Production of Bio-Mimetic Nanoparticles for the Detection of Ascorbic Acid

INTRODUCTION

Background

Nanotechnology has emerged as a central field in the modern sciences since it enables manipulation of matter at nanoscale, where materials exhibit distinct physicochemical properties, unlike their large scale artifacts [1,2]. The size of nanomaterials is normally defined as 1-100 nm and is recognized by excellent optical, electronic, catalytic, and surface properties. These characteristics prove them to be very valuable in application in fields like sensing, catalysis, energy storage, biomedicine, and environmental monitoring[3].

Among such applications, chemical and biological sensing has gained especial prominence because analytical processes are increasingly required with a high level of speed, sensitivity, low cost and portability. Conventional techniques in analysis are accurate but may require complex equipment, elaborate sample preparation and trained personnel. Conversely, nanomaterial sensors are easy to operate, sensitive, and offer immediate feedback, which is essential in point of care and on-site analyses [4].

Nanostructured metal oxides and oxyhydroxides have seen high levels of research in the recent times due to morphology, surface area, and the chemical durability along with the redox properties [5]. These substances play a key role in sensor creation, especially in colorimetric detection packages where the detection process is run by the surface-mediated redox reactions.

The Importances of Ascorbic Acid

Ascorbic acid (AA) is one of the most important consumable water-soluble vitamins that plays a significant role in the human body [6]. AA serves as a potent antioxidant because it donates electrons to deactivate the presence of free radicals and reactive oxygen species; thereby protecting the elements of cells against oxidative damages [7]. Ascorbic acid is very important for overall health. Ascorbic acid in the body would be abnormal, and in this case, it causes diverse health complications. Deficiency may cause scurvy which is characterized by fatigue and bleeding gums, anemia and suit slow healing of wounds, but excessive intake may result in gastrointestinal complications and the development of kidney stones. This is because ascorbic acid is a critical

determinant in biological functions and therefore, the need to measure the ascorbic acid in biological fluids, drugs, food, and beverage accurately is critical. This leaves development of reliable, fast, and sensitive systems of AA detection as an important research field [8,9].

The Redox Chemical of Ascorbic Acid

Ascorbic acid is a powerful reducing agent, which is easy to give electrons in redox reactions due to its enediol structure. Oxidation of AA in water based environment results to dehydroascorbic acid after transferring two electrons [10]. Such a redox property renders AA extremely reactive to the metal ions and metal oxide surfaces, as the basis to its detection concept of redox-based sensing probes. In colorimetric assays, ascorbic acid usually stimulates or suppresses the oxidation of chromogenic substrates because it is going to eat up reactively or reduce metal centers. The extent of color change is directly associated to the concentration of ascorbic acid, thus can be quantitatively measured [11].

Non-electronic Techniques of Ascorbic Acid Detection

Other types of analytical methods that have been reported to determine ascorbic acid include the high-performance liquid chromatography [12], capillary electrophoresis [13], spectrophotometry, fluorescence [14] and electrochemical sensor [15]. Although these methods are very sensitive and a good source of accuracy, they are always associated with some disadvantages including high cost of operation, time consuming, and they are not mobile. Although electrochemical methods are sensitive, they are subject to a number of problems such as electrode fouling and contamination on other materials. The use of fluorescence methods involves labelling reagents and advanced machinery. Such drawbacks have led to exploration of other detection schemes including colorimetric sensing [16].

Colorimetric Detection

Colorimetric type of detection is based on chemically observed color changes due to chemical reactions or catalytic processes, and this type of detection can be identified with a naked eye or measured with a UV-vis spectrophotometer [17]. The procedure usually entails an oxidation reductions reaction integrated with the sensing material that leads to alterations in the intensity of the absorbance or wavelength.

Colorimetric sensing boasts of various major advantages, including ease of operation, instant response, low costs, less equipment needed, and real-time and on-site analyses. Such features

render the colorimetric methods especially inviting when using them in biomedical diagnostics, environmental analysis, and food safety [18].

Nanomaterials in Colorimetric Sensing

Due to their high surface volume ratio, discrete catalytic properties and potential to serve as the imitator of specific enzymes, referred to as nanozymes, nanomaterials enhance the efficacy of the colorimetric sensors significantly [19]. Metal oxide nanozymes catalyze the oxidation of chromogenic substrates which exhibit strong and persistent colorative signals. Nanostructures in a single dimension such as nanowires offer some added advantage, such as directional electron transport, structural stability and efficient diffusion of analytes. Such characteristics make sensors more sensitive and responsive [20].

MnOOH and Manganese-based nanomaterials

Nanomaterials based on manganese have attracted increasing attention due to different oxidation states ($Mn^{2+/}Mn^{3+/}Mn^{4+}$), eco-friendliness, and strong redox activity [21]. Of them, manganese oxyhydroxide (MnOOH) is particularly outstanding since it has a layered crystal structure, chemical integrity, and catalytic properties. Some of the polymorphic forms of MnOOH are α-MnOOH and γ-MnOOH that possess distinct crystal structures and surface features [22]. The polymorphs influence the rate of electron transfers and catalytic activity both of which are vital in sensing roles.

MnOOH Multilayer Nanowires

Nanostructures of MnOOH may be produced in various shapes, including nanoparticle, nanorod, and nanowire shapes. Among these, the multilayer nanowire designs have hierarchical porosity, increased active surface area, and enhanced mass transfer. The multilayer structure contributes to an increase in structural stability and the presence of plenty of reactive sites, which facilitates an equivalent level of interaction with the ascorbic acid molecules. This causes a peak catalytic activity and improved colorimetric response.

Compared to other Metal Oxide Sensor

A variety of metal oxide nanomaterials like CeO_2,Fe_3O_4, and Co_3O_4 have been reported to be used in the colorimetric technique. Although these materials exhibit good catalytic performance they have disadvantages in the form of increased cost, complex synthesis or reduced stability. By comparison, the MnOOH has some advantages, including reduced toxicity, ubiquitous source,

strong redox capability, and facile production via hydrothermal technology. Its high sensing properties are also enhanced by the fact that it has a multilayer nanowire structure which makes it a better choice in terms of AA detection [23,24].

Hydrothermal Synthesis and Its significance

Hydrothermal synthesis is a versatile technic wherein nanostructured materials are obtained through the direction of temperature and pressure in water-based solutions [25]. In this method, the size, background, and crystallinity of particles can be controlled in a precise way. Hydrothermal synthesis in the case of MnOOH nanowires promotes directional crystalline growth and multilayered structures resulting in uniformly crystalline and uniformly formed structures, which are perfect to use in sensing [26].

Ascorbic acid Detection by MnOOH

Colorimetric assay on ascorbic acid with MnOH nanowires is mostly based on redox reaction involving AA and the centers of manganese. Ascorbic acid decreases Mn^{3+} to decrease oxidation levels, which alters catalytic oxidation of chromogenic substrates leading to a particular color change being observed [27].

Application in Real Samples

The colorimetric sensor derived in terms of MnOOH is applicable in the detection of ascorbic acid in actual samples, such as human serum, juices, and pharmaceutical samples. High recovery rates and low RSD indicate the reliability and reproducibility of the sensor [28].

Research Gap and Motivation

Despite all the relevant studies on colorimetric sensors based on metal oxides, the literature has limited itself on the study of MnOOH that focuses on multilayer nanowire sensors to detect ascorbic acid. The need to have better sensor sensitivity, selectivity and ease of use continues to lure demand. This research aims to address this gap to develop a MnOOH multilayer nanowire based colorimetric sensor that offers an excellent analytical property.

Objectives of Current Research

The following can be also referred to as the primary aims of the research:

Synthesizing MnOOH multilayer nanowires via hydrothermal techniques Characterization of structural and morphological properties of nanowires Development of sensitive colorimetric detection of ascorbic acid, evaluating the analytical activity in case of real-life experiments.

Biochemical use of Ascorbic acid

At the molecular level, ascorbic acid forms an important cofactor in a range of enzyme reactions, especially reactions involving hydroxylation. It plays a role in the collagen synthesis process of the enzymes in keeping the iron and copper ions in their reducing state at their active sites like the prolyl and the lysyl hydroxylases [29]. This biochemical effect gives one a rationale why physiological systems need to have adequate concentrations of AA.

Immuno regulation is also played by Ascorbic acid through improving the functions of the immune cells like boosting their leukocyte activity, their chemotaxis and also their protection against oxidative effects. Additionally, AA is involved in conversion of dopamine to norepinephrine formation and also, in the production of neurotransmitters. All these numerous biological processes emphasize the necessity of the proper way of determination of AA in clinical diagnosis.

Ascorbic Acid Degradation and Stability

Ascorbic acid is a naturally volatile material that tends to disintegrate whenever it comes into contact with light, heat, oxygen and alkaline pH environment. It is observed that in both pharmaceuticals and food product AA degradation will cause major nutritional depreciation. Consequently, one of the aspects that will be critical is to monitor AA levels in processing, storage and distribution [30]. The other problem of analysis is that AA is unstable, measurements may not be accurate due to the fact that it is easily oxidized. Colorimetric sensing with the use of quick redox approaches are particularly applicable in the listed problems due to the fact that they can be applied to detect PAQs at a earliest stage prior to severe degradation taking place.

Analysis issues in the detection of Ascorbic acid

It is not very simple as the ascorbic acid is selectively determined, and occurs as a result of the presence of the other substances like, glucose, uric acid, dopamine and other antioxidants which are commonly found in the biological and food samples. These substances will have the tendency of behaving alike in regard to redox reactions and thus they may give untrue positive results in the traditional sensing systems [31]. Nanomaterial colorimetric sensors have high selectivity to dependent on surface chemistry on the one hand, and catalytic selectivity and regulated reaction

kinetics on the other. The nanowires of MnOOH, multilayer are selective to react with AA since their redox potential is good and sticks on the surface.

Surfaces and Crystal Structures of MnOOH

The crystal structure of MnOOH consists of MnO_6 octahedra which are shared at their edges forming the layered structures. There are hydroxyl groups present on the surface which are crucial in adsorption and redox processes. Hydrogen bonding and electrostatic interactions with ascorbic acid molecules are enhanced by these functional groups [32]. Furthermore, defects at the surface, oxygen vacuity and mixed states enhance catalytic activity through electron exchange. Such structural principles render MnOOH a good nanozyme in colorimetric sensing.

Influence of Morphology on the Sensing Performance

The shape of nanomaterials are also crucial in determining their sensing properties. Nanowires with one dimensional orders enable direct transition routes of transport of electrons and contribute to reducing the possibility of recombination of charges. Also, nanowires in multiple layers increase the surface area of operation and diffusion of the analytes inside the sensing matrix [33]. MnOOH multilayer nanowires are sensitive, respond faster, and have higher stability when compared to the nanoparticles and bulk materials are particular importance is the fact that these benefits are particularly useful in low values of AA.

UV- visible Behaviour and Optical Properties

Colorimetric sensing applications of MnOOH nanostructures heavily depend on optical characteristics of the nanostructure. MnOOH exhibits clear-cut absorption patterns of the UV-visible spectrum and is attributed to d and d transitions and charge-transfers. These absorption characteristics are altered in the presence of ascorbic acid, and they produce spectral changes. Quantitative measuring of vitamin C can be done by monitoring the shift of absorbance intensity at specific wavelengths. The procedure is both very sensitive and reproducible and simple to apply.

Economic and Environmental Relationships

Materials that are sensitive to the environment and are relatively cheap must be put into consideration in the creation of sensors. Among the metals, manganese is a good substitute of the noble metals since it is abundant, cheap and less toxic. MnOOH-sensors are therefore a green alternative to the colorimetric sensors that require gold or silver. Further, the hydrothermal

synthesis process principle utilizes low energy consumption/power and does not employ hazardous compounds, an additional factor to enhance the sustainability of the intended sensing platform.

Point-of-Care and On-Site Analysis Relevancy

Colorimetric sensors based on MnOOH multilayer nanowires designed as a multilayer nanowire is suitable to use in terms of point-of-care diagnostics and to analyze on-site due to the simplicity of their structure and a short response time. The semi-quantitative analysis is made possible by the change in color which is visible and should not require complex equipment as can be beneficial in resource limited settings. The sensing method can be integrated within portable devices, paper based systems and the test kits and hence it can be used in a broad scope of applications.

Future of MnOOH-Based Colorimetric sensor

The MnOOH-based colorimetric sensors of the future could be focused on surface modification, composite formation, and adapting them to digital imaging capabilities that would enhance the sensitivity and selectivity. Improved stability and performance would be obtained through the use of of MnOOH with carbon materials or carbon-based polymers. It is hoped that with improvements in the field of nanostructure engineering and sensing modals more analytes of both biological and environmental importance will be monitored by MnOOH based sensors in the future besides ascorbic acid.

General importance of the current study

The invention of MnOOH multilayer nanowires and their use to detect ascorbic acid is a step in the right direction towards nano meter-based sensors. In essence, this new system provides simplicity, sensitivity, selectivity, and sustainability; it is able to overcome the existing weaknesses in the traditional methods of detection. The study adds new layers to a rapidly growing area in the nanozyme-based colorimetric sensors and provides a solid foundation on the direction of future studies and applications.

Comprehensive review of the development of colorimetric sensors

The design of a colorimetric sensor should be carefully done with consideration of materials to be used, reaction mechanism, generation of signal, and the analysis ability of the sensor. The sensing material replaces the natural enzymes in the system with nanozyme which has advantages of stability, lower cost and has a long shelf life. MnOOH nanowires multilayers are successful nanozymes due to the presence of redox-active surfaces and resistant structures.

The nanozyme is used in a typical colorimetric sensing experiment, where the oxidation of an oxidisable chromogenic substrate resulting in a visible color change takes place. The presence of ascorbic acid interferes with such catalysis process by decreasing the thereby oxidized species or metal centers hence changing the intensity of color. This is an effective method in the detection of antioxidants because it is an indirect method.

Redox reaction Kinetics in Colorimetric Systems

The quick response and sensitivity of colorimetric sensors are crucial in nature of kinetics of a reaction. The faster rate at which the electronic transfer occurs between analyte and the sensing material leads to a faster rate of signal generation. The kinetics of MnOOH multilayer nanowires are favorable because of their 1D shape and a high concentration of active sites. The multilayer structure is favorable to effective diffusion of ascorbic acid molecules to minimize mass-transfer limitation. As a result, the sensor has a quick response, which is especially useful in the real-time and onsite analysis [34].

Effects of pH and Conditions of Reaction

The colorimetric sensors are very sensitive to the pH, temperature or duration of the reaction. In acidic medium the stability of AA is the highest in acidic environments and the optimize activity of MnOOH occurs in slightly acidic to neutral environments. Consequently, adjustment of the pH should be determined with much caution in order to achieve the best sensitivity and consistency. The temperature also helps to affect the rates of the reaction and the formation of color. Favorable reaction conditions are mild as it assists in ensuring sensor stability and it also inhibits both the degradation of the analyses, as well as the sensing material. These aspects are essential towards development of a reliable and strong sensing system [35].

Mechanism of Selectivity To Ascorbic Acid

Selectivity is of utmost importance in sensor performance especially in complex real world samples. The selectivity of MnOOH multilayer nanowires to ascorbic acid associates with the favorable coordination of redox potentials between atop Mn and AA. Other common interferents do not interact as strongly or as fast resulting in little interference. Surface hydroxyl groups and defects on MnOOH are an additional factor that enhances the selective adsorption of AA molecules. Such a selectivity naturally reduces the need to make additional changes to the surfaces or complex punishment of the samples.

Merit Figures for Analysis

The performance of colorimetric sensors is usually based on the analysis capabilities through the consideration of such factors as linear range, detection limit, sensitivity, reproducibility, and stability. The range of sensitization of MnOOH sensors is large, and the limit of detection is more than that of other types of sensors because of the good allosteric activity and high colorimetric formation. The long-term stability of the MnOOH is due to the influence of the long-lasting stability of MnOOH, and the reproducibility is ensured by the structural stability of multi-layered nanowires. These are highly appreciated aspects in application of analysis in practice.

As opposed to Enzyme-Based Sensors

Colorimetric sensing is commonly applied to natural enzymes like peroxidase that cause issues of being expensive and unstable besides being susceptible to environmental variations. The sensors based on nanozymes are the solution to these problems and can be characterized by a higher level of work durability and flexibility. The MnOOH multilayer nanowires are enzyme-like catalysts with no drawbacks to the biological enzymes and therefore it is ideal to carry out a routine analysis and to use it in the field.

Simple Detection Platforms Integration

Colorimetric sensing is easy and thus can be incorporated in low cost detection methods like paper based, test, and portable kits. The MnOOH nanowires can be put on other surfaces without losing its functionality so as to incorporate sensing tools that were convenient to operate. These platforms come in handy especially when it comes to point-of-care diagnostics, food quality, and environmental analysis where the principle will have to make a quick and at-the-playground diagnosis.

Societal and industrial Relevality

Since the proper determination of ascorbic acid is crucial to the field of healthcare, it is also crucial to the food and the pharmaceutical industry. The beverages, enriched food, and vitamin C supplements would require the application of the reliable methods of analysis to ensure quality. The colorimetric sensors monitored periodically are replaced by the MnOOH colorimetric sensors, which are cost effective. Nutrition and disease diagnostics can be evaluated by the early diagnosis of AA in a medical workplace. The suggested sensing platform is hence highly important to the society and the industry.

Restrictions and Scope of the Study

Despite all promising outcomes of the MnOOH multilayer nanowires-based sensor, there are certain drawbacks. There are still issues to be addressed such as matrix effects in complicated samples and stability of long term storage that require development. These challenges will be overcome, which will enhance the application of the sensor. Although the given work concentrates on colorimetric sensing of ascorbic acid, the sensing method can be modified based on the other antioxidants and biomolecules with appropriate changes.

Final Statements of the Introduction

This comprehensive introduction provides the basis of developing MnOOH multilayer nanowires and incorporating it into the colorimetric determination of ascorbic acid. The studies filled a critical gap of mixing the components of nanomaterial science, redox chemistry, analytical chemistry, and biomedical relevance to provide an easy, reliable, and inexpensive way of sensing AA. This is built-upon in the subsequent chapters, including providing detailed experimental procedures, characterization results, and analytical evaluation which put together demonstrates that MnOOH multilayer nanowires offer potential to be a powerful colorimetric sensing platform.

The analysis of Ascorbic Acid in history

The analytical determination of ascorbic acid has been a topic of great interest in the chemistry and biochemistry disciplines over a long period of years. Incidentally, initial titrations on the basis of Iodine or dichlorophenolindophenol reagents were used. In spite of being simple methods, these were not sensitive and selective, particularly with complicated mixtures. With the improvement of instrumental analysis, the spectrophotometric and chromatographical techniques were invented which increased the accuracy of detection by great margins. However, such techniques were frequently accompanied by high-tech machinery and highly skilled work forces. With the development of nanotechnology, the development of contemporary sensing techniques that unite the aspects of sensitivity and simplicity of application has occurred, which is the most significant change in the analysis of ascorbic acid.

Development of Colorimetric Sensing Technologies

Colorimetric sensing developed over the simple dye-based schemes to advanced platforms that make use of the nanozymes. Conventional colorimetric methods were based on organic dyes and enzymes where there were problems with stability and limitation to operating conditions. With the

emergence of nanomaterials colorimetric sensing has received a new dimension due to the availability of stable, tunable, and highly active catalytic materials. Colorimetric sensor In colorimetric sensors, nanozymes have demonstrated excellent results in comparison to the conventional enzyme-based systems. They are durable and versatile thus they are well suited to a wide range of analytical applications including antioxidant detection.

Basic Concepts of Nanozyme catalysis

Nanozymes are cage materials which replicate the functions of enzymes. These nanozymes have the inorganic structure unlike the natural enzymes which can be denatured and destroyed. They have surface-active sites as well as mixed oxidation states and defects that give them catalytic properties. MnOOH multilayer nanowires serve as peroxidase-like nanozymes, which facilitates in the electron transfer reactions that are important in the colorimetric sensing. Multilayer structure increases the catalytic activity by adding the active sites and increasing their accessibility.

Thermodynamics Redox reactions in MnOOH

Thermodynamics should also be taken into account in order to understand the principle of redox-based sensing. The MnOOH reduction oxidation potential of the $Mn^{3+/}Mn^{2+}$ pair is similar to that of ascorbic acid making it to react easily in electron transfer reactions. This favorable thermodynamic orientation permits the selective elimination of MnOOH by AA that is vital in the detection of MnOOH by the colorimetric method. There is also the reproducibility of the sensing performance which is increased due to the stability of the redox system.

Determination of Surface Defects and Oxygen Vacancies

Oxygen vacancies and surface impurities highly affect the catalytic behavior of the metal oxide and oxyhydroxide nanomaterials. These defects are active electron transfer and adsorption sites in the case of MnOOH multilayer nanowires. Redox flexibility and rapid reactions with ascorbic acid molecules are enhanced by the presence of oxygen vacancies. It is also possible to regulate the conditions of synthesis, thus increasing the density of defects, and this increases sensing abilities.

Introduction to Biosensors

Biosensors are a type of analytical tools, which combine a biological recognition element and a physicochemical transducer to determine a certain analyte present. They convert a biological response into a measurable signal which may be electrical, optical, thermal or colorimetric. This invention, since the beginning of their development has led to the revolution of the science of

analysis because these biosensors result in quick, sensitive, and selective detection of biological and chemical molecules. Biosensors have wide application in medical diagnostics, in environmental monitoring, food safety, pharmaceutical analysis, and biotechnology. Their high throughput of real-time data with limited sample preparation makes them attractive particularly in point-of-care applications as well as on-sites.

Principle of Biosensors

The principle idea of a biosensor comprises of an interaction between an analyte and a biological recognition element. It is in this interaction that a physicochemical parameter is changed which is dissolved into a detectable signal by a transducer. Otherwise, an analyte in a standard biosensor system either interacts with or binds the bioreceptor, resulting in the transfer of electrons, a change in mass, heat generation, or optical variation. This variation is in turn amplified and transformed by the transducer to an output signal which can be measured.

Components of Biosensors

A typical biosensor is a combination of three main elements:

1. **Bioreceptor**: It is the biological part that will allow a particular interaction with the target analyte. Bioreceptors consist of such examples as enzymes, antibodies, nucleic acids, cells, and biomimetic materials, such as nanozymes.
2. **Transducer**: Transducer is the component that transforms the biorecognition event to an event that can be measured. Such transducers can be either electrochemical, optical, piezoelectric, thermal or colorimetric.
3. **The Signal Processor and Display**: The signal processor and display module reads the produced signal on the available transducer and shows the results in a format that can be readily understood (say; the absorbance readings or the color change observable).

Attributes of a perfect Biosensor

To reach a reliable analytic performance, the ideal biosensor has to have the following main characteristics:

- High sensitivity and low level of detection
- Great specificity of the analyte
- Quick response time
- Good ability to reproducibility and stability

- Wide Linear detection range
- Very cheap and also easy to produce
- Skill in real sample analysis

The colorimetric sensors based on MnOOH satisfy most of these conditions due to their high level of redox energy, solid structure, and an easy detection mechanism.

History of Biosensors Development

The idea of biosensors dates back to the year 1962, when Leland C. Clark Jr. designed the first enzyme biosensor that developed into a glucose biosensor. The innovation was a historic milestone that formed the basis of the modern technology of biosensors. The next decades were characterized by the rapid development of biosensors, development of the immunosensors, DNA sensors, and entire cell biosensors. In the early 21 st century, there was a major advancement as nanotechnology was incorporated and this led to better sensitivity, miniaturization and multifunctionality.

Types of Biosensors

The biosensors may be grouped by the nature of the bioreceptor, or the transduction mechanism. Classification on the basis of Bioreceptor.

Classification on the Basis of Bioreceptor

- **Enzyme-based biosensors**: Use enzymes in order to selectively detect substrates
- **Immunosensors**: They rely on the affinities between antigens and the antibodies
- **DNA/RNA biosensors**: This identifies genetic material via the hybridization process
- **Cell-based biosensors**: Entire cells serve to identify biosensors

Nanozyme based biosensors Incorporates nanomaterials that can replicate the action of an enzyme, e.g. MnOOH

Transducer-based classification

- Biosensors are electrochemical sensors
- There are optical-based biosensors
- Piezoelectric technology is used in biosensors
- Thermal change biosensors
- Colorimetric based biosensors

The given research falls into the category of the colorimetric biosensor based on nanozymes.

Applicability of Biosensor Concepts to the Current Research

Understanding the principles, components and features of biosensors is very important in designing effective sensing platforms. The colorimetric sensor proposed in this paper, the MnOOH multilayer nanowire, can be considered as the paradigm of biosensor technology, even though this sensor does not rely on traditional enzymes of body. The present paper remarks on how the integration of nanozyme catalysis and colorimetric signal transduction can be employed to demonstrate how modern biosensors can be highly performing without being complicated or delicate.

Making the connection between Biosensors and Colorimetric Nanozyme Systems

Nanozyme-based sensors serve as a link between traditional biosensors and entirely chemical sensors, offering enzyme-like precision without the issues of biological instability. MnOOH multilayer nanowires are a new type of functional nanomaterial that can effectively perform biosensor functions. This combination underscores the interdisciplinary nature of current research, merging materials science, analytical chemistry, and biosensor technology into a cohesive sensing platform.

Literature Review

Shicheng Hou et al. (2018) introduce a colorimetric detection system of ascorbic acid, using a catalyst of copper–rhodium nanozyme (Rh-Cu). This nanozyme is effective in oxidizing TMB to the blue oxidized form and ascorbic acid which is a strong reducing agent returns the oxidized TMB to be colorless. The concentration of ascorbic acid is determined by the extent to which this color changes. Critical parameters of the operations including the level of nanozyme, concentration of TMB, pH and temperature were optimized to increase sensitivity. In this case, the technique provides an easy and inexpensive way of quantitatively analyzing ascorbic acid in different samples [36].

Siqi Guo et al. (2019) offers a new method of identifying ascorbic acid (AA) by using a PtRu nanozyme that has a high oxidase-like activity. RuCl 3 and chloroplatinic acid were mixed with PVP (3:1, 1:1, 1:3) to produce three PtRu nanozymes. TMB was used to test their catalytic activity, and the catalytic efficiency of the sample A (3:1 ratio) was the most efficient. The best catalytic factors, such as temperature, PH, and the concentration of both the TMB and nanozyme were later established. When working under these optimal conditions, the system was good at detecting AA in a linear range of 0.01 to 0.27 mM [37].

Qianqian Chen et al. (2019) presents a colorimetric method of ascorbic acid (AA) detection using multi-metal cubic nanozymes, which is a Prussian blue analogue formed by co-precipitation between copper, cobalt, citrate, and ferricyanide. These nanozymes show peroxidase-like activity that can be regulated by different factors like pH, nanozyme concentration, the amount of H 2 O 2 and amount of the substrate, TMB. The best performance of the assay is under the following conditions: pH 4.0, 12 uL of nanozymes, 20 uL of 20 mM TMB, and 35 uL of 200 mM H 2 O 2. The ascorbic acid can be accurately identified under the given conditions in the linear range of 0 to 60 mM and the working range of 10 to 120 mM [38].

The invention introduces an innovative method of detecting ascorbic acid in living plants with the help of a microelectrode biosensor. This method represents a microelectrode array and this array is interconnected to a signal acquisition unit, and electrochemical workstation. The sensor is calibrated with ascorbic acid standards in order to come up with a mathematical model that can be used to relate the amount of electrical current with the concentration levels. After the calibration, the biosensor is put into the plant to collect real-time data, thus, allowing to evaluate ascorbic acid

levels. The system will be designed in such a way that it can be portable, easy to use, applicable in the field and can also be continuously monitored [39].

The current invention is connected to a color developer, the way it is synthesized, and used. A cobalt oxyhydroxide nanosheets suspension is mixed with an aqueous solution of 3,3,5,5'-tetramethylbenzidine to make this color developer. The molar ratio of cobalt oxyhydroxide nanosheets to 3,3', 5,5'-tetramethylbenzidine is kept at 2:1 in this formulation. This agent is used in detection of ascorbic acid, and it is very sensitive with low detection threshold. [40].

The invention introduces a simple colorimetric system of ascorbic acid detection using the Ce(IV)/TMB system. Under this protocol, Ce (IV) oxidizes TMB to produce a blue TMB imine salt. When ascorbic acid is introduced, this blue compound is reduced to change its color and this color is linked to the concentration of ascorbic acid. This method creates a linear relationship in which measures will be possible to swiftly and efficiently detect a real-time solution. It is highly sensitive (detection limit of 0.08 0M) and has very good selectivity and anti-interference [41].

Chuan et al. (2021) used the Light-on Colorimetric Assay of ascorbic acid (AA) is a sensitive label-free colorimetric assay that utilizes Fe-MIL-88. AA leads to the occurrence of hydroxyl radical, which enhances the colorimetric reaction of TMB-H 2 O 2. It is a simple, cost-effective, and precise technique that can be detected as low as 0.5 -40 5M with 0.12 5M limit of detection when applied to serological tests [42].

Fahime et al. (20024) developed β-Mn2V2O7 nanoparticles capable of functioning as natural enzymes for the generation of low cost, high performance colorimetric sensors. These nanoparticles generate ROS which aids o-phenylenediamine (OPD), to change the color. Because vitamin C (ascorbic acid, AA) can halt this oxidation by hindering ROS, the sensor senses AA according to the reduction in color intensity, which is recorded by UV-vis spectroscopic techniques. It is simple, sensitive, and suitable for AA detection. it has a very low detection limit (0.47 μM, range: 1–10 μM) and works effectively in fresh juice [43].

Guangliang et al. (2025) designed and tested a quick and economical colorimetric sensor based on β-Mn 2V2O7 NP mimics of tardy enzyme for o-phenylenediamine (OPD). Since vitamin C (AA) abrogates this oxidation by scavenging OH• radicals, the sensor detects AA based on the sighted color intensity decrease with UV-vis spectroscopy. It has a detection limit of 0.47 μM (ranging from 1–10 μM) and performs favorably in fresh juice, making it sensitive, selective, and effective even in complex samples for AA detection [44].

A dual-signal sensor for ascorbic acid (AA) was established based on TSPP and DCIP. While TSPP fluorescence was quenched and the solution was turned blue in the presence of DCIP, AA reversed this effect and restored the fluorescence, while the color of the solution changed to green. The sensor was sensitively able to detect AA and it functioned in real samples. Thus, it was found to be useful as a portable visual detection tool [45].

Lu et al. (2024) designed Co-PB nano cubes as a stable oxidase mimic for H_2O_2-free colorimetric detection. The enzyme cascade transforms the AAP to AA, inhibiting TMB oxidation so AA (1.67 μM) and ACP (0.0266 U/L) can be detected. The approach was highly effective in both beverages and human serum, providing a speedy, sensitive, and reproducible detection platform [46].

Dong et al. (2023) fabricated Mn_3O_4@p-rGO nanocomposites for rapid, H_2O_2-free colorimetric detection of ascorbic acid (AA). The compound produces $^1O_2$ with an oxidase-mimicking property to oxidize TMB to its blue product, which can be reduced by AA for detection. The sensor, which can be used in juices and has a detection limit of 0.278 μM (detection range: 0.5–80 μM), is a straightforward and stable tool for testing food and diagnosing disease [47].

A peroxidase-mimicking IrO2 nanozyme on graphene oxide (GO) was fabricated. Its H_2O_2-mediated catalyzed TMB oxidation is inhibited by ascorbic acid (AA), thus a colorimetric AA detection method with a detection limit of 324 nM is realized. The assay was then utilized on real samples [48].

Preparation of single-atom Fe on N-doped graphene (Fe-CNG) exhibiting remarkable oxidase- and laccase-mimetic activities. It produces active oxygen species to enhance TMB oxidation, which can be suppressed by ascorbic acid (AA) and hydroquinone (HQ). It enables selective colorimetric detection of AA (0.048 μM) and HQ (0.025 μM). This strategy was recently validated for the determination of AA in vitamin C, thus paving the way for nanozyme-based sensors [49].

A cupper-based material (Cu-ICA) was prepared in an aqueous solution and exhibited excellent aqueous stability and oxidase-like catalysis of TMB oxidation. When combined, it allows colorimetric detection of ascorbic acid (0.5–5 μM, LOD: 0.1304 μM) and glutathione (0.5–4 μM, LOD: 0.097 μM). With its stability and efficiency, Cu-ICA may be promising for detection of reducing small-molecule compounds in aqueous systems [50].

The detection of ascorbic acid (AA) on a nickel-doped hydroxyapatite (Ni-HAp) biosensor with peroxidase-like activity was developed due to its change from blue green to colorless. It had a 2–

47 mM detection range and a limit of 0.11 mM, and worked in physiological solutions, providing an easy way to detect AA [51].

This research elucidated the oxidase-mimicking activity and reusability of Pt NPs with various morphologies. Compared with 5 nm Pt NPs, the self-assembled 30 nm Pt NPs exhibited higher reusability owing to their stacked structure that improves the transport of substances around the NPs. Detection limits of 131–152 nM was recorded for ascorbic acid using a colorimetric method with Pt NPs. The study shows that Pt Nano enzymes can be effectively reused in bioanalysis applications while modulating their functionality via facile synthetic routes[52].

Single-atom nanozyme (Fe-N-C SAzyme) was prepared through pyrolysis, exhibiting high oxidase-like activity for TMB oxidation. It facilitated two colorimetric based assays one for detection of ascorbic acid (AA) (0.1–2 μM, LOD: 0.1 μM) and second one for glutathione (GSH) detection (1–10 μM, LOD: 1.3 μM). This work provides a framework for designing functional SA zymes with the potential to broaden their application in biosensing [53].

For the quick (30 s) and sensitive fluorescence detection of ascorbic acid (AA), the study created (magnetic PBA-functionalized UiO-66) nanoparticles with a linear range of 5.0–60μM and a detection limit of 2.5μM. Selective AA detection in actual samples, such as vitamin C tablets, is made possible by the dual-functional nanoparticles' combination of hydrophilicity and boronate affinity. This technique provides a straightforward, quick, and accurate way to analyze trace AA in food and medicine [54].

By utilizing template less growth, a core-shell Fe_3O_4/CoFe-LDH hybrid was synthesized and then used as a peroxidase mimic for colorimetric AA detection. It exhibited excellent catalytic activity towards TMB oxidation, allowing detection of AA across 0.5–10 μM with a lower limit of detection at 0.2 μM. This magnetic hybrid can be easily recycled and has potential applications in biosensing, environmental monitoring, and medical diagnostics [55].

Xiufeng et al. (2024) used the microwave-assisted hydrothermal approach to create Mo-doped MnO_2 nanoflowers (Mo-MnO_2 NFs), which showed increased oxidase-like activity to oxidise TMB into blue oxTMB. Ascorbic acid (AA) and isoniazid (INH) both inhibit the process, making it possible to detect them with LODs of 0.579 μM and 0.436 μM, respectively. Drug monitoring and antioxidant sensing in actual samples appear to be promising applications for this sensitive and selective colorimetric platform [56].

Mishra et al. (2023) demonstrated an improved peroxidase-like activity of a Fe/Co-doped porphyrin MOF (PCN-224-Fe/Co) for the development of highly sensitive and specific colorimetric detection of ascorbic acid (AA) with a limit of detection of 0.2 μM [57].

A convenient smartphone-based colorimetric sensor using Zn/Co-derived nanozymes (BiM-ZIF-C) was developed for the fast detection of ascorbic acid (AA). They are synthesized via room-temperature pyrolysis and display robust peroxidase-like activities. Visual, colorimetric, and smartphone-assisted modes for the detection of AA with detection limits of 2 mM, 2.01 μM, and 2.26 μM, respectively. This method was validated with vitamin C tablets and provides a cost-effective and portable solution for food safety and health diagnostics [58].

Maria et al. (2025) developed simple, portable colorimetric sensing strips for the fast on-site detection of ascorbic acid in food and achieved the 5 mg/kg detection limit and 15–150 mg/kg linear range. Validated in juices and baby food, it provides an efficient alternative for quality control, at low cost [59].

Rocky et al. (2023) developed a dual-mode AgNCs@PEI sensor for ascorbic acid (AA) detection by fluorescence quenching and color change. The characteristic has high sensitivity, is executed in a hydrogel state, and exhibits accuracy in urine specimens, which executes its practical use [60].

Ziqi et al. (2024) constructed a PtTeNi-based nanozyme for sensitive colorimetric determination of ascorbic acid (AA), which the detection limit is as low as 2.94 μM. The system demonstrated high stability and accuracy in the analysis of AA in commercial samples, making it suitable for antioxidant assessment [61].

The invention introduces the way of quantitatively detecting ascorbic acid. This method makes use of the redox reaction between manganese dioxide nanosheets and ascorbic acid which causes the color to change in the manganese dioxide nanosheet solution. The change is then determined by placing an ultraviolet spectrophotometer that enables the quantitative determination of ascorbic acid. This is an easy and effective method with a high sensitivity at low cost [62].

It is an invention in nanomaterials and analytical chemistry, specifically, a color developer that determines the presence of ascorbic acid and a technique to determine its concentration. The mass ratio of cobalt oxyhydroxide, thiocyanate and Tween 80 is (9-18 μg): (2-10 mg): (1-10 mg). The distinguishing characteristics of this chromogenic agent are its low cost of production, high accuracy and speed of detecting ascorbic acid [63].

Yasunao et al. (U.S. Patent No. 4,303,409) presented a system of a complex of a chelating agent and a polyvalent metal ion in a higher oxidation state, an indicator that is able to react with the metal ion in a lower oxidation state, and a buffering agent. The given composition and test strip are used to analyze ascorbic acid in body fluids using colorimetric methods [64].

The sensor invented by (Kim Jong-seong et al. 2018) is a sensor composed of a graphene oxide-quantum dot complex, which is specially designed to detect ascorbic acid through photoluminescence, and a means of measuring this sensor. To be able to properly demonstrate the correlation of the quantum dot quenching reactions with the concentration of ascorbic acid, this composite sensor employs a sheet of graphene oxide, which is characterized by a high surface area and a high electron transfer potential. It is very sensitive and can determine the concentration of ascorbic acid as low as 568 pM. The sensor can be used to easily identify the concentration of ascorbic acid by measuring the intensity of emission in aqueous solutions [65].

The vitamin C is detected by the use of special material in the application. It consists of an ascorbic acid finding material of a kit. It is made of a certain formula, ca0.2Sr0.8Er3F11. It emits green and red light when struck by a laser of 808 nanometers. When it combines with ascorbic acid, the color of green fades and the color of red becomes stronger. This transition assists in the rapid and precise measurement of the quantity of ascorbic acid without having to touch it [66].

Yan Fang et al. (2022) just recently provided an enhancement of the creation of an AuRu nanozyme in the field of analytical chemistry. The methodology involves adding NH3BH3BH3 and having a reduction reaction, and subsequent sequential filtration, washing and freeze-drying to produce the AuRu nanozyme. This nanozyme can detect ascorbic acid at a concentration of 10-170 μM and the detection limit of the nanozyme is 2 μM. It also shows a high level of resistance to interference caused by dopamine, glycine, and phenylalanine compounds. Applications of nanozyme have been done to measure the concentration of ascorbic acid in pharmaceutical formulations with satisfactory results [67].

Fang Yanjun et al. (2021) presented an innovation in the field of analytical and detection technology, namely the process of ascorbic acid identification, as well as a detection preparation and kit. It is an innovation that uses cobalt oxyhydroxide nanosheet together with a 3,3,5,5'-tetramethylbenzidine detecting system, blue carbon dot detecting system. In this arrangement, the concentration of the target is aligned with the color space value thus making it easier to detect. Characteristic of the method, it has a high speed of detection, a high level of efficiency, real-time

communication, and sensitivity, which means that there is a great potential of application in field detection [68].

Ning SUI's et al. (2016) invented ways of stabilizing gold nanoparticles (AuNPs) in aqueous solutions with tripolyphosphate (P3O105). The AuNPs were also stable on the presence of, Cr2O72-, Cr(VI), but with the presence of Cr3+, Cr(III), aggregation was accomplished by metal-ligand interaction. The aggregated AuNPs were coloured differently as compared to the dispersed ones. Ascorbic acid (AA) was identified by the development of a colorimetric assay involving the use of AuNPs-Cr(VI). In the presence of AA, the Cr (VI), was reduced to form Cr(III), leading to aggregation of AuNPs. The essay exhibited good selectivity and linear response between 0.2 and 10 -1M and had a 0.15 -1M detection limit [69].

Nanozymes are necessary in the sphere of food analysis, but there are some difficulties caused by the complexity of materials and methods of their production. The design of Cu-GSSG nanozymes was carried out in an environmentally friendly manner, which is based on the detoxification mechanism of glutathione in living organisms. The nanozymes were incorporated into filter paper to make a smartphone-compatible sensor that could be used to detect ascorbic acid (AA). Operating in similar fashion to peroxidase enzymes, Cu-GSSG nanozymes had a detection limit of 2.9 μM and a linear range of 10-300 μM, which are outstanding anti-interference features of detecting AA in prickly pear juice and supplements. The method has a high potential to conduct quick screening of AA in fruits and supplements based on this bio-inspired method [70].

Ascorbic acid is a vital human nutrient though overdose may be dormant ensuring analytical strategies that are laboratory independent. A hand-held colorimetric detector has been created to measure ascorbic acid in-field through the reduction of phosphorus heteropoly acid in the creation of a blue compound. The heteropoly acid is bound to a polymer template in the presence of menthol as extractant to increase the stability. The sensor was proven to be reliable with the detection limit of 5 mg/kg and a linear range of 15-150 mg/kg. It determined the ascorbic acid in fruit juices and baby food with the 88-110% recovery resulting in possible on-site screening [71].

Ascorbic acid (AA), which is vital in fruits, is consumed to eliminate pathological conditions and oxidative stress such as cancer and cardiovascular diseases. A colorimetric sensor with assisted technology was designed in the detection of AA in fruits by the use of polydopamine-Fe (III) (PDA-Fe^{3+}) nanozymes. The oxidation of 3,3′,5,5′-Tetramethylbenzidine (TMB) with the hydrogen peroxide (H_2O_2) is catalyzed by these nanozymes to give blue colored oxidized TMB (

oxTMB). AA lowers the color of the oxTMB to blue. The sensor has linear range of 1.00 to 150 μM and a detection limit of 50.0 nM to 50.0 nM. The AA sensor built in this smartphone allows the user to obtain the results of the AA levels of fruits in a short time by using an application; the results of RGB showed that AA was detected in the fruits, such as kiwifruit, banana, and strawberry, indicating the usefulness of the sensor in assessing the nutritional status of fruits [72].

A Smartphone-digital image-based (DIB) processing L-ascorbic acid (L-AA) quantification colorimetric sensor on cotton thread is presented. Our thread based microfluidic analytical device (μTAD) is a redox reaction involving Prussian Blue (PB), whose blue color is decreased by L-AA. Conditions were optimized using central composite design with regards to sensor fabrication. The sample incubated in the microdevice (30 Unmarried units of food) exhibits a high level of linearity (R 2 = 0.993), low detection limit (0.18 mg L -1) and recoveries in the range of 87.2% to 106% with fruit pulps. There were no significant differences at the level of 95% confidence as compared to iodometric titration which confirms the reliability of the method [73].

Boron and nitrogen co-doped carbon dots (B, N-CDs) were produced by hydrothermal processing of 3-amino phenylboronic acid (3-APBA) as the precursor at a yield of 82.5%. The B, N-CDs exhibited a constant fluorescence emission with a quantum yield of 51.4 per cent in different conditions such as exposure to 4 M NaCl, storing 120 days, and exposure to 100 o C. Based on the boronate affinity of the boric acid groups fixed on B, N-CDs and the cis-diol structure of ascorbic acid (AA), a 41 nM limit of detection fluorescence assay of AA was developed by the interaction of the two molecules. Another assay involving hydroperoxide (H_2O_2)-boric acid redox reaction was also developed, with 4.5 nM detection limit. The samples of food could be subjected to detect trace AA and $H2O_2$ effectively with recoveries ranging between 82.5% and 115.75% [74].

Green spectrofluorometric (FL) techniques that are affordable improve the safety of ascorbic acid (ASA) formulation quality control. Complexity, slow response times, and costly equipment requirements are obstacles for ASA analysis. In this work, a microwave-assisted method is used to create natural carbon quantum dots (NACQDs) from pumpkin seed peels (PSPs). Transmission electron microscopy, scanning electron microscopy, energy-dispersive x-ray spectroscopy, and Fourier-transform infrared spectroscopy were used to characterize the NACQDs. When ASA caused fluorescence quenching, these NACQDs functioned as FL probes [75].

A core-shell Au@PdNi nanozyme was synthesized through co-precipitation of Pd (2+)and Ni (2+) onto Au cores. The oxidase (OXD)-like properties showed activity of 194.2 U mg. The high

activity resulted from the core-shell structure's extensive surface area, nickel enhancing current density, and Au core-PdNi shell interaction improving binding kinetics. The Au@PdNi nanozyme shows exceptional performance detecting ascorbic acid (AA), with high sensitivity and selectivity. A portable electrochemical sensor achieved satisfactory recovery in fruit sample analysis [76].

Dual-readout acid phosphatase (ACP) assay is a technique that has been devised to detect acid phosphatases using fluorescence and colorimetry. The technique involves aggregation-induced emission (AIE) in glutathione-protected gold nanoclusters (GSH-AuNCs) by the use of Ce(III) ions and the oxidase action of Ce(IV) ions. Ce(IV) ions produce blue oxidized 3,3,5,5-tetramethylbenzidine (oxTMB) using oxygen in the company of Ce(IV) ions as catalysts. ACP splits L-ascorbic acid-2-phosphate (AAP) into ascorbic acid (AA) and Ce(IV) to Ce(III) ion, and inactivates oxidase activity. GSH-AuNCs in the presence of Ce(III) ions causes an increase in fluorescence, which is an effect of AIE. This platform has unique detection limits of 0.101 U/L and 0.200 U/L, which are applicable in analysing ACP in human serum and screening inhibitors, are an ideal method of analysing ACP [77].

Ascorbic acid, which is necessary in the synthesis and metabolism process cannot be produced internally and therefore must be acquired in the diet. The loss in storage causes food concentration, which requires sensitive photoelectrochemical (PEC) biosensor detecting. The lack of PEC materials working in long wavelengths with low bias voltages is a setback in the biosensor development. The photocurrent of a 3,4,9,10-perylenetetracarboxylic dianhydride (PDA) self-assembly rod material is enhanced by ascorbic acid in the presence of light (630 nm) and a voltage (0 V) on the electrode vs. Ag/AgCl. This is a self-driven PEC biosensor with a linear response to 5 $\mu M.L^{-1}$ to 400 $\mu M.L^{-1}$, and the detection limit of 4.1 $\mu M \cdot L^{-1}$, providing a wide linear range and the capability to use in wine samples [78].

Methods & materials

Hydrothermal Method

Another widely employed technology in the synthesis of crystalline materials, in particular, nanostructured metal oxides, hydroxides, and oxyhydroxides, is the hydrothermal approach. This is a method used in which chemical reactions are carried out in a high pressure, closed-container, referred to as an autoclave, with water present under high temperatures, typically above 100 0 C. Hydrothermal synthesis provides high accuracy of particle size, shape, crystallinity and phase composition which is essential in the application of catalysis, sensing, and energy storage. Hydrothermal techniques have become popular during the last several decades to generate nanomaterials of consistent shape, high surface area, and defined structure, which is crucial in biosensing and catalytic applications [79-80].

Principle of Hydrothermal Method

A method that is used is hydrothermal synthesis where chemical reactions take place in high temperature and pressure in water-based solutions. Autoclave pressure: Interacts with autoclave used material to raise its solubility, allows an autoclave-supersaturation rate-controlled contraction to begin nucleation and accelerates diffusion to favour homogeneous crystal growth [81,82]. This method typically involves dissolution of the precursors into water, adjustment of the pH, encasing the mixture in an autoclave, and heating the mixture over a set period of time. Crystalline product can then be collected through filtration, rinsing and drying (following cooling) [83].

The Hydrothermal Method has the following benefits

The hydrothermal method has a number of major advantages:

Low-temperature synthesis: It is possible to synthesize crystalline materials at fairly low temperatures (100 °C to 250 °C) without involving high-temperature calcination [84].

Morphology control: Morphology and size of particles can be very accurately controlled by manipulating reaction conditions in terms of pH, temperature, and concentration of precursors [85].

High crystallinity: The hydrothermal process conditions support coming up with crystals without defects, enhancing the catalytic and sensing properties [86].

Environmentally friendly: The reliance on organic chemicals, which are harmful to the environment, is reduced as the primary solvent is water [87].

Scalability: This approach is flexible to be scaled down to laboratory use all the way into large-scale production [88].

Variants of Hydrothermal Processes

The hydrothermal synthesis can be classified into a variety of categories: The traditional hydrothermal procedure is using water as a solvent in an autoclave. Microwave-assisted hydrothermal technique makes use of heating through microwaves in order to obtain a fast and uniform synthesis. In the template-assisted hydrothermal method, surfactants or structure-directing agents are used to form nanowires, nanotubes, or more than two layers. The traditional hydrothermal synthesis is commonly applied in the production of MnOOH multilayer nanowires due to its repeatability, simplicity and capacity to produce quality end-products [89].

Materials

The reagents were analytical grades and were not taken any other purification.

Manganese triacetate dihydrate (134.05mg) was acquired at alfa aesar, Sodium hydroxide (0.6g) was acquired at Omicron and Hydrogen peroxides(H_2O_2) was acquired at Sigma Aldrich.

Synthesis of MnOOH multilayer Nanowires

The 134.05 mg of $C_6H_{13}MnO_8$ were then dissolved in 20 mL of distilled water with magnetic stirring at ambient temperature. This solution was vigorously stirred by spinning 30 minutes. NaOH was then added to the solution at room temperature and vigorous stirring was performed further over 30 minutes. It was stirred by vigorously shaking the mixture again (another 30 minutes), followed by the dropwise addition of 5 mL of H_2O_2, then placed in a Teflon-lined stainless-steel autoclave. The autoclave was incubated at 200 °C during 12 hours then allowed to cool to room temperature. The product obtained was repeatedly rinsed with the deionized water and absolute ethanol, then dried in a vacuum oven at 60 °C for 4 hours [90].

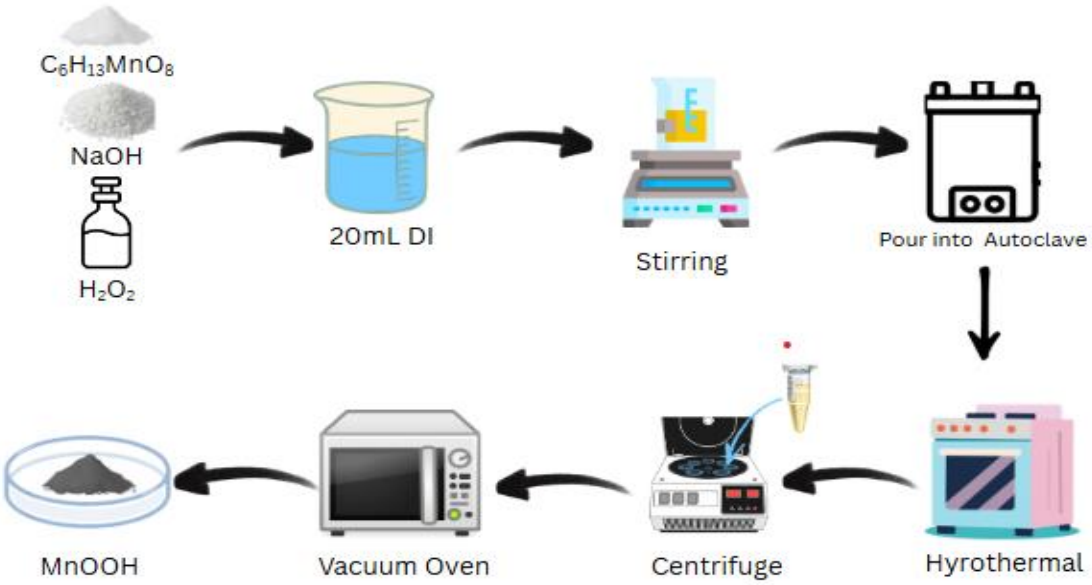

Figure 1: Hydrothermal synthesis of MnOOH Multilayer Nanowires

4- Chapter Result & Discussion

X-ray Diffraction

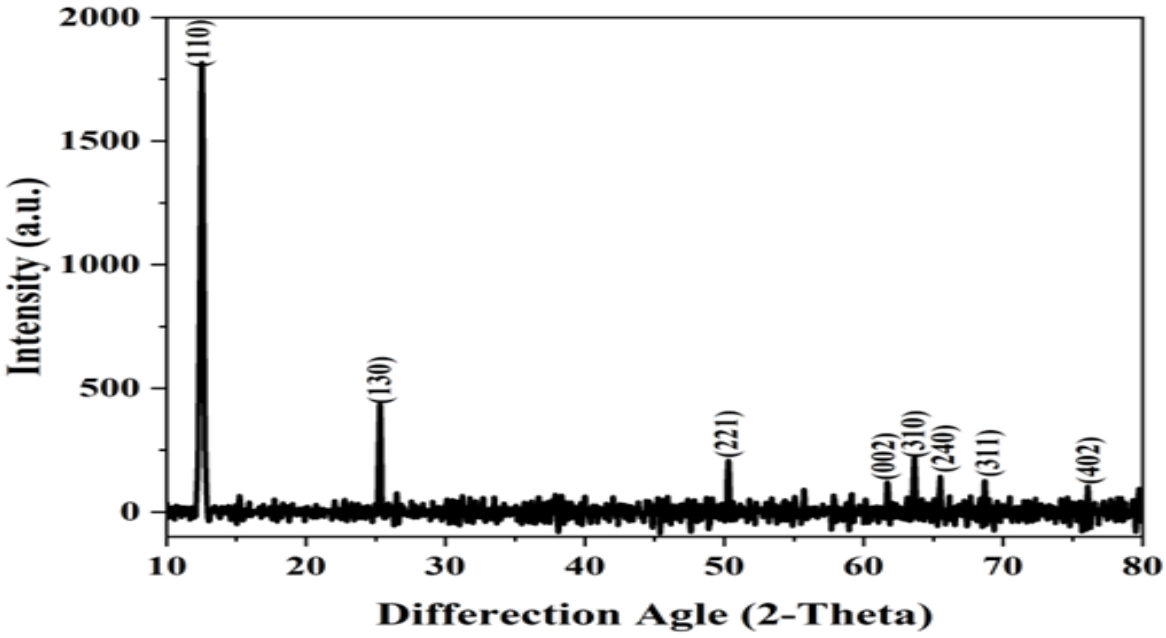

Figure 2: XRD Spectra of MnOOH

X-ray diffraction (XRD) was utilized to study the crystalline structure of the MnOOH nanowires that had been prepared by the use of the Cu K alpha radiation (length = 1.5406 A). The XRD pattern showed well-crystallized material as it had the following diffraction peaks indicating the presence of a crystal material at 2 theta- angles of 12.53, 25.30, 50.21 61.69, 63.59, 65.42, 68.68, and 75.94. The spacings between the planes (d-values) of such peaks were measured with Bragg law and these were in correlation with the standard orthorhombic MnOOH in the literature. Further analysis of the diffraction peaks was done by assigning Miller indices (hkl) to the different crystal planes that contributed to each of the reflections. The high peaks at 12.53 0 and 61.69 0 were explained as a result of the (110) and (002) planes, respectively, which prefers the growth orientation to the [001] direction, which is an expected behavior of one-dimensional nanowire morphology. Higher angle reflections of 50.21 ° (221), 63.59 ° (310), 65.42 ° (240), 68.68 ° (311), 75.94 ° (402) among others verified the orthorhombic crystal structure and reflected on the good crystallinity of the nanowires. Further peaks were not observed and the purity of MnOOH synthesized phase was confirmed.

The crystal size was calculated by determining the **Scherrer Equation**:

D= $\mathbf{K\lambda/\beta\cos\theta}$

Where;

$'D'$ is the size of particle

$'K'$ is Scherrer's constant=0.94

$'\beta'$ = 2.57 = which is the FWHM (full width half maxima) value.

$'\lambda'$ is X-ray wavelength (Cu-Kα) =1.5406 A°

$'\theta'$ is the angle of diffraction

Then;

D= $\mathbf{0.94\lambda/\beta\cos\theta}$

The mean size of crystallites constituting the Nanowires produced by the synthesis of MnOOH is 21.5nm, which determines the size of a crystal by the Debye-Scherrer equation.

Fourier Transform Infrared Spectroscopy (FTIR)

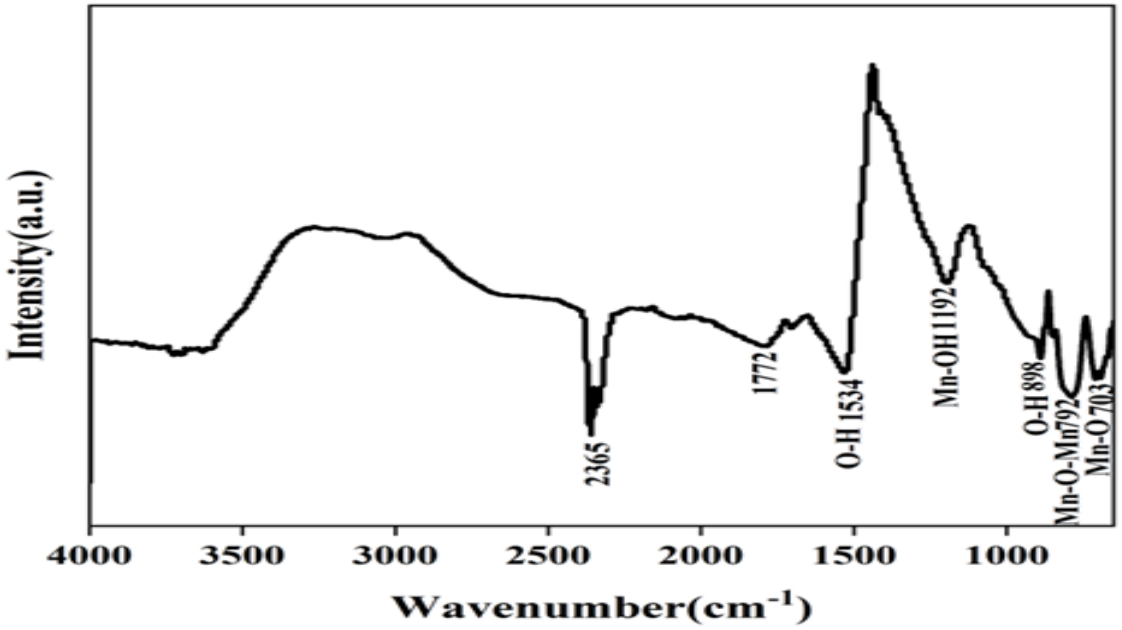

Figure 3: FTIR spectrum of MnOOH

In order to identify the functional group and bonding properties of the synthesized MnOOH nanowires, Fourier Transform Infrared (FTIR) spectrum of the synthesized MnOOH nanowires was obtained (Figure 3). The spectrum had different distinct absorption bands. The presence of a broad band in 3200-3400 cm^{-1} is suggestive of the O-H stretch which might be attributed to

adsorbed water or hydroxyl group in the MnOOH nanowires. The Mn-O and Mn-OH bending vibrations are associated with the 703 cm^{-1}, 792 cm^{-1}, and 898 cm^{-1} peaks, so the MnOOH phase was created. The high peak at 1772 cm^{-1} may be due to C=O of surface-bound species or small impurities and high peak at 2365 cm^{-1} is characteristic of adsorbed CO2 of the atmosphere.

Field Emission- Scanning Electron Microscopy (FE-SEM)

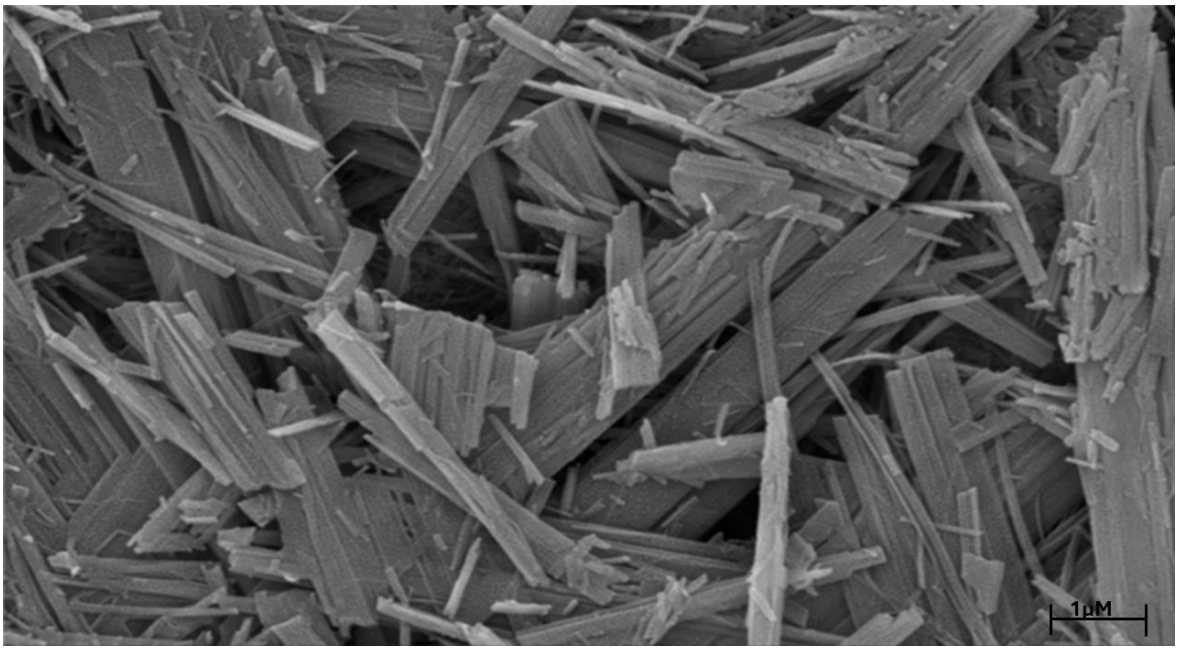

Figure 4: FE-SEM Image of MnOOH

To investigate the surface morphology of the MnOOH multilayer nanowires that were synthesized, an approach that was used was field-emission scanning electron microscopy (FE-SEM), as shown in Figure. In the SEM picture, there is a vivid demonstration of the network of rod-shaped nanowires an orientation of which is not selective as well as an interconnected architecture three-dimensional. Nanowires are regularly dispersed and have elongated morphologies with microscale lengths and nanometer scales of thickness, which implies a homogeneous nucleation process and regulation of growth throughout the synthesizing process. At increased magnifications, observations show that the nanowires have rough and layered surfaces thus defining that they are in multilayers. It is these crossed links of these nanowires that create numerous inter-wire chambers and open apertures creating a very porous structure. The morphology offers a high electroactive surface area, easy diffusion of electrolyte and analyte molecules, and improves electron transfer kinetics, which individually leads to the improved electrochemical performance of MnOOH multilayer nanowires in the sensing of ascorbic acid (AA).

Raman Spectroscopy

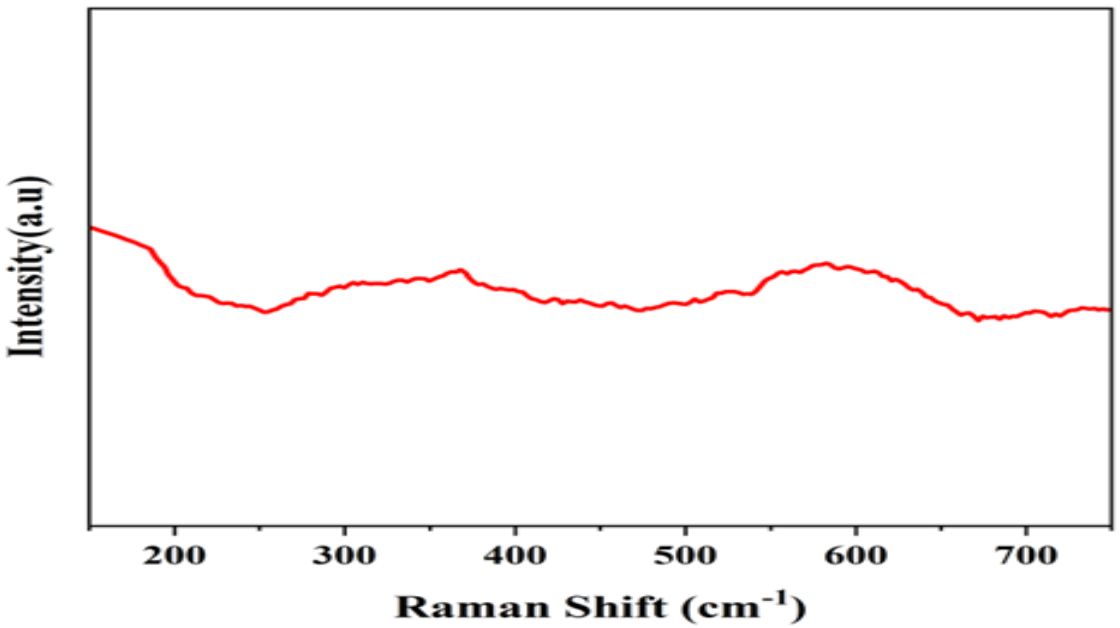

Figure 5: Raman Spectra of MnOOH

The vibrational mode of Mn-O lattice and embodying the crystal structure were identified by taking the Raman spectrum of the prepared MnOOH nanowires (Figure 5). Its spectrum had certain distinctive peaks at 367 cm $^{-1}$, 582 cm^{-1} and 610 cm $^{-1}$ which can be attributed to MnO. The peak at 367 cm $^{-1}$ is highest because it is linked with Mn-O bending vibrations through which is related to octahedral coordination of the Mn^{3+} ions to form complex and MnOOH. Mn -O stretching vibrations are attributed to bands with 582 cm $^{-1}$ and 610 cm $^{-1}$ and existence of Mn -O bonds, as well as an orthorhombic crystal structure is verified. The Raman findings are verified by the literature descriptions of the orthorhombic MnOOH along with being in line with the XRD data. The sharpness of the MnOOH nanowires is shown by its fact that they have sharp peaks and the fact that the Raman bands are sharp is its indication that the structure is free of serious defects.

Zeta Potential

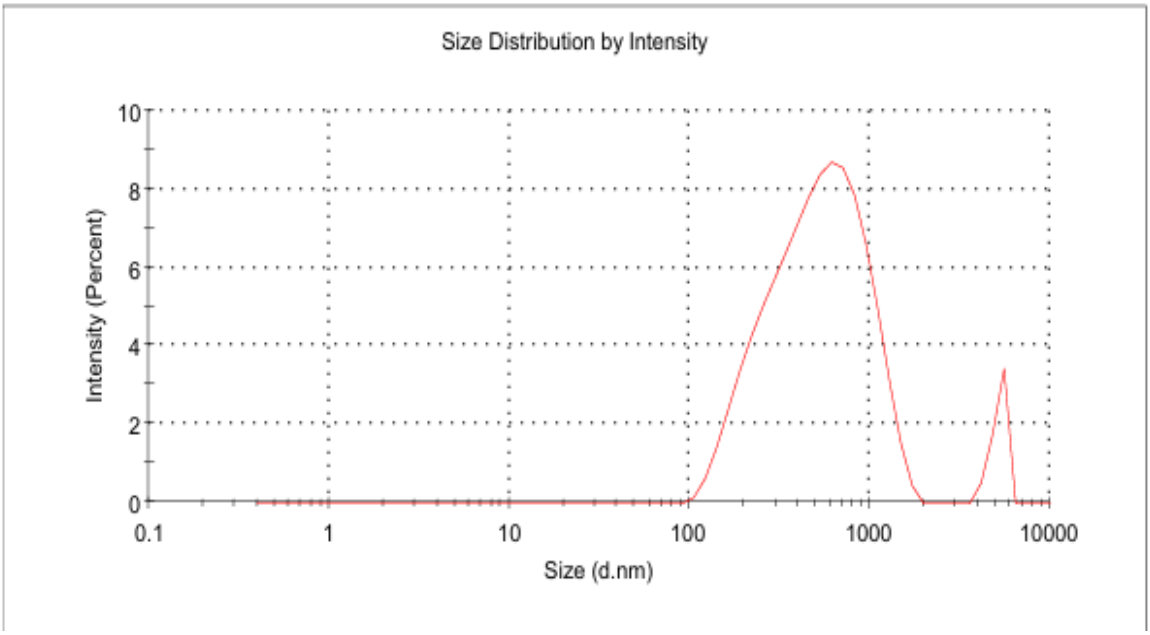

Figure 6: Zeta Potential values of MnOOH in DI-Water

The electrophoretic mobility (μ n) of the MnOOH nanowires was calculated using Henry equation that relates the movement of the particles within an electric field to their zeta potential:

$$\zeta=(3\eta\mu\_e)/2\varepsilon\ f(\kappa a)$$

Here, we consider aqueous systems, and the Smoluchowski approximation was applied which simplified the equation to:

$$\zeta=(\eta\mu\_e)/\varepsilon$$

This calculation was automatically done with the Malvern Zetasizer Nano ZS by considering the dispersant nature of the sample.

In order to determine the surface charge and colloidal stability of MnOOH nanowires in water, the zeta potential measurements were conducted. The zeta potential of the MnOOH nanowires was found to be negative and equal to -47.1 mV, which indicated a high level of electrostatic repulsion of the particles and high stability of the suspension. The reason behind this strong negative surface charge is the occurrence of the hydroxyl groups on the surface of the MnOOH particle that give a

total negative charge and hinder aggregation of particles, therefore creating the fine dispersion in water.

Dynamic Light Scattering (DLS) Particle Size Analysis

The images of the FE-SEM, used to represent the results of the particle size distribution analysis, showed the size distribution of the MnOOH nanowires to be rather narrow and homogenous, and the average size of the particles is 521.1 nm. Most of the measured particles are concentrated around this mean value which means that the growth was also consistent and the factors that led to the synthesis were also controlled. The observed distribution pattern shows that multilayer nanowires bundles were formed rather than single nanowires.

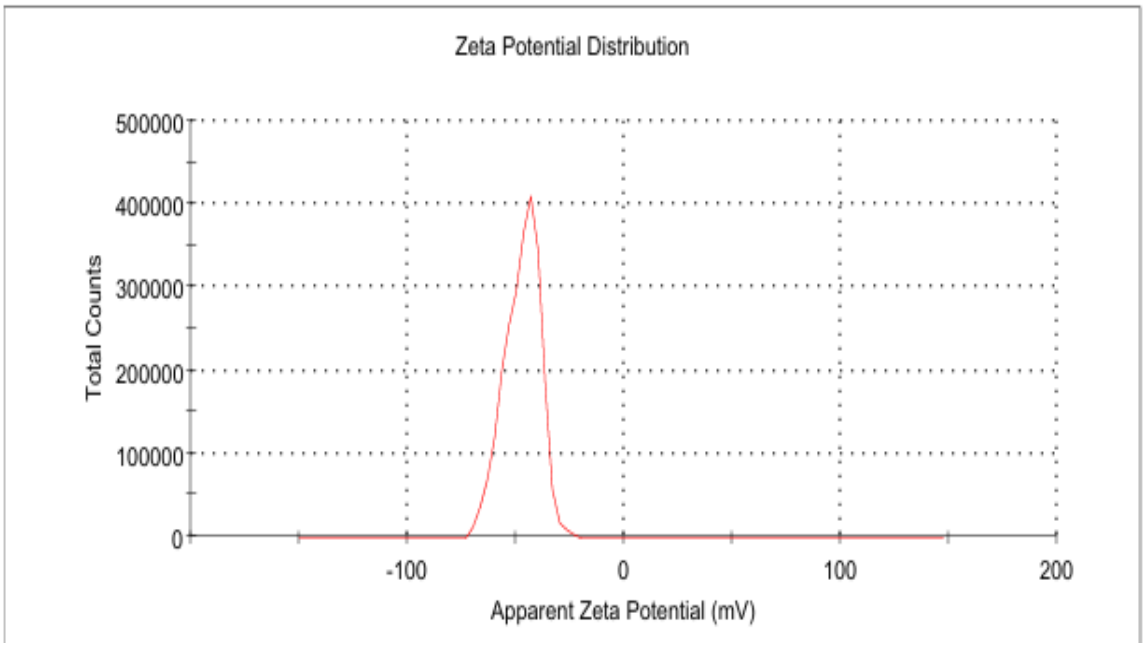

Figure 7: DLS size distribution of MnOOH nanowires

Optimization and the Mechanism of Colorimetric Detection of Ascorbic Acid using MnOOH nanowires Biosensors

Stock Solution preparation for Selectivity Studies

In a bid to determine the selectivity of the proposed colorimetric sensor to ascorbic acid (AA), stock solutions of both AA and other possible interfering substances were prepared under the same condition. The choice of these interfering analytes was due to the fact that they are representative of the biomolecules and ions that can be found in the real samples. Stock solutions (10mM) of ascorbic acid (AA), dopamine (DA), urea, uric acid, cholesterol, glucose, fructose, cystine, glycine, potassium chloride (KCl), and calcium hydroxide [$Ca(OH)_2$] were prepared by dissolving the corresponding amount of the compound in 5 mL of deionized (DI) water. All solutions were prepared fresh beforehand in order to avoid degradation or oxidation especially on AA and DA. The ready stock solutions were in turn diluted accordingly to suit the colorimetric experiments. The above solutions were used to examine selectivity and anti-interference property of the developed colorimetric sensing system, where color response of AA was compared with others biomolecules and ions under identical conditions of experiment.

Colorimetric Sensor Preparation

Colorimetric sensor was designed with the help of a simple solution-based method, where MnOOH was used as a catalyst, and 3,3′,5,5′-Tetramethylbenzidine (TMB) was used as a chromogenic substrate. At first, an exact amount of MnOOH was weighed and put into an Eppendorf tube that was clean. In order to maintain the necessary reaction conditions, sodium acetate buffer was placed to the tube to hold the needed pH level to the sensor system, which is essential in the activity of MnOOH in catalyzing the reaction and the successful oxidation of TMB. After the addition of the buffer, the mixture was stirred by gentle vortexing or shaking to distribute the suspension of the MnOOH particles evenly. A new and clean TMB solution was then added in the reaction mixture whilst mixing the two. Combination of MnOOH and TMB led to catalytic oxidation of TMB to form a clear blue colored product which formed the foundation of the colorimetric response. The equilibrating sensor system at room temperature took a constant amount of time to make sure that the catalytic reaction was complete. The resulting intensity of color was then observed or with UV Visible Spectrophotometry. The present MnOOH-TMB system offered a good and viable platform in which future colorimetric detection could be conducted. The selective detection of ascorbic acid

(AA) was done using the fabricated sensor in an analytical way. When AA was introduced, the color of the oxidized TMB was also reduced to its colorless color and the intensity of the color was observed that significantly reduced. The process of reduction enabled the quantitative determination of AA by variations in absorbance or visible color to show that the sensor can be used to conduct colorimetric sensing.

Colorimetric Ascorbic acid Detection

Visual Response of MnOOH-TMB System Visual Colorimetric

Research paper examined colorimetric determination of ascorbic acid (AA) at a MnOOH TMB system of sensing based on a redox change in color. The sodium acetate buffer was first added to stabilize the pH then 3,3′,5,5′-Tetramethylbenzidine (TMB) was added. MnOOH was an effective catalyst in the oxidation of TMB in the absence of AA and oxidized TMB (ox-TMB) was formed, with a characteristic dark blue color. This blue color ensured that the oxidation of TMB was successful and MnOOH was catalyzed. When AA was introduced into MnOOH-TMB system the dark blue color faded to a large extent. The higher the concentration of AA, the less the intensity of the color became until it was light blue. This color transformation was concentration-dependent and without a complicated instrumentation, showing the ability of the system to be able to visually detect AA.

Redox Reaction Mechanism

As the mechanism behind the detection of ascorbic acid (AA) by the colorimetry method, there is a cascade of oxidation-reduction reactions within the MnOOH-TMB system that dictate this process. At the first step, MnOOH promotes the oxidation process of TMB that results in the ox-TMB formation causing a blue color. The following reaction can be used to express this sequence:

Oxidation of TMB

$$\text{TMB (colorless)} \xrightarrow{\text{MnOOH}} \text{ox-TMB (Dark blue)}$$

Ascorbic acid is a strong reducing agent and transfers electrons easily to ox-TMB. The introduction of AA changes ox-TMB to its colorless form, whereas the oxidation of AA changes it into dehydroascorbic acid (DHA). It leads to a blue color loss in this reduction process.

Figure 8: Photograph of Oxidation of TMB

Reduction By Ascorbic Acid:

$$\text{ox-TMB (blue)} + \text{AA} \longrightarrow \text{TMB (colorless)} + \text{DHA}$$

The net redox process results in the decrease of absorbance strength of ox-TMB which is the axial redox indicator of AA detection. The high reducing capacity of AA enables it to interfere easily with the MnOOH-TMB oxidation equilibrium thereby making it easily differentiated among other analytes.

Figure 9: Reduction By Ascorbic Acid

The Anti-Interference Performance and selectivity

The selectivity of the MnOOH -TMB colorimetric sensor to ascorbic acid (AA) was evaluated through response in relation to the presence of many possible interference substances (e.g., dopamine (DA), glucose, fructose, cholesterol, urea, uric acid, cystine, glycine, potassium chloride (KCl), and calcium hydroxide [$Ca(OH)_2$]. They were chosen in order to depict frequent biomolecules and inorganic compounds that may co-exist with AA in real samples. In the selectivity test, each substance was added in equal portion to the pre-existing MnOOH -TMB detection system under identical conditions. After the addition of AA, the dark blue color faded rapidly and the color turned noticable, which showed the presence of oxidized TMB was successfully reduced by AA. On the contrary, there was no observed color change or response when dopamine or glucose or fructose or cholesterol or urea or uric acid or cystine or glycine, KCl ,$Ca(OH)_2$ was added to sensing system. The specific dark blue hue of oxidized TMB did not change, which indicated that these substances were not able to lead to the reduction of ox-TMB in the given experimental conditions. Markedly, even the redox-active dopamine failed to lead to any visible color change, water, amino acids, and compounds containing nitrogen did not any how interact with MnOOH-TMB system. Likewise, ions of KCl and $Ca(OH)_2$ inorganic origin did not cause any reaction and proved the high stability of the sensing platform to ionic species and the

absence of effects of interferences. The fact that the following interfering substances did not give any response indicates the high specificity and great selectivity of the MnOOH to ascorbic acid by the MnOOH sensor. The high reducing ability of AA is the major cause of such selectivity since AA can effectively reduce oxidized TMB, but the other materials do not have sufficient reducing power to elicit a colorimetric change.

Figure 10: Photograph of Selectivity of Ascorbic Acid against 12 Amino Acid

The benefits of Developed Colorimetric Biosensor

The MnOOH -TMB -based colorimetric sensor is characterized by many advantages, including user-friendliness, rapid response rate, high sensitivity, and capability of visual detection of the changes. It is simple to manufacture and does not involve the use of expensive machinery in the process of its manufacture, and therefore can be used in normal analysis. Also, the color difference between reduced and oxidized forms of TMB is highly specific, which allows the easy interpretation of the results. The stability and performance of the sensor is also enhanced by the use of MnOOH as a catalytic agent. The redox-based detection system will ensure a steady and reliable output which is essential in the real world.

Practical Significance

The MnOOH-TMB sensing system is sensitive with great selectivity and specific colorimetric response, which makes it promising to be used in the detection of AA in real-life samples such as foodstuff and biological fluid samples. The fact that this method only requires AA to be visibly spotted without the use of complicated tools makes this technique particularly attractive to point-of-care and on-situ applications. Overall, the novel colorimetric sensor is a stable, sensitive, and specific procedure in the detection of AA and that it can be used as a prospective area of analytical and biosensing activities.

Condition Enhancement of Detection

Optimization Exercise Conditions Colorimetrically

In order to get a strong, stable, and easily identifiable colorimetric reaction to detect ascorbic acid (AA), experimental conditions were adjusted stage by stage refined systematically. These were the concentration of sensing material, TMB concentration, pH of solution and analyte concentration. This optimization process was carried out by changing one parameter at a time when the rest of the conditions stayed constant.

Improvement of material Potentization

A solution of the sensing material of 1 mg mL^{-1} concentration was prepared. This stock sample was diluted to obtain the desired working concentrations, and the colorimetric results were determined by measuring the quality of the blue color generated on the oxidation of TMB. The color development was light at low concentration of the material as few active catalytic sites were available. The concentration of the material also increased gradually with the intensity of the blue color. Further concentration however did not result in significant color enhancement and was thus indicative that the catalytic activity had reached equilibrium level. The concentration of the material that was selected as the best was 0.005mg mL^{-1} because the level produced a clear, consistent and reliable color change that was easy to detect visually.

Figure 11: Photograph of the color change after adding 0.005mg/1mL Material

Maximization of TMB Concentration

The 10mM TMB solution was made and diluted to form the various working solutions. Visual evaluation of the effect of TMB concentration on color development was determined. In less concentration of TMB, a slight blue color was visible because of undergo of oxidation. The intensity of color improved slowly with the increase in the TMB concentration. Nonetheless, high levels of TMB caused high background coloration, which might decrease the visual acuity. Therefore, it was decided to choose a concentration of 0.5 mM TMB as the best showing a bright and clear blue color with a little interference of the background.

Figure 12: Photographs of color change of adding various concentration of TMB

Optimization of pH

The pH of the reaction atmosphere is significant in identifiability of the effectiveness of colorimetric sensors in particular those that may need to oxidize the chromogenic reagents namely TMB, consistency of reaction intermediates and redox nature of TMB, and consequently the color intensity and steadiness. The pH effect was therefore investigated extensively to establish the situation of successfully undertaking the colorimetric detection.

The pH effect on the outcome of colorimetric response at a broad pH range of 3 to 11 has been explored in the paper using appropriate buffer solutions. The other conditions of the experiment like material and TMB concentration were kept constant to rule out the possibility of color intensity change because of the difference in pH condition. The colorimetric response was measured by the visual degree and the clearness of the blue color formed when the TMB oxidized.

The color change was considerable and different at acidic conditions. Worth mentioning is that the emergence of a dark and strong blue color was recorded at the pH of 3 and 4 meaning that the oxidation of TMB was best favored under these exceptions. These latter excess coloration values fixing on these pH values suggest the augmented catalytic efficacy of sensing material, and high exchange of electrons between reactants. The acidic environment was investigated with pH 3, the strongest color with a blue clear, uniform, and stable color. The pH 4 was therefore selected as the best pH to apply in future colorimetric researches.

The strength of color was gradually reduced as the pH changed towards a near neutral value. This reduction in color development is associated with a drop in the catalytic activity and recycling of the provision of protons, which is needed in TMB oxidation. Since, proton concentration influences the oxidation of TMB, low concentration of protons in a higher pH rate has an adverse effect on the production of the oxidized blue product. It means that the sensing system cannot work in mildly acidic and neutral conditions because the intensity of the color in this pH range is lower. On the other hand, the change of color was not observed in the pH that was neutral (between 7 and 11) and alkaline respectively. These environments combined with the fact that the samples were not blue in color demonstrates that oxidation of TMB was strongly inhibited. This can be attributed to the instability of the oxidized species of TMB, as well as the lower catalytic activity of the sensing the material in alkaline conditions. What is even greater, alkaline conditions can prevent the formation of complexes of charge transfer and may affect the reaction pathway to be used and

develop the colors properly. A low colorimetric response was therefore encountered in the sensing system in this range of pH.

The fact that the colorimetric reaction highly relies on the pH concentration may be seen as a definite indication that the acidic environment is significant to the effective functioning of the sensing platform offered. The faster color development in the more acidic condition serves to confirm that the conditions of acidic reaction kinetics and the stabilization of the formulated oxidized TMB product can be developed to provide clear and strong visual indicator. Based on this finding a pH 4 was chosen as the optimal pH, and therefore all the further colorimetric based detection experiments were done at pH 4.

The optimization research of the pH point shows that acidity of reactions is significant in achieving the optimum intensity of color, sensitivity, and clarity of the eyes. The results attest that pH regulation is a major factor to be taken into consideration to obtain valid and stable colorimetric detection. In addition, the ideal acidity levels determine the ideal environment under which the sensitive detection of the analytes can be done.

Figure 13: Photograph of the color change at various pH

Ascorbic Acid Analyte Optimization

The maximization level of ascorbic acid (AA) was done through the development of a stock solution of 10 mM ascorbic acid (AA) followed by progressive dilution of the stock solution to different working concentrations of millimolar and nanomolar concentrations of AA. The change of the visible color using the blue hue of the oxidized TMB was measured by visual observation. With a concentration of AA as high (10 mM to 1 mM), the reaction mixture was clear and colorless,

which indicates that all the oxidated TMB was reduced by the excess of AA. On these levels, the strong reducing ability of AA rapidly counteracted the oxidized chromogenic species resulting in the fading of the blue coloration. When the solution was diluted into even smaller molarities (micromolar 5moles/L) and nanomolar (nM) shares, the gradual reduction of the blue color was observed rather than its complete elimination. This fading increased with high concentration of AA in this lower concentration which demonstrated an inhibitory effect of AA on the colorimetric reaction that was concentration dependent. It was possible to differentiate the AA concentration through observation in progressive and noticeable change in the µM and nM concentration ranges. These results indicate that at high AA levels, color formation is completely inhibited; but under low AA levels (µM-nM) the blue color gradually fades in a controlled manner, and this is the best range of the colorimetric detection being sensitive. Therefore, nanomolar concentration range was selected as the optimal range of operation in order to carry out analysis in other studies.

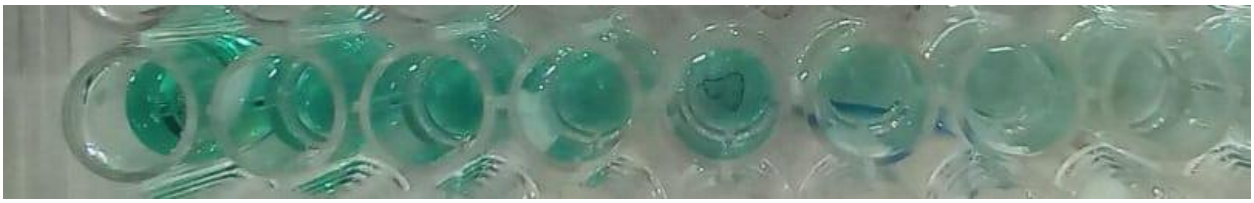

Figure 14: Photograph of the color change after adding various concentrations of AA

Parameters Optimization of UV- Vis Spectrophotometry

Material Concentration (UV-Vis Study) Optimization

A stock solution with the sensing material (1 mg mL^{-1}) was diluted to form working concentrations of 1 to 25 µM to determine the optimal concentration of this material to use in the UV -Vis detection. All the concentrations were subsequently added to the reaction mixture of TMB and a buffer with the optimal pH, and the UV-vis spectra were taken. Absorbance at 652 nm, which is the wavelength of oxidized TMB (oxTMB), was studied. The absorbance was minimal at lower concentrations of the material (1-5µM) meaning that not all of the catalytic active sites were sufficient to oxidize TMB. The higher the concentration of the material (10-20 µM), the higher the absorbance was and it was also a sign of better catalytic activity and therefore more product was made with ease which was blue oxTMB. The absorbance stabilized beyond 25 µM and indicated that the catalytic sites had been saturated and additional development in the concentration of material made no significant contribution to the oxidation of TMB. On the basis of these results,

the optimal concentration of a material used was selected (about 1-20µM) to conduct UV -Vis experiments. This concentration gave optimal absorbance, discrete spectral maxima,and reproducible results, which was in line with the pattern of the visual colorimetric experiments.

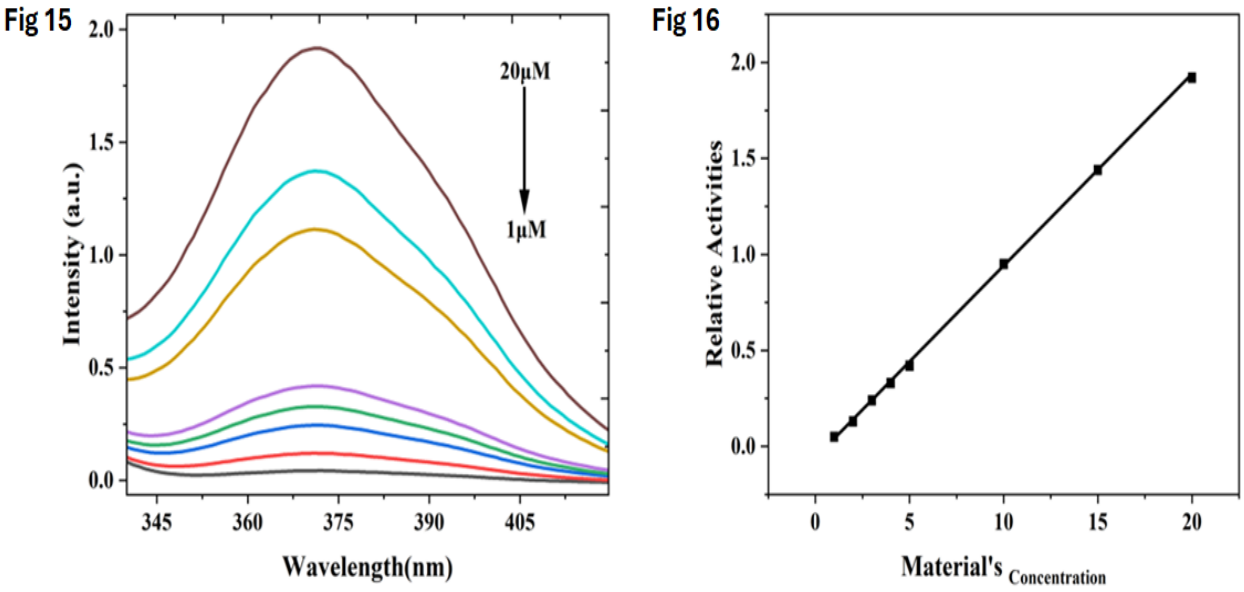

Figure 15: UV-vis absorption spectrum of MnOOH, Figure 16: Linear calibration curve of MnOOH

TMB Concentration (UV-Vis Study) Optimization

TMB concentration effects of the UV-vis absorbance of the oxidized TMB (oxTMB) were evaluated by 10mM stock solution which was diluted to working TMB ranges of 0.1 mM to 0.5 mM. The reactions were carried out with optimized material concentration based on the selected pH conditions and the absorbance was taken at around 652 nm. The absorbance at the low concentration of 0.1 mM was not so high implying that not all the TMB oxidation had taken place. The more TMB concentration was present, the higher the absorbance rose which was an indication that an increase in oxTMB was made. The maximum absorbance was observed when the concentration was 0.5 mM and the value above 0.5 mM was not tested as in the range of 0.5 mM, there was a strong absorbance value with a sharp peak and low background. These results obtained made TMB concentration of 0.5 mM to be considered the best in UV-vis experiments since the spectral intensity and reproducibility reached its maximum and in line with the tendencies of the colorimetric observation on the eyes.

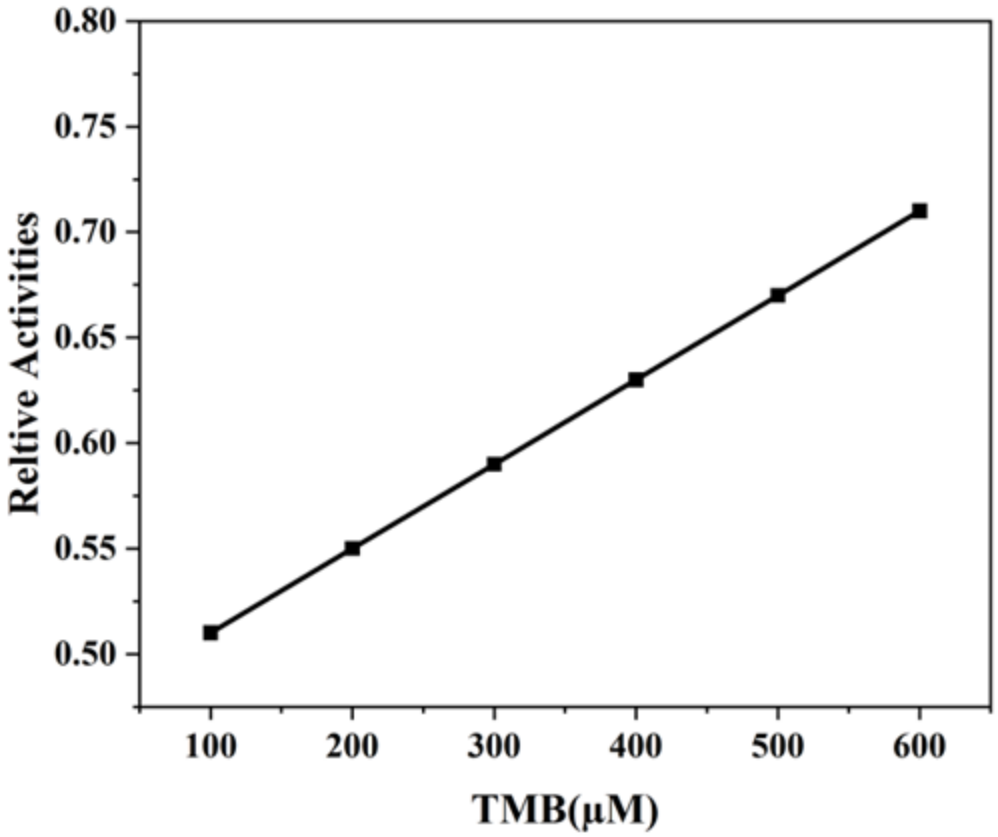

Figure 17: UV-vis absorption spectra of TMB

PH (UV-Vis Study) Optimization

The pH dependence upon the UV-Vis absorbance TMB (oxTMB) of oxidized TMB was studied, with an appropriate range of pH (3 to 11) with suitable buffer solutions. The reaction mixture consisted of 5 ug of the sensing material and 0.5 mM TMB, and the absorbance solutions were measured at some point of around 652 nm. The maximum level of absorbance was observed under an acidic environment and especially under pH 3 and pH 4, which implies that TMB oxidation is most efficient in an acidic environment. The absorbance decreased steadily as the pH approached the neutral, meaning that the catalytic power of the material decreased and the formation of the oxTMB was not as efficient. The absorbance in the pH range of 7-11 was lower indicating that very little oxidation of TMB was possible and the colorimetric reaction was inhibited. Based on such discoveries, it was decided that the pH 4 was the best condition to use to conduct UV- Vis experiments due to the high absorbance of solutes, distinct spectral peaks as well as consistent values, which compared with the tendencies of the colorimetric researches.

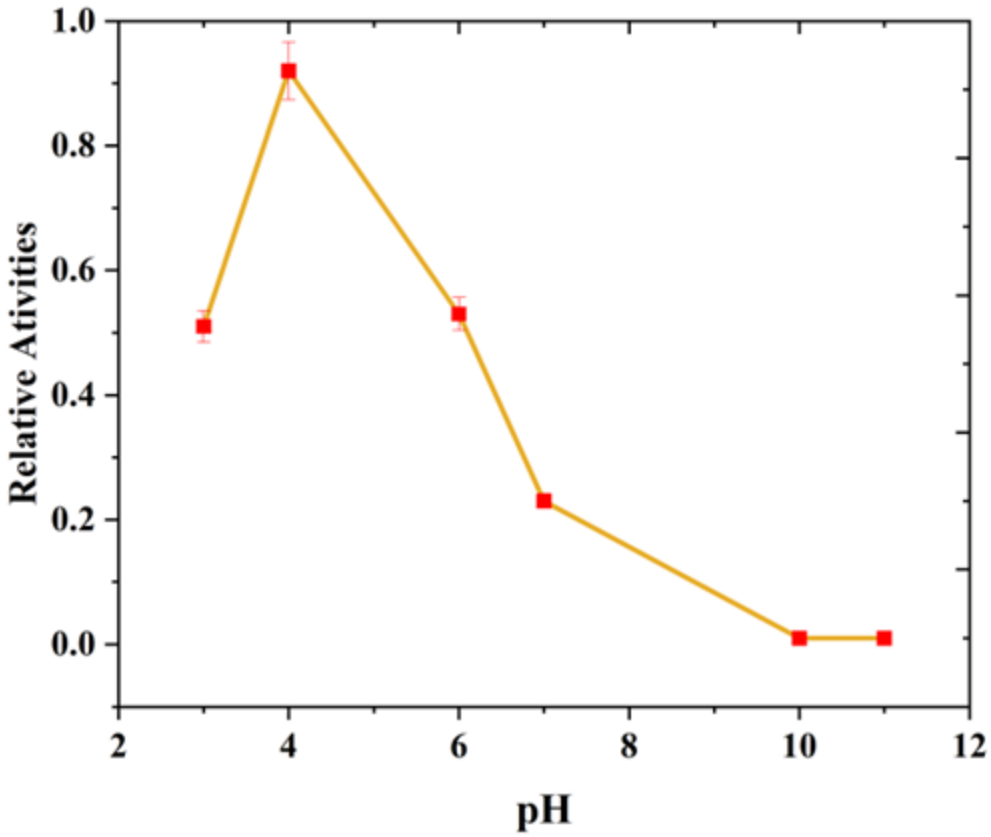

Figure 18: UV-vis absorption spectra at different pH

Optimization of Ascorbic Acid (AA) Analyte Concentration (UV-Vis Study)

An ascorbic acid (AA) stock solution of concentration of 20 nM was first prepared and subsequently diluted using a series to gain the following working concentration: 2 nM, 2.5 nM, 3 nM, 4 nM, and 5 nM. The absorbance of the reaction mixture of the UV- Vis was recorded at an approximate 652 nm in ideal conditions under varying conditions that comprised of 0.5 mM TMB and 5 uM sensing material and pH 4. In the lowest concentration of AA (2 nM), the absorbance was large at 652 nm indicating a small amount of reduction of oxidized TMB (oxTMB). The absorbance gradually went down as the concentration of AA rose between 2.5 nM and 5 nM, which showed that oxTMB was reduced by AA in a concentration dependent manner. This absorbance decrease demonstrates that under low nanomolar conditions AA is a good inhibitor of TMB oxidation and accordingly, a linear change in absorbance is measured. The results indicate that the organic UV Vis system developed is very sensitive to small concentrations of AA and will give a quantitative response of 2 to 5 nM. The same outcome is displayed in the colorimetric data whereby a gradual disappearance of the blue color could be physically observed as the

concentration of the AA grew in the same range. Following these results, the maximized detection limit of AA by UV-Vis spectrum analysis is 2-5 nM and all the reactions were performed in the proposed conditions of 0.5 mM TMB, 5 uM material and at pH 4.

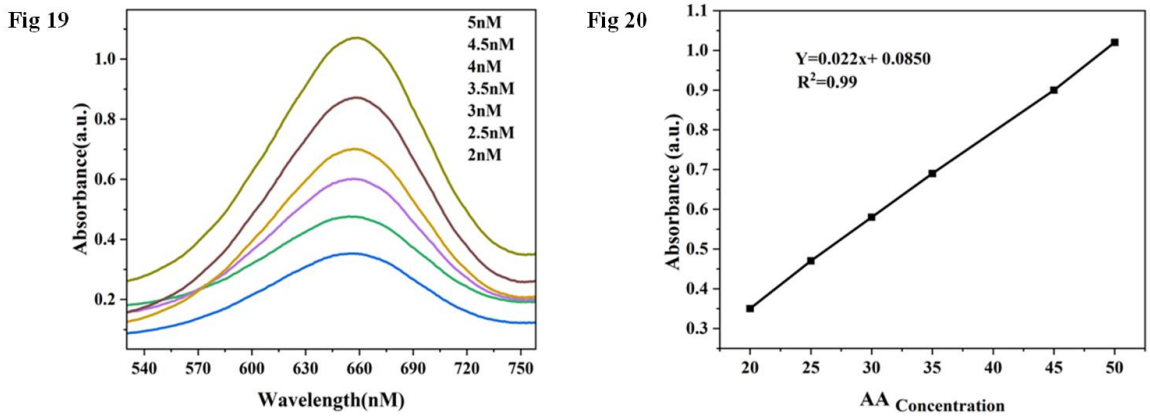

Figure 19: UV-vis absorption spectra of analytes, Figure 20 Calibration curve of analyte

Actual Sample Analysis

In order to gauge how the newly developed sensing platform can be practically used and relied on as a detector of ascorbic acid (AA) real samples were analysed using lemon juice and orange juice as typical food samples. To minimize the effects of the used matrix, the standard addition method was applied to ensure that the offered approach was accurate.

Lemon Juice Sample Analysis

The lemon juice was used as a representative of real-life sample to determine the appropriateness of the proposed sensing technique to real world sample. The fresh lemon juice was first filtered to remove pulp and solid particles. In analyzing, the juice was diluted by adding 1 mL of lemon juice of 9 mL of deionized (DI) water to create a ten-fold dilution of the juice. This was a diluted solution that was used in unspiked and spiked measurements to minimize the effects of the matrix.

To determine the natural ascorbic acid (AA) content in the sample, the diluted lemon juice sample without spikes was initially tested under some optimized experimental conditions. An observable reaction was observed, and this supported the presence of AA in the lemon juice.

Accuracy was then analyzed by use of the standard addition method. Lemon juice sample was diluted and known quantities of AA standard solution were spiked into the sample, which was prepared using 20 nM AA stock solution. 20 µL, 35 µL and 50 µL volumes of the diluted sample

were added individually. The colorimetric/UV -Vis reaction was measured at the end of each spiking, and the respective AA concentration was calculated on the basis of calibration curve.

The percentage recovery of the lemon juice sample in the additions of 20, 35 and 50 µL appeared as 99.9%, 100.08% and 100%, respectively. The rates of these recoveries are almost 100%, and this fact demonstrates the high accuracy level of the supposed method and a mild interference of the sample matrix.

Sample Analysis of Orange juice

Orange juice was also taken as a case study to estimate the efficacy of the suggested sensing technique. First, fresh oranges juice was filtrated in such a way that the particles and pulp are eliminated. To examine the sample the solution has been diluted by 1 mL of orange juice combined with 9 mL of deionized (DI) water and this gave a solution ten times. The approach followed on the basis of this dilute sample to warrant minimum interference of the matrices was performed by the medium of unspiked and spiked measurements.

On the one hand, the dilute version of orange juice free of spikes was also investigated according to the optimized experimental conditions in the effort to find the naturally occurring content of ascorbic acid (AA). The observation and apparent reaction indicated the availability of the AA in the orange juice sample.

The accuracy was determined using the standard addition technique. It was followed not only with the release of the large volumes of the diluted orange juice sample but also with the addition of 20 nM AA stock solution (20 µL, 35 µL and 50 µL). The colorimetric/UV -Vis response was recorded after each individual addition, and the AAs concentration was determined through the already known calibration curve.

The corresponding volumes of spiking got a recovery value of 98.0%, 101.11% and 97.98% of the orange juice sample. These recovery results indicate that the recovered and added values of AA values are strongly correlated and thus the suggested method of sensing offers a high degree of precision, as well as, less matrix effects in orange juice samples.

Discussion of Recovery Result

The recovery rates for both lemon and orange juice samples fell within an acceptable range of about 98–102%, demonstrating the high accuracy and reliable reproducibility of the developed sensing platform. The close match between the added and recovered AA concentrations indicates

that the typical components found in fruit juices do not significantly interfere with the detection process.

Conclusion

An effective, touchy and economical colorimetric and UV-visual spectroscopic identification platform has been planned in an attempt to sense ascorbic acid (AA). This is identified as the oxidation of TMB treatment of 3, 3, 5, 5 treatment of TMB and consequent development of blue color, which is the by-product of the oxidation of TMB, referred to as ox-TMB that is reduced by AA thus making the color golden in color. It would allow both visual determination and quantitative UV-visualization. This was done by fitting such parameters as content of material, TMB concentration, pH and concentration of analyte to achieve optimum sensing performance. It was concluded that the most appropriate conditions will be the concentration of the material 5 μM, 0.5 mM TMB, low pH (3-4) with which will be formed a certain color transformation. The system was very pH-specific because the color aesthetic was formed at acidic pH state and minimal or no indication at neutral and alkaline pH (pH 7-11). Under these ideal conditions the UV-vis absorbance at 652 nm went down with increasing amount of AA. The procedure displayed a linear relationship at 2-5 nM which had a limit of detection (LOD) and a limit of quantification (LOQ) value of 1.18 nM and 3.59 nM, respectively, and therefore, a good performance analytically. A.A was the particular substance that was very sensitive to the sensing system with minimal interference of other substances like dopamine, glucose, cholesterol, urea, uric acid, amino acids and metal ions. It was demonstrated that diluted lemon and orange juice (1mL juice+ 9mL deionized water) had good applicability because the dilution recovery values ranged 97.98-101.11. The sensing strategy that has been developed is colorimetric/UV -Vis, in which it is simple, fast-reaction, low-cost, high-sensitivity, accurate, and may be regarded as a promising method to be applied in the determination of ascorbic acid in food samples. The results provide the foundation of designing mobile sensing platforms to carry out routine examination.

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