the electrochemical nature of the SMX interaction. These analytes were measured at the electrode surfaces in ABS 0.5 M with a pH of 7.4.

Sulfamethoxazole Surface Imprinted Core-Shell Magnetic Nanoparticles were used in the CV technique to obtain qualitative data on electrochemical behavior patterns of various SMX concentration levels, including the rapid location of redox potentials of electroactive varieties and the easy evaluation of the effect of media on the redox potentials. CV measurements were taken using a start potential of -0.4V, a stop potential of -0.4V, an upper vertex potential of 1 V, a lower vertex potential of -1.2V, a scan number of 0.5V, and a scan rate of 0.2V/s.

The DPV approach involved a DPV analysis that ranged from 0.0V to 0.8V. The following parameters were used in this methodology: step 0.005V, interval time 0.5s, modulation time 0.05s, and modulation amplitude 0.025V.

Measurements were made in a redox solution of 5mM$[Fe(CN)_6]^{3-/4-}$ containing 0.1M KNO_3 using the EIS method in the frequency range of 100kHz–0.1Hz. The following parameters were used to do the measurements: initial applied frequency 100kHz, last applied potential 0.1Hz, number of frequencies 10 per decade, amplitude 0.01V, and estimated time 12207s.

CHAPTER IV

Findings and Discussion

Cyclic Voltammetric Measurements of Samples

Electrochemical analysis can assist, validate the effective synthesis of SMX MIP-decorated Fe_3O_4 MNPs by providing information on the peak current of MIPs and NIPs. To demonstrate the electrochemical activity of the analytes at the surface of the electrode, cyclic voltammogram is performed at an NIPs electrode and a MIPs electrode in the redox solution of 5mM$[Fe(CN)_6]^{3-/4-}$ 0.1M of KNO_3 was added to the mix. Figure 12 indicates that the SMX compound has greater peak currents in MIP and smaller peak currents in non imprinted polymer (NIP). The positions of the analytes' redox reactions potentials, were also detected from the CV analyses of the SMX. Low current peak heights were seen in the reduction peaks, which were also seen in the reverse scan. Increased electroactive surface area caused by the Fe_3O_4 nanoparticles is thought to be the cause of this phenomenon. Furthermore, the inclusion of Fe_3O_4 nanoparticles in the MIP aids the electron transport, improves current responsiveness, and promotes analyte adsorption and enrichment on the electrode surface, hence aiding the oxidation process.

Figure 12

Cyclic Voltammograms Shows the Response of SMX MIP and SMX NIP in Redox Solution that contains 5mM[Fe(CN)$_6$]$^{3-/4-}$ of 0.1M KNO$_3$, Scan Rate: 0.20 V/s

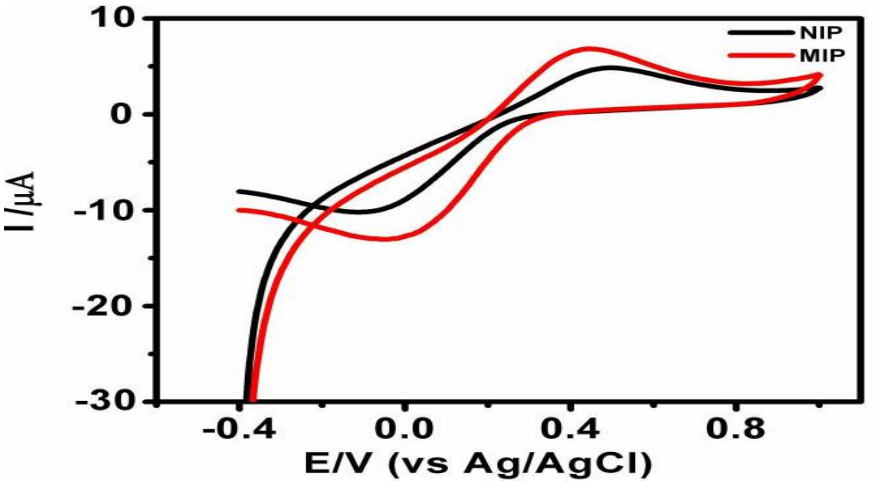


Figure 13, 14, 15 shows a procedure comprised of multiple CV cycles in different working solutions that contain 2 ml of each antibiotic mixed with ABS 0.5 M, pH 7.4 buffer solution that was used to investigate the cyclic voltammetric behavior of SMX, CEPH, AMX respectively, at the MIP/CPE, NIP/CPE electrodes with a Scanning Potential range -0.4 to 1.0 V. The peak value at the MIP altered electrode was higher but also bigger than the peak current at the NIP adjusted electrode, indicating that MIP contains selective binding sites, as shown in the Figure. As a conclusion, the MIP is used as a chosen recognition element in the sensor's fabrication. DPV approach was used to investigate the rebinding tests utilizing naked and modified CPE. In comparison for other methods, peaks acquired using DPV are better defined, have a higher sensitivity to low analyte concentrations, and have a reduced background current.

Figure 13

Cyclic Voltammogram that Shows the Oxidation and Reduction Behavior of SMX by CPE, Modified MIP/CPE and NIP/CPE in ABS 0.5M, pH 7.4 Buffer Solution; Scanning Potential Range-0.4 to 1.0V, Scan Rate=0.20V/s in SMX.

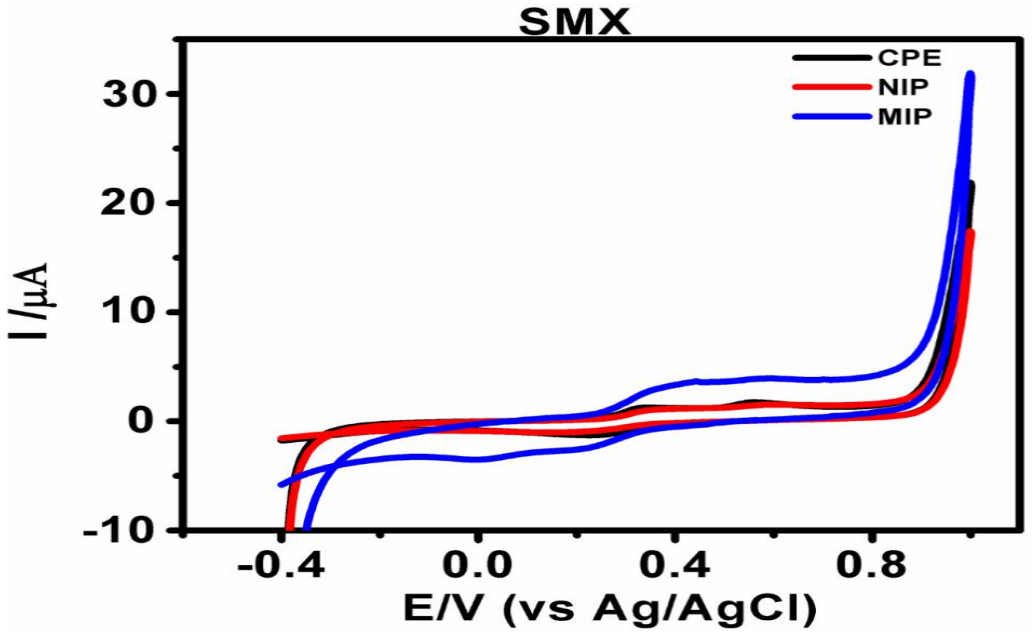


Figure 14

Cyclic Voltammogram that Shows the Oxidation and Reduction Behavior of SMX by CPE, Modified MIP/CPE and NIP/CPE in ABS 0.5M, pH 7.4; Scanning Potential Range -0.4 to 1.0V , Scan Rate=0.20V/s in CEPH.

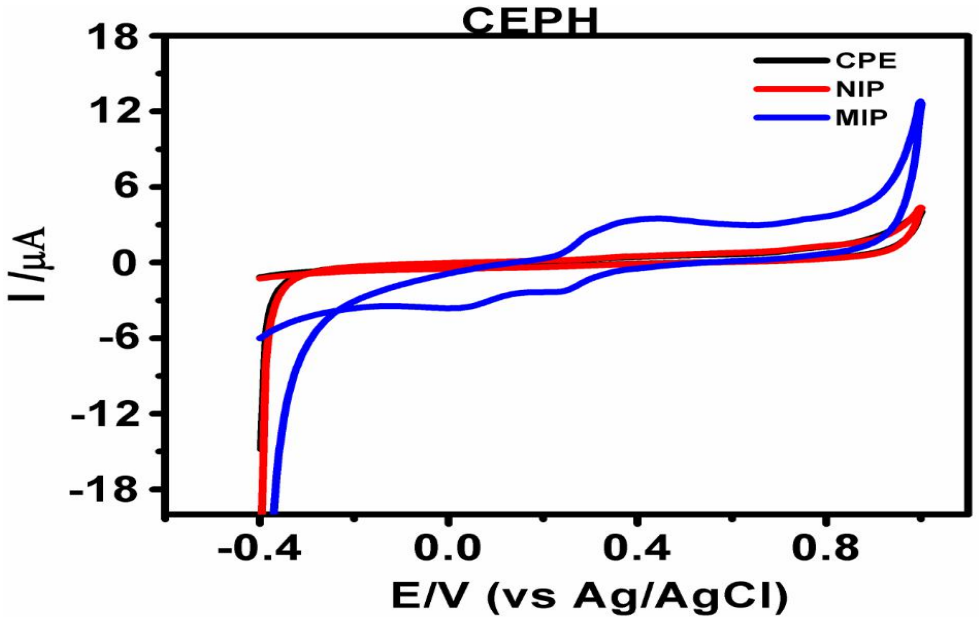
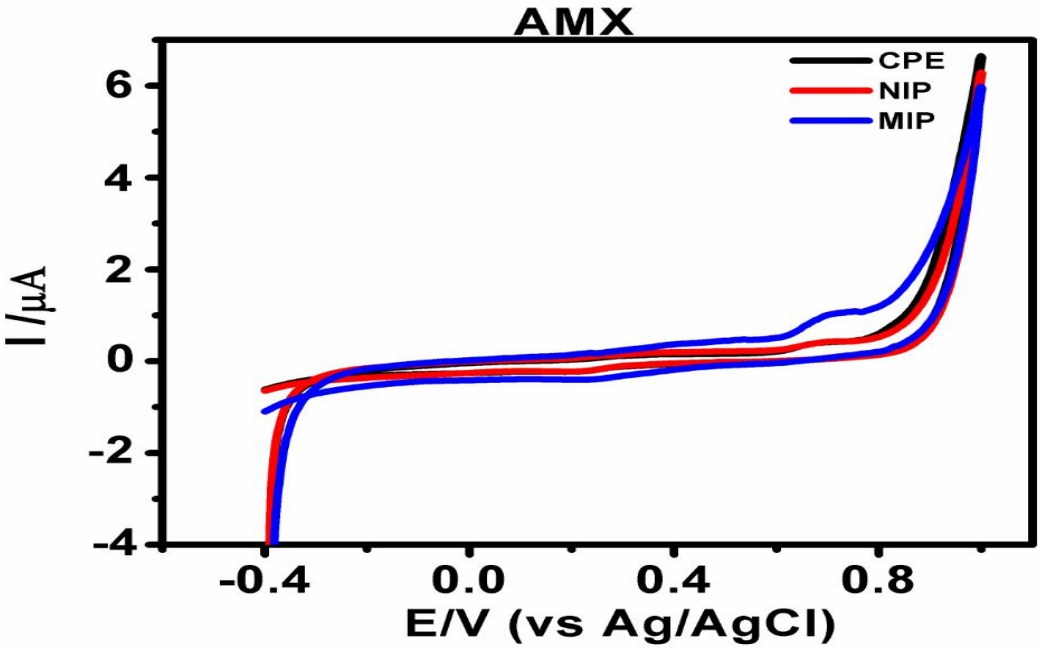


Figure 15

Cyclic Voltammogram that Shows the Oxidation and Reduction Behavior of SMX by CPE, Modified MIP/CPE and NIP/CPE in ABS 0.5M, pH 7.4 Buffer Solution; Scanning Potential Range -0.4 to 1.0V, Scan Rate=0.20V/s in AMX.



Differential Pulse Voltammetry of Samples

Figure 16 shows a comparison of the DPV reactions of SMX/ MIP modified carbon paste electrodes and SMX/ NIP electrode measurement to better understand the electrochemical behaviors at MIP Fe_3O_4NPs/CPE, in ABS of 0.5M, solution at pH 7.4; scan rate from 0.0 V to 0.8 V, pulse amplitude=0.025 V, This measurement shows a very much characterized peak at 0.46 V for SMX MIP at Fe_3O_4NPs/CPE with a current of 0.042 µA While the peak current of SMX NIP/CPE is 0.021 µA, the peak current obtained at MIP Fe_3O_4 NPs modified CPE for the Sulfamethoxazole was seen to be higher than the resulting anodic current from NIP/CPE. Thus, the high electrocatalytic effect of MIP Fe_3O_4NPs/CPE could be credited with the existence of MIP Fe_3O_4NPs within the CPE, the sensitivity and the working electrode's outer layer was increased, however, electron transport between Sulfamethoxazole and MIP Fe_3O_4 NPs/CPE was accelerated.

However, in Figure 17, the DPV measurement was also used for the electrochemical analysis at different concentrations of SMX at Fe_3O_4NPs modified CPE and SMX NIP/CPE, and it displays the inset of the DPV at various concentrations of SMX (20 mM to 100mM) in ABS 0.5M, pH 7.4 buffer solution. For NIP and MIP With an increment of the concentration starting from 20 to100 mM of SMX, it been noticed that the current signal response of Sulfamethoxazole increased, and it was higher in the SMX MIP/CPE than the SMX NIP/CPE. This indicates that there is a direct relationship between the concentration and the current signal and because the NIP doesn't have the cavities for the SMX selectivity. As can be seen in Figure 18, A the concentration of SMX was ranged from 5mM to 100 mM, and it was made to find the lowest limit of detection, for Figure 18, B The formula was calculated from plots Ip for the different 8 concentrations as follows; Ipa (μA)=0.138C(M)-0.100, (R^2=0.9981).

Figure 16

DPV Voltammograms for SMX MIP and SMX NIP Obtained Under the Optimized Parameters in ABS 0.5M, pH 7.4 Buffer Solution with a Scan Rate from 0.0V to 0.8V. A Peak at 0.46V for SMX MIP at Fe_3O_4NPs/CPE with a Current of 0.042μA While the Peak Current of SMX NIP/CPE is 0.021μA.

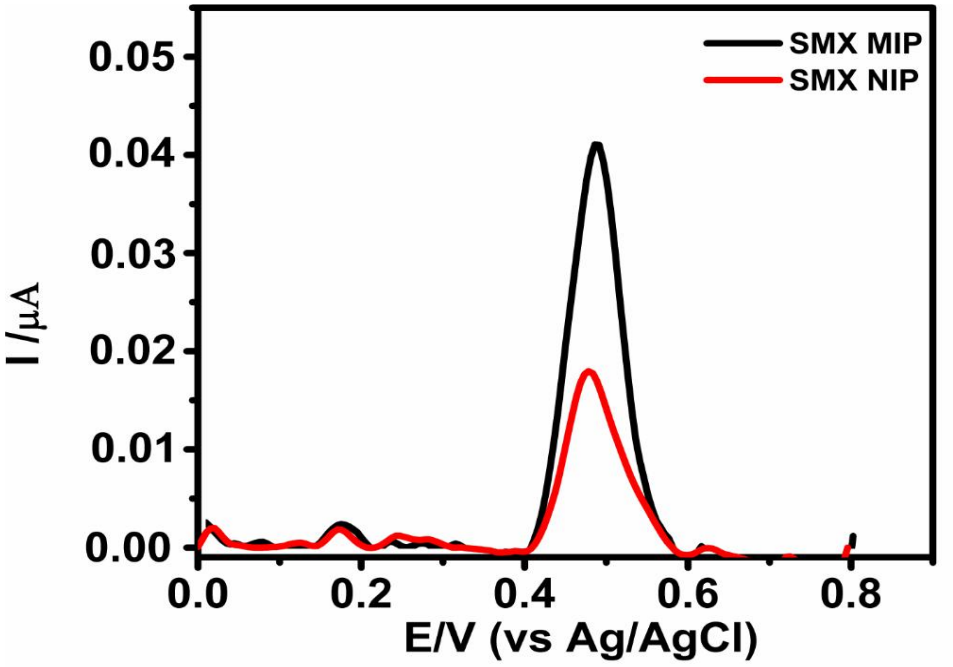


Figure 17

A Inset DPV Voltammograms for the Determination of SMX/MIP and SMX/NIP Obtained Under the Optimized Parameters in 0.5 M ABS pH 7.4 Buffer Solution, Containing SMX in a Range of 20 to100 mM.

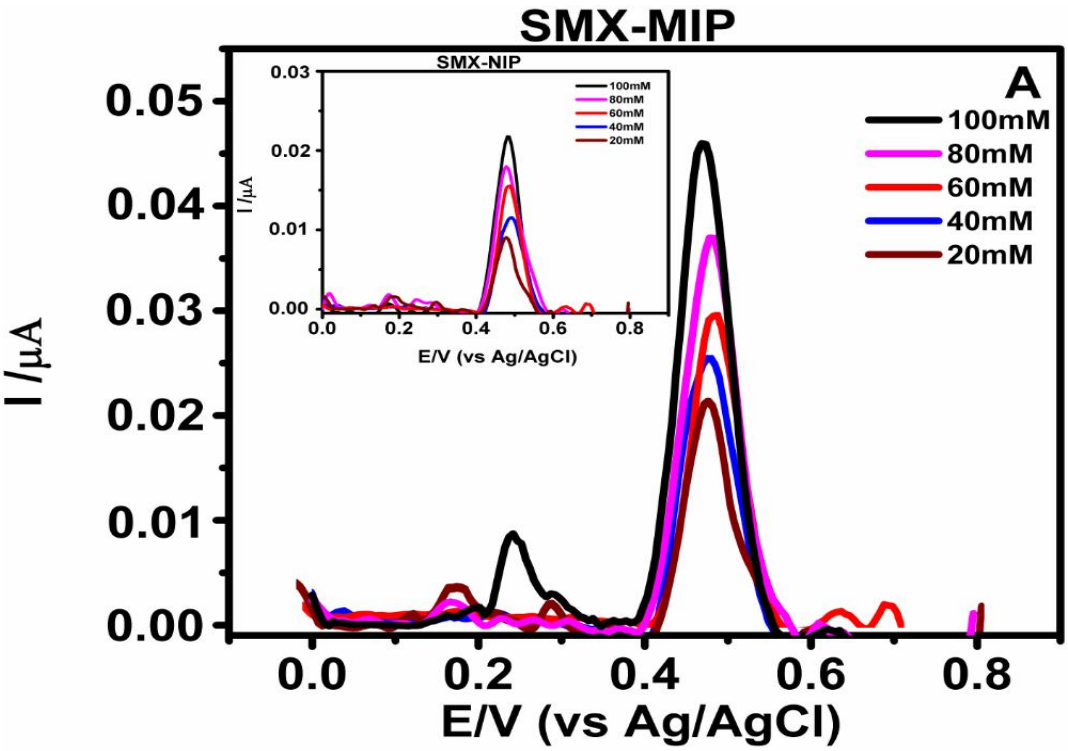
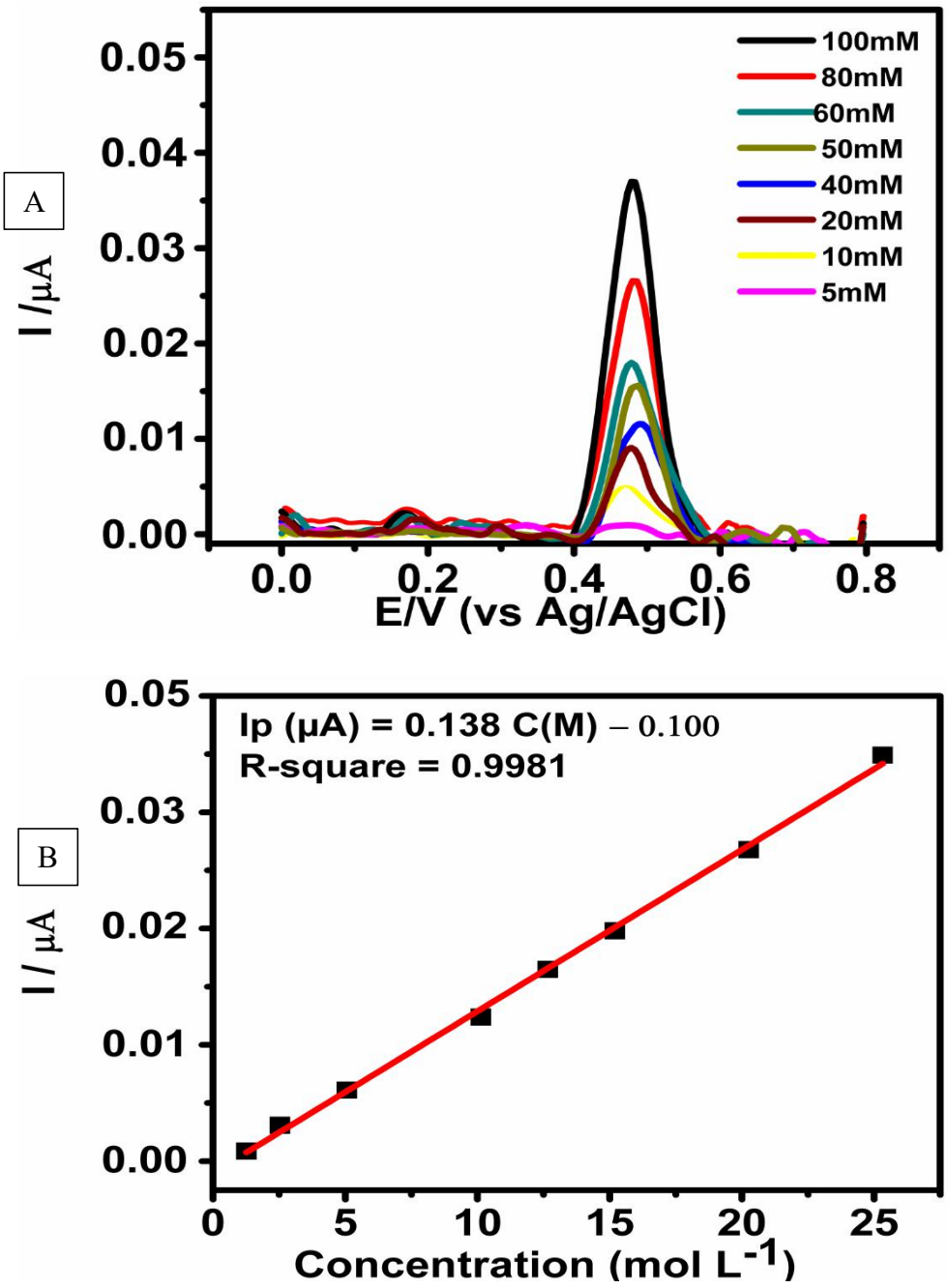


Figure 18

A) DPV Voltammogram for the Determination of SMX Obtained Under the Optimized Parameters in 0.5 M ABS pH 7.4 Buffer Solution Containing SMX in a Range of 5 to100 mM B)Linear Dependence of the Peaks Current with SMX Different Concentrations. The Regression Equation was Calculated from Plots Ip Versus Concentration as Follows; Ipa(μA)=0.138C(M)-0.100, (R^2=0.9981).



Electrochemical Impedance Spectroscopy of Sulfamethoxazole Sample

Electrochemical impedance spectroscopy (EIS), as previously mentioned, is a useful instrument for examining changes during such a recognition event, at a user interface. Faradaic measures, have been claimed to have enhanced sensitivity. The usual impedance data, on the other hand, are represented by the storyline of Nyquist with a semicircular element at higher frequencies that corresponds to the interfacial charge-transfer operation, at lower frequency region, there is a straight component that corresponds to the diffusion operation. Faradaic EIS measurements were carried out in this study using printed carbon electrodes into a solution which contains 5mM$[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KNO_3 with frequency spectrum of 0.1Hz to 100 kHz and a voltage amplitude of 0.001mV. In addition,the disappearance and existence of various levels of SMX (2 mM to 10 mM) in the frequency range of 100kHz to 0.1Hz , the experiment was conducted out in water from the tap with MIP-adjusted Fe_3O_4 MNPs.

EIS was used to investigate the behavior of MIP, NIP, and CPE, as shown in Figure 19, because it investigates the electrode-solution interference characteristics considering the mechanism of electrochemical reactions between the redox solution and the electrode. MIP, NIP, and CPE have R_{ct} values of 17.01 k, 21.86 k, and 25.42 k, respectively. The highest R_{ct} value for CPE implies that electron transmission between the oxidizing solution and the electrode contact is slow. However, the electron's slowest pace transport middle of the electrode interface and redox chemical reagent showing the greatest R_{ct} result for CPE. On the other side, the R_{ct} values held at MIP suggests quick charge transfer. The findings indicate that nanoparticles may assist electron passage between the electrode surface and the redox solution, hence improving electroconductivity. As a result, the Fe_3O_4 nanoparticle proved to be an excellent choice for building an electrochemical sensor for SMX measurement.

The collected findings were evaluated using the equivalent circuit of Randles , as shown in Figure 20. R_s: resistance of electrolyte, R_{ct}: (electron) charge of resistance transmission , C_{dl}: the ratio of interface , and Z_w : effective resistance of Warburg in the similar circuit. In each SMX concentration, different values were obtained. In the lack of SMX, the electrode's R_{ct} is 40k; this low value can be

because of the free-electron transport from the surface of the electrode to the solution. The analyte raises for the presence of the R_{ct} values because the solution that contains SMX binds to MIP via its recognition sites or cavities. Across a wide range of SMX concentrations, the magnitude of the shift in R_{ct} increases between 2 mM and 10 mM, as illustrated in Figure 21 in the inset. The R^2 value is 0.994, the slope is 110.504, and the intercept is 7.236.

Figure 19

EIS Measurements were Performed Through Printed Carbon Electrodes in 5mM$[Fe(CN)_6]^{3-/4-}$ Solution Containing 0.1M KNO_3 using MIP, NIP and CPE.

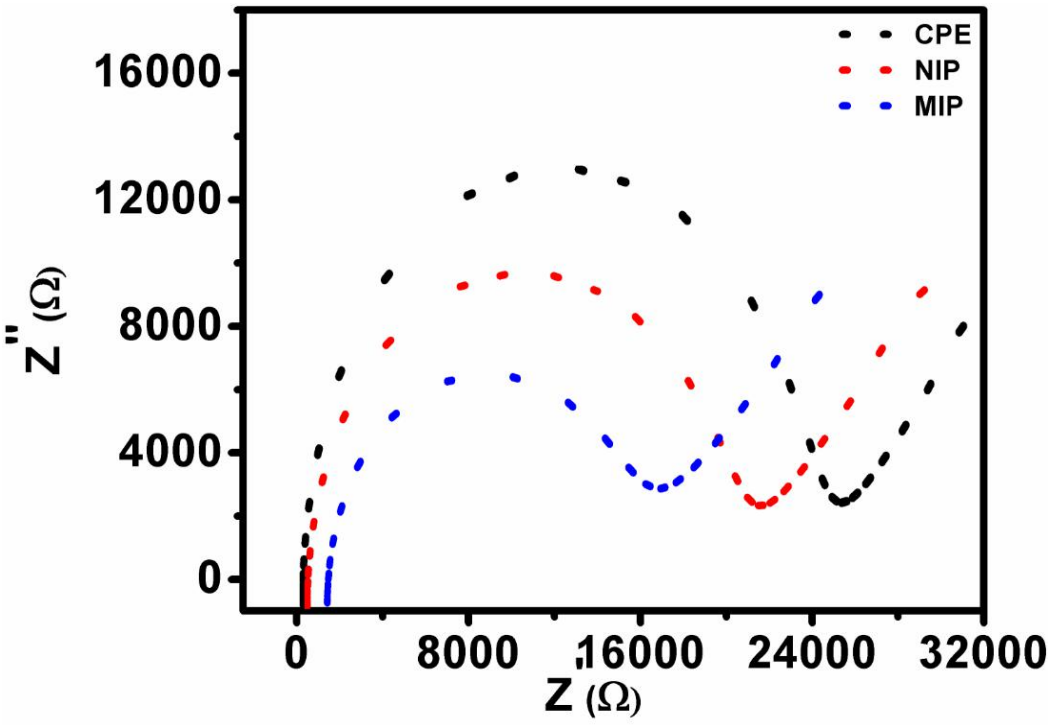


Figure 20

MIP-Decorated Fe3O4 MNP Modified CPE Nyquist Plots in Different Concentrations of SMX (2mM to 10 mM) using EIS Across the Frequency Spectrum from 100kHz to 0.1Hz and the Equivalent Circuit of Randles Model for the Impedance Data.

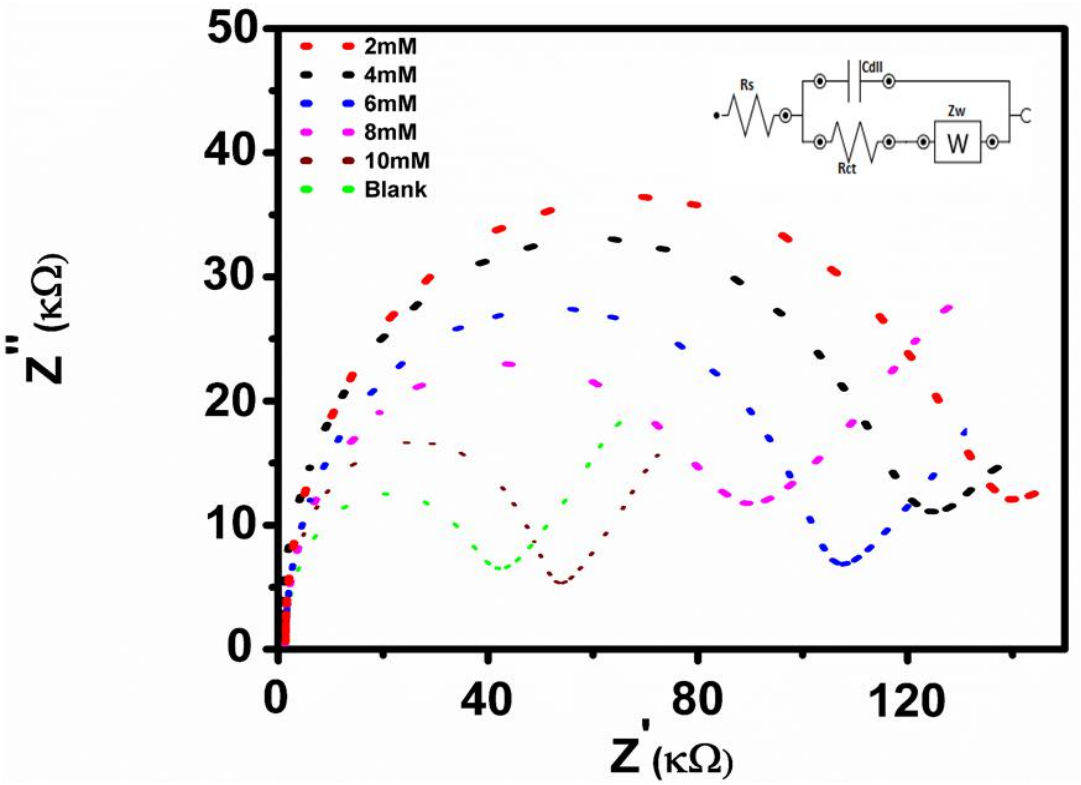
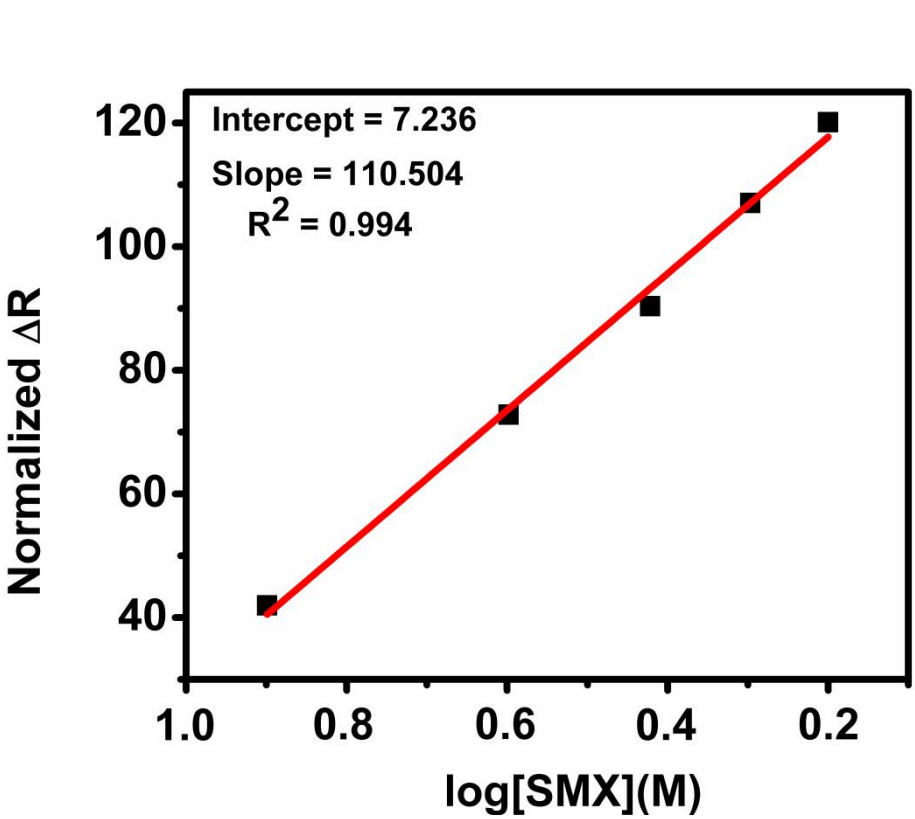


Figure 21

A Magnitude Calibration Curve for the Difference in R_{ct} of SMX Concentration in a Wide Range Between 2 mM to 10 mM in the Inset. the R^2=0.994 and the Slope=110.504, and the Intercept=7.236.



Specificity of the Sensor

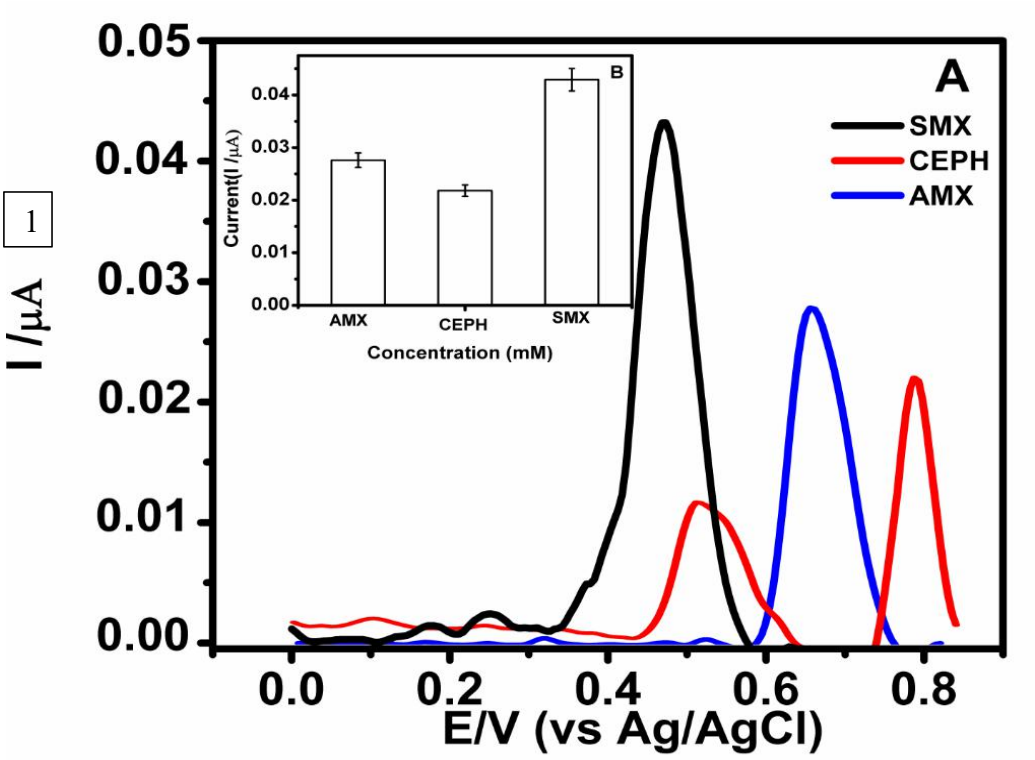
The performance of the MIP-adjusted $F_{e3}O_4$ NPs was contrasted to the (NIP) composite, and two antibiotics (AMX and CEPH) were used as interferents to determine their specificity and selectivity.

In complicated samples, selectivity is a critical metric for SMX detection. Decorated Fe_3O_4 MNPs modified electrodes displayed good selectivity for target molecules due to the existence of imprinting cavities. The concentrations were (0.79103 M for SMX, 0.58103 M for CEPH, and 0.55103 M for AMX) as indicated in Fig.22.1, and a strong oxidation signal was seen. Furthermore, as shown in Figures

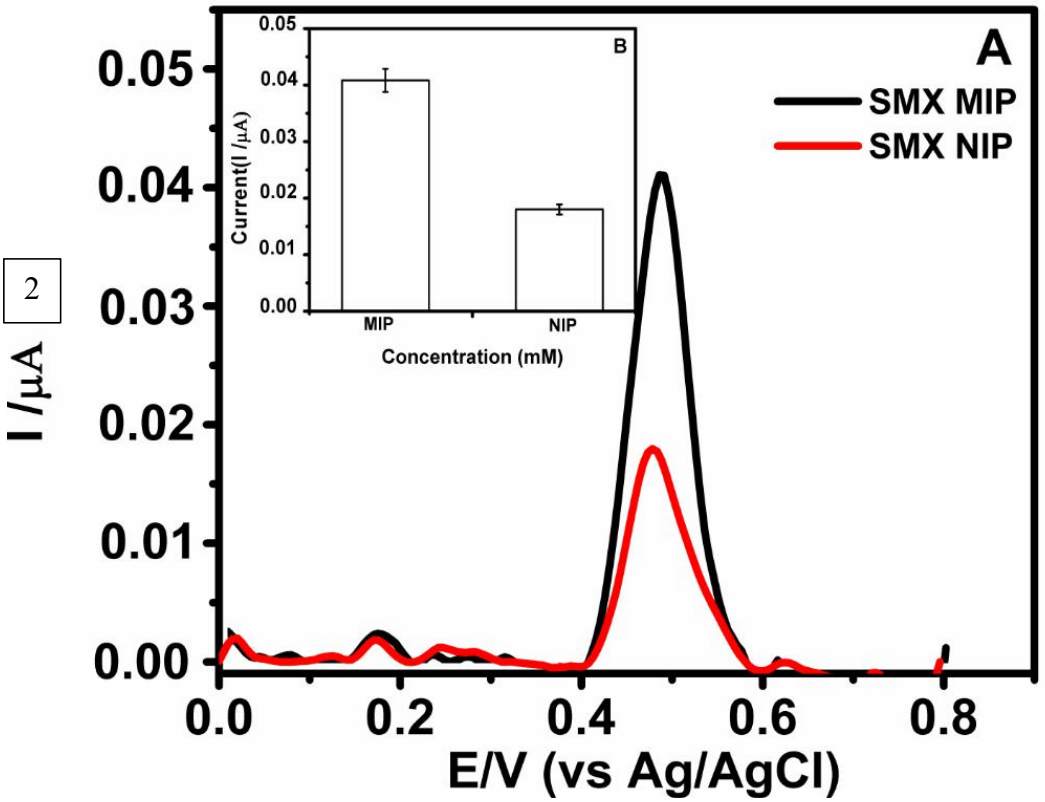
22.2 and 22.3, peak current changes following MIPs and NIPs capture for these samples at various concentrations (0.2103– 0.6103 M) for figure 22.2 and (20 mM to 100 mM) for figure 22.3 and showed no significant differences after NIPs capture in the various concentrations. MIPS, on the other hand, exhibited fluctuations in peak current. As a result, the imprinted holes with adequate diameters provide homogeneous functional groups for SMX molecule specific identification. As a result, it is clear that the sensor has a high selectivity for SMX detection.

Figure 22

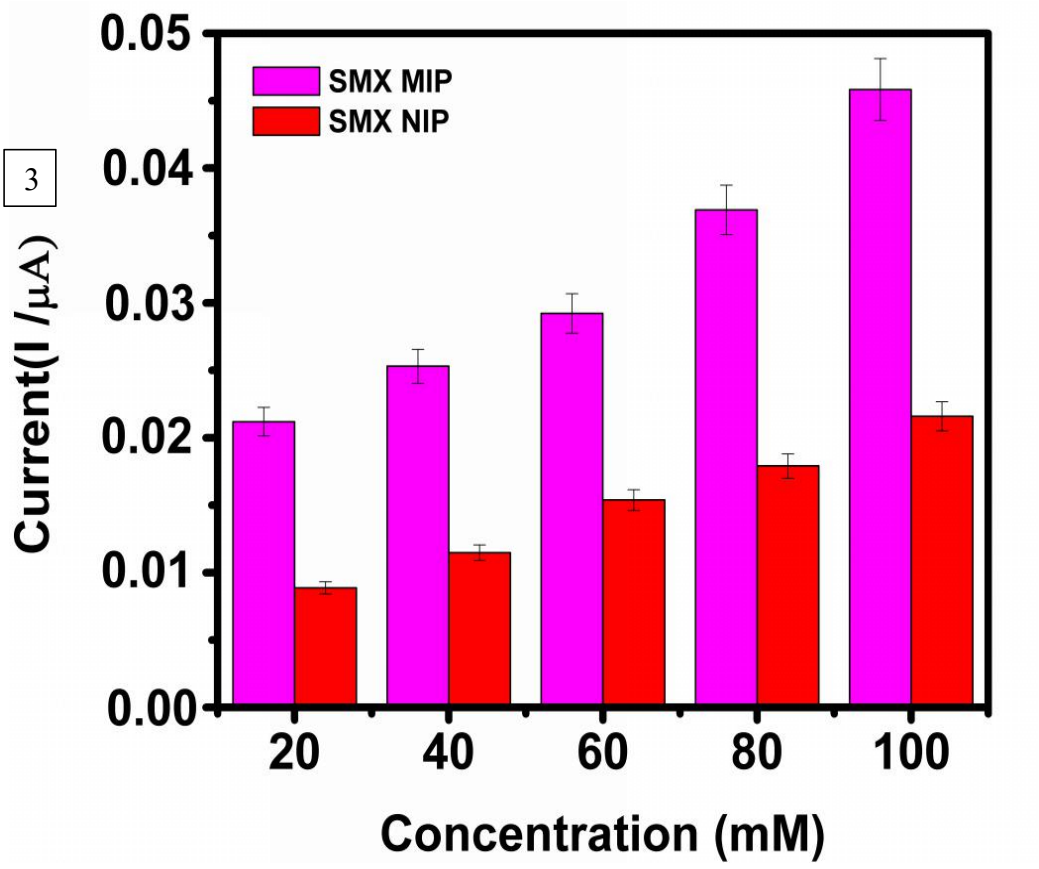
The Results were Obtained by Applying MIPs and NIPs with Determining Optimal Conditions to the Real Sample. 1)DP Voltammogram for SMX, CEPH and AMX 2)DP Voltammogram for Sulfamethoxazole MIP and SMX-Non Imprinted Polymer 3) Histogram for SMX/MIP and SMX/NIP in Different Concentrations.



2


A
SMX MIP
SMX NIP
B
Current(I /μA)
MIP
NIP
Concentration (mM)
I /μA
E/V (vs Ag/AgCl)

3


SMX MIP
SMX NIP
Current(I /μA)
Concentration (mM)

Detection of Sulfamethoxazole in Tap Water Samples

This electrochemical detection method based on MIP/Fe_3O_4 NPs/CPE detects SMX in tap water samples in order to evaluate its applicability in a real aquatic sample. The SMX content in the tap water, on the other hand, has been found to be too low for this sensor to detect. As a consequence, SMX was discovered at three distinct quantities in the samples. The analytical findings for the samples spiked with 0.2–0.6 mM of SMX are shown in Table 1. The recovery ranges from 98.0 to 103.7 percent, showing that the proposed assay can reliably identify SMX in real-world samples.

Table 1.

The Recoveries for SMX Spiking from Tap Water Samples.

	S/Value of SMX(mM)	Detection(mM)	Recovery(%)
Tap Water	0	Undetected	-
	0.2	0.2076	103.7
	0.4	0.3944	98.6
	0.6	0.6041	100.7

Table 2 compared the proposed sensor's functionality to that of other electrodes for Sulfamethoxazole. The MIP sensor obviously outperforms or is pretty similar to the mentioned electrodes of (LOQ), (LOD), and material used in the sensor's construction.

The SMX employed in this research was tested at doses ranging from 0.02 to 0.1 M. For the DPV Response, Formula (1) and (2) were used to derive the LOD and LOQ of SMX making use of peak currents, (Lister, 2005).

LOD = 3.3 x SA/$\dot{\alpha}$ **(1)**

LOQ = 10 SA/$\dot{\alpha}$ **(2)**

SA represents (calibration curve's slope), while the $\dot{\alpha}$ represents the (response's standard-deviation). Oxidation signals of SMX gradually rose when the concentration of SMX was raised from 0.02 M to 0.1 M.

Table 2.

Limit of Detection (LOD) and Limit of Quantification (LOQ) Reported for the Detection of SMX Compared to Other Methods Used.

Composites	Methods	LOQ (M)	LOD (M)	Ref.
MIPs/Fe_3O_4	DPV	6.45788 x 10^{-8}	2.1311 x 10^{-8}	In This Study
MIPs/Fe_3O_4	EIS	0.6598 x 10^{-8}	0.2177 x 10^{-8}	In This Study
MIP/BDD	CV,SWV,Raman Spectroscopy	-	24.1 x 10^{-8}	Zhao, et al (2015)
MWCNT SbNPs	Fifield Emission Gun, SEM, CV	-	2.4x10^{-8}	Cesarino, et al (2013)
SMX/MIP	Taguchi	-	1.2 x 10^{-9}	Arvand , Alirezanejad (2012)

CHAPTER V

Conclusion and Recommendations

Fe_3O_4 nano-particles have remarkable mechanical properties and a very large surface area, Sulfamethoxazole Surface Imprinted Core-Shell Magnetic Nanoparticles for selective identification of SMX were effectively manufactured. This work shows that several electrochemical measuring techniques may be used to assess the response of the Sulfamethoxazole Surface Imprinted Core-Shell Magnetic Nanoparticles carbon paste electrode. The developed voltammetric electrode is a promising screening sensor with a highly selective Sulfamethoxazole determination. The Sulfamethoxazole Surface Imprinted Core-Shell Magnetic Nanoparticles/CPE sensor was shown to be more sensitive, and had greater capacities for Sulfamethoxazole than the non-imprinted polymers (NIPs). Furthermore, MIPs were also discovered to be more diverse than NIPs. Template concentration, temperature, and cross-linking percentage were all examined during the imprinting process to corroborate these findings. Moreover, the given sensor has a low detection limit and has shown to have several advantages, including single-use, simple sample preparation, good analytical performance, low cost, and environmental friendliness.

The electrode had high electrocatalytic activity when it came to MIP oxidation for SMX. These analytes were measured at the electrode surfaces in ABS 0.5 M with a pH of 7.4. For the CV study, a scan rate of 0.20 V/s was used. From 0.0 V to 0.8 V, the DPV analysis was performed. EIS measurements were carried out in a redox solution of 5mM$[Fe(CN)^6]^{3-/4-}$ with a frequency spectrum of 100kHz–0.1Hz, including 0.1M KNO_3. When MIP levels in SMX and NIP samples are compared, MIP (as peak height) may be regarded a special measure for adulteration screening. This electrochemical technique is quite useful as a screening tool because of all of these alternatives. Sulfamethoxazole was effectively determined in water samples using the sensor, with recovery values ranging from 98.6 to 103.7 percent.

It would be advantageous to expand the study, because climatic circumstances, production, storage, and the application matrix may impact SMX,

and it would be also beneficial to take a different real sample such as a tissue or a muscle in poultry for more selective determination of the concentrations of SMX in it.

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APPENDICES

Appendix A

Turnitin Similarity Report

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