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Review Article

Superoxide Electrochemical Sensors and Biosensors: Amperometric, Impedimetric Sensors and Enzyme-Based Electrosensor Applications

Omar Shaker Kamal [1] | Nourhan Abdel Salam Saleh [2]

[1, 2] Department of Medical Device Engineering Technology, The Bilad Alrafidain University, Iraq.

Abstract:

The ever-increasing demand for sensors, coupled with the quick speed of technological advancement, has created a vast and dynamic world of sensors. The agricultural, food, and oil sectors, as well as environmental and medicinal applications, all make extensive use of electrochemical sensors due to their low cost and ease of use in detecting changeable analytes. The low theoretical detection limits that arise from the differences in the Faradaic and nonFaradaic currents, as well as the variability of the reporting signals (e.g., voltage, current, overall power output, or electrochemical impedance) are the two primary reasons why electrochemical sensing is so popular. Also covered is the part nanoparticles play in the development and study of electrochemical sensors. We hope that the data given here will inspire researchers to keep digging into the topic of electrochemical sensors. In medical applications, electrodes are useful for detecting superoxide and nitric oxide, two species with very short half-lives. They can open the door to a spatially and temporally resolved study that sheds light on the physiological function and interplay of the two radicals. Electrodes that have been modified with cytochrome c or superoxide dismutase as recognition elements are predominantly used in superoxide sensing. The method relies on either direct electrochemistry of proteins or the detection of products of superoxide breakdown to function. The majority of the electrodes used for NO measurement have been upgraded with gas-permeable membranes. By reducing the applied electrode potential, transition metal complexes have been used to improve NO electrocatalysis. Electrodes incorporating hemoproteins is a more recent development. Using the unique interaction between NO and the haem group, NO sensing can be achieved by direct protein-electrode interactions. Both the extracellular and intracellular regions of living cells contain extremely low amounts of superoxide and hydrogen peroxide, and these species have a limited half-life due to the presence of a complex antioxidant system that rapidly consumes them. Living cell production of superoxide and hydrogen peroxide makes real-time monitoring of these compounds a significant challenge. Cells can continually create superoxide or hydrogen peroxide, which can be monitored using biosensors and electrochemical sensors that are appropriately built. Therefore, they are in a good position to finish analytical procedures that provide endpointsand/or indirect evidence of these species' overproduction by cells. Despite the abundance of reports on electrochemical sensors for hydrogen peroxide and superoxide, there is a dearth of literature detailing their application in cellular biology. Additionally, the majority of these articles do not detail comprehensive research but rather projects that only go as far as a proof of principle.

Keywords: Biosensors, Superoxide, Amperometric, Impedimetric Sensors, Applications

Introduction:

Active sensing materials and signal transducers are the building blocks of sensors. Transmitting the signal without amplification due to a selected chemical or a change in response is the job of these two crucial components in sensors. You can convert the electrical, thermal, or optical signals produced by these devices to digital signals so you may process them digitally. Using these output signals is one approach to sensor classification. Electrochemical sensors are superior to their counterparts because, unlike other types, they are able to detect materials within the host without harming the host itself. In contrast, chemical sensors and biosensors are the two main types of sensors. By their ability to detect biochemical substances, such as biological proteins, nucleotides, and tissues, biosensors can be classified according to their sensing characteristics. An electrode's active sensing material should catalyze a process involving biological chemical compounds in order to generate output signals in these sensors [1, 2]. A new category of sensors, electrochemical biosensors, have emerged as a result of combining the two approaches; these sensors use electrochemical methods in their creation and operation. Choosing and creating an active substance is no easy feat. Any substance that can serve as a catalyst for the detection of an analyte or an ensemble of analytes can be used as an active sensing material. Numerous electrochemical and biosensor applications have benefited from the plethora of new materials and devices made possible by recent advances in nanotechnology. Simply said, by constructing nanostructures, one can influence the basic characteristics of materials independently of their chemical make-up. Thus, the alluring realm of low-dimensional systems, in conjunction with present advances in the manufacture of functional nanostructured arrays, may play a pivotal role in the emerging patterns of nanotechnology. In addition, nanostructures are essential to the operation and integration of nanoscale devices due to their dual usage as efficient electron transporters and optical excitation agents. Nanosystems are essential to the operation and integration of these nanoscale devices since they are the smallest dimensional structures that may be utilized [3-5] for efficient electron transport. Due to the quantum confinement effect and their tunable electron transport properties and high surface-to-volume ratio, even small perturbations have a large impact on their electrical properties. There has been a lot of interest in these nanostructures, especially on nanowires, in recent research. There are two main ways to create these nanostructures. The first is the "bottom-up" strategy, which involves building larger structures from smaller ones through self-assembly. The alternative is the "top-down" method, which involves building multifunctional nanoscale structures by first shrinking massive systems [6]. Compared to carbon nanotubes (CNTs), these nanowires offer two key benefits when applied to biosensing devices. First, by adjusting the synthesis conditions and making use of established doping techniques, the material properties can be fine-tuned. Second, nanowires may make use of a wide range of functionalisation and blocking chemistries due to the native oxide layer that arises on their surface. There is currently a plethora of information available in the literature on the design of electrochemical sensor devices, the selection of appropriate sensing materials for appropriate analytes, and their numerous applications. The research on nanowires used in biosensors is the most intriguing of them.

Electrochemical Sensors:

In its definition, the IUPAC states that a chemical sensor is "a device that converts chemical data, ranging from the concentration of a single sample component to complete composition analysis into an analytically usable signal". The two primary components of any chemical sensor are the receptor and the physicochemical transducer. Different receptors can interact with analytes in different ways; they can be simple surfaces that have been activated or doped, or they might be sophisticated (macro)molecules. A device is

called a biosensor if its receptor is biological in nature, such as DNA, antibodies, or enzymes. The recognition event is transformed into a preset output signal when the receptor interacts with the analyte. In order to prevent false-positive results caused by chemical species that could interfere, it is essential for sensors to retain a high level of selectivity for the target analyte. The transducer is an essential part of sensors because it takes the signal produced by the interaction between the receptor and the analyte and turns it into a value that can be read. This means that biosensors and chemical sensors alike can be categorized as either affinity-based or catalytic. Different from catalytic sensors, which use catalytic activity to produce a signal (e.g., enzymatic, DNAzyme, or functionalized surfaces that can undergo redox reactions under specific conditions), affinity-based devices depend on extremely specific interactions between the receptor and analyte (e.g., utilising the specific affinity of ssDNA and aptamers), antibodies and antigens, or host and guest interactions). Optical, gravimetric, or electrochemical approaches are among those that can be used to monitor the recognition events, among others [6, 7]. The most popular kind of sensor is the electrochemical sensor, which has a number of benefits over its competitors, including a low detection limit (as low as picomoles), high speed, and inexpensive sensing equipment. From standalone benchtop units to completely integrated wearable sensors, electrochemical sensors are available in a wide range of form factors. A chemical sensor's main function is to provide precise, real-time data about the chemical make-up of its immediate environment. In a perfect world, this kind of technology wouldn't mess with the sample at all and could react continuously and reversibly. These devices work by coating a transduction element with a layer that may be identified chemically or biologically [8, 9]. A target analyte's contact with a recognition layer generates an electrical signal, which electrochemical sensors use to derive analytical information. Environmental monitoring can make use of a wide range of electrochemical devices, each tailored to a certain analyte, sample matrix, and level of sensitivity or selectivity needed. Electrochemical sensors, among others, can be either amperometric or potentiometric, depending on the type of device. In chemical and biological identification, amperometric sensors can detect electroactive species.

Theories, investigations, and applications of electrochemical and biosensors for superoxide:

The reduction of dioxygen to water requires four electrons and produces three main byproducts: superoxide radical (O2•_), hydrogen peroxide (H2O2), and hydroxyl radical (•OH). Of these byproducts, only hydrogen peroxide is stable enough to accumulate significantly in neutral water. It has long been acknowledged as a byproduct of dioxygen's biological reduction and a potential cause of dioxygen's toxicity. O2 •_ and •OH, on the other hand, are radical intermediates in dioxygen reduction that, under typical circumstances, have finite lifetimes and, as a result, have only lately been seriously considered. Nowadays, everyone agrees that dioxygen's toxicity is mostly due to the superoxide radical, and that the body's main defence mechanism against this radical is superoxide dismutases (SODs). Both the protonated and ionised forms of univalently reduced oxygen are referred to as the hydroperoxyl radical (HO2 •) and O2 •_, respectively. As its pKa is 4.8, HO2 • is considered a weak acid. Two reactions—the univalent reduction of dioxygen and the univalent oxidation of H2O2—can generate the O2•_ radical. Hydrogen atoms or electrons hydrated from photolysis, radiolysis, or ultrasonication of water can chemically reduce dioxygen, or electrochemical reduction in aprotic solvents or alkaline aqueous media can also produce O2•_. The oxidation of H2O2 by ceric ions and the reduction of dioxygen by carbanions, reduced dyes or flavins, catecholamines, ferredoxins, or hemoproteins [38, 39] are other possible sources. Mass spectrometry, optical spectroscopy, conductimetry, and electron-spin-resonance spectroscopy (ESR) are some of the physical approaches that have been used to identify the

O2•_ radical. Reactive oxygen species (ROS) include O2•_, a principal species that is produced in biological systems as a reduced intermediate of molecular dioxygen in large amounts. The role of O2 •_ as a chemical involved in cell/cell signaling and as a component of the host defence mechanisms is crucial. A rather low and undetectable endogenous physiological concentration of O2 •_ results from a disproportionation under normal physiological conditions caused by non-catalytic or enzymatic processes. Traumatic brain damage is associated with an increase in O2 •_ activity [9-11]. It is hypothesized that ischemia-reperfusion, hypoxia, and O2 •_ contribute to the development of cancer, ageing, and progressive neurological illnesses like Parkinson's. Quantitative data on the O2•_ level in various in-vitro and in-vivo models is now crucial for understanding the pathophysiology and diseases associated with ROS and free-radical biochemistry because of the significant roles it plays in biological processes. It is relatively difficult to detect the concentration of O2 •_ due to its short lifetime, low concentration, and strong reactivity. While O2 •_ isn't particularly reactive on its own, it can become H2O2 by reacting with other biomolecules or being rapidly disproportionated in the presence of SODs. In the presence of metal ions like Fe2_ and Cu2_, the Fenton reaction can convert H2O2 to the most powerful radical, the hydroxyl radical [10-13]. Chemical or enzymatic reactions typically result in relatively low steady-state concentrations of O2•_ because spontaneous dismutation reactions are so fast. Despite being direct and unambiguous, the physical methods for detecting O2•_ are generally determined to be insensitive since they are limited to measurements of steady-state concentrations. It was necessary to use a high concentration of the reactants and work at elevated pH in order to suppress the dismutation reaction and obtain detectable levels of O2•_ when studying the O2•_ production by xanthine oxidase using the EPR method, for the reasons mentioned above. There are integrative chemical approaches that are both simple and very sensitive for detecting O2•_. An indicating scavenger can be used to trap O2•_ in those ways.

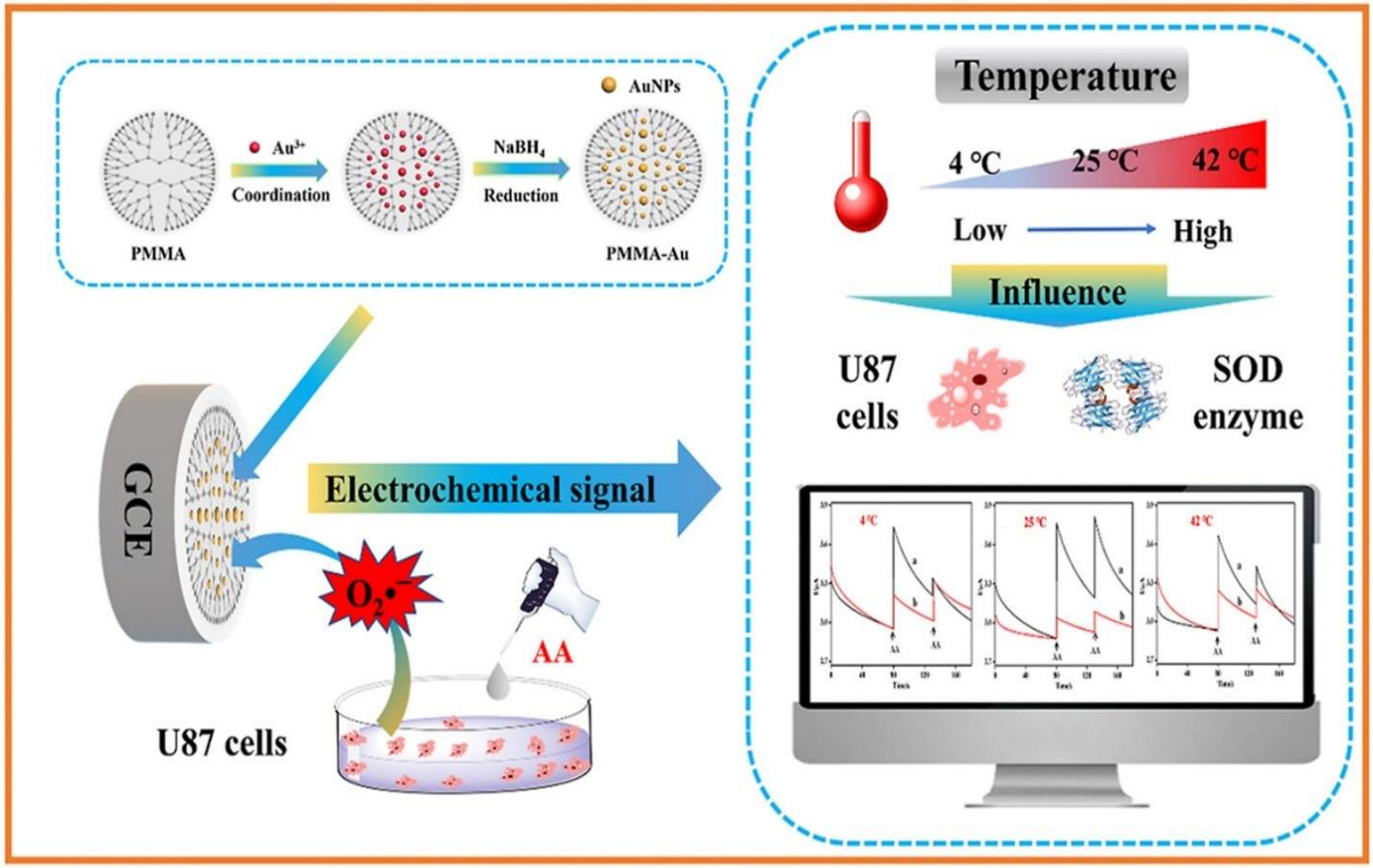

Figure 1. superoxide Dismutase Enzyme Activity.

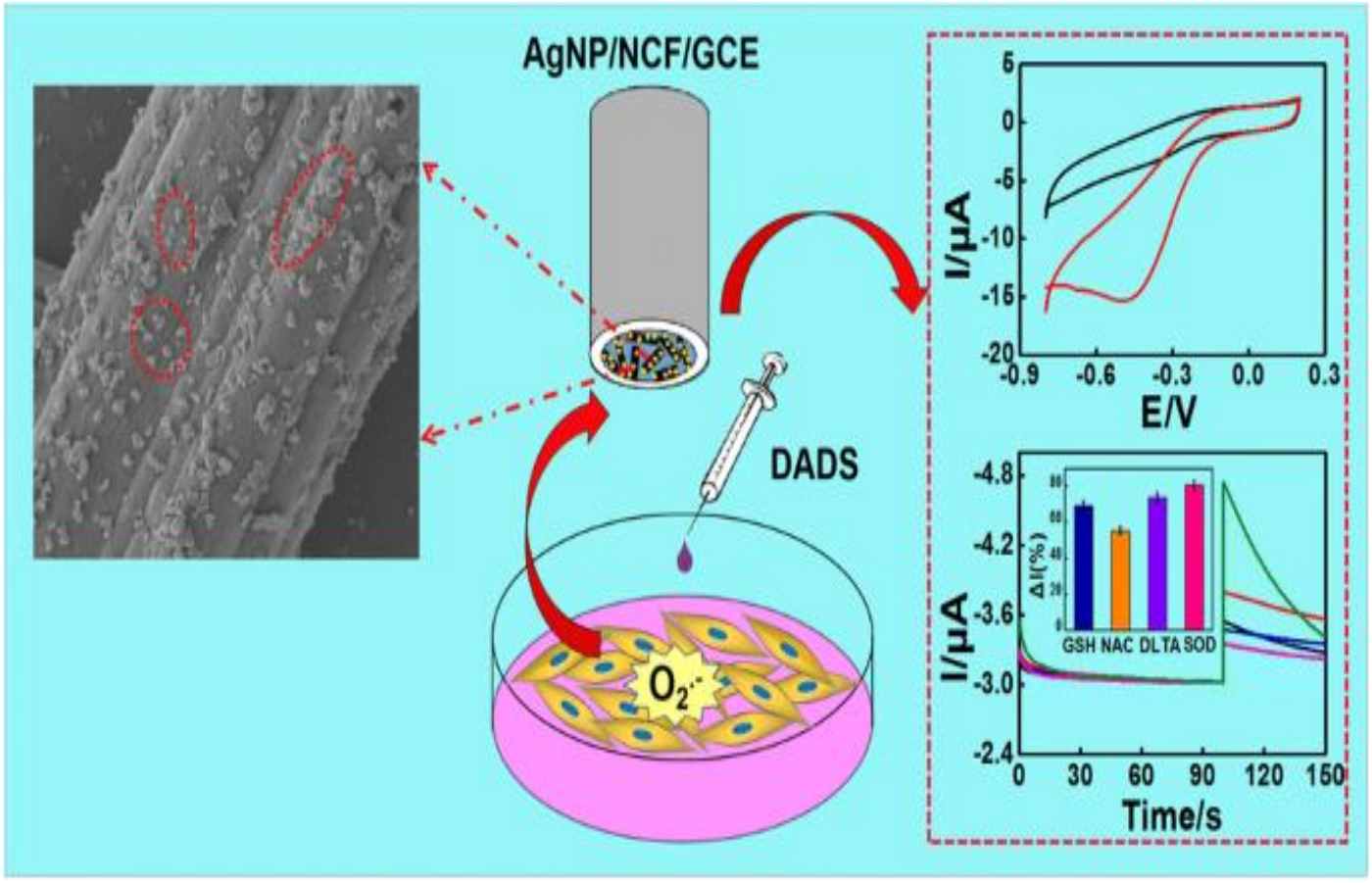

Figure 2. An ultrasensitive electrochemical sensor based on cotton carbon.

Biosensors with enzymes other than SODs:

Proteins and enzymes such as tyrosinase, galactose oxidase, haemin, and cytochrome c (Cyt. c) have been used to build enzyme-based biosensors for the O2•_ measurement, alongside a family of SODs. To demonstrate the analytical mechanism of these O2 •_ biosensors, we will utilize Cyt. c in this context. In order to build a biosensor using Cyt. c, the biosensor electrode typically has Cyt. c immobilised on it so that it can mediate electron transport between the electrode and O2•_. Cyt. c (Fe(II)) is reoxidised on the electrode at a potential of 0.15-0.25 V (versus Ag/AgCl) when the O2•_ radical lowers the immobilised Cyt. c (Fe(III)) to Cyt. c (Fe(II)). This highlights the critical role of Cyt. c's electron transport to the electrode. Electrodes commonly employed in electrochemistry, like glassy carbon, gold, and platinum, could not easily achieve such an electron transfer. The platinized activated carbon electrode (PACE) was able to facilitate electron transfer, and this property has since been utilized for the detection of O2•_. A planar Au electrode had a lower sensitivity to O2•_ than the Cyt. c-modified PACE electrode, which had an evaluation of 34.0 (μA cm_2)/(μM_1). Because PACE is a very porous material that can bind a big quantity of Cyt. c., its increased effective surface area was thought to be responsible for the improvement in sensitivity compared to the flat Au electrode. At Au electrodes modified with self-assembled monolayers of, for instance, N-acetyl cysteine, 4,4-dithiobipyridine, or 3,3-dithiobis (sulfosuccinimidylpropionate), the electron transport property of Cyt. c can also be achieved. In order to facilitate the electron transfer of Cyt. c., these modifiers were used as promoters. Effective electron transmission between Cyt. c and the electrode, followed by biosensing of O2•_, often requires a free orientation of the molecule without denaturation [14-16], from a practical application standpoint. In this instance, the formation of self-assembled monolayers of alkanethiols onto Au electrodes is noteworthy due to the fact that they can both aid in the direct transfer of electrons from Cyt. c and shield the electrode from potential interferences in the solution. Hence, a Cyt. c-based amperometric

O2•_ biosensor has been constructed using the self-assembled monolayer (SAM) of HS(CH2)10COOH confi ned on the Au electrode. Further development of this direct electron transfer property has led to its application in O2 • biosensing. Mixed SAMs, which include both short and long alkanethiol SAMs, have also been used to facilitate the electron transfer of Cyt. c. Examples of such compounds are 3-mercaptopropinic acid and 3-mercaptopropanol [96]. Campanella et al. used haemin to mediate electron transport between Cyt. c and a carbon paste electrode, resulting in an O2•_ biosensor based on Cyt. c. For continuous O2•_ measurement at _0.8 V versus SCE, the analytical current was the haemin (Fe(II)) oxidation current. O2•_ biosensor with a detection limit of 0.2 mM and a response time of 2 minutes. There was a three-day shelf life for the biosensor. A more recent method for biosensing O2•_ involved a multilayer Cyt. c-modified electrode; the most sensitive of these electrodes was those with six layers of Cyt. c. Despite the practicality of electrochemical biosensors based on Cyt. c for measuring O2 •_ in biological samples, it is well-known that Cyt. C is not an O2 •_specific enzyme. For instance, similar to peroxidase, Cyt. c possesses intrinsic enzymatic activity for reducing oxidants such as H2O2 and ONOO_. Despite reports that the peroxidase activity of Cyt. c can be regulated by electrode design, the non-specific catalytic activity of Cyt. c does limit the implementation of Cyt. c-based electrochemical biosensors for selective measurement of O2•_ in biological systems. Because SODs are enzymes that catalyse the dismutation of O2•_ into O2 and H2O2 with high activity and specificity, they would be an excellent alternative to be used in this regard. There has been recent research and development of electrochemical biosensors based on SOD for the selective and sensitive measurement of O2•_.

Electrochemistry of SODs:

Reciprocal electron transfer between redox proteins and an electrode is notoriously difficult to achieve due to the presence of a thick insulating protein shell surrounding the active regions of the proteins and enzymes. The direct electron transfer capabilities of SODs have also been a long-standing challenge to realise. Motivating research in this area has been the realization that knowledge about direct electron transfer is crucial for developing third-generation biosensors based on SODs for O2•_and, more importantly, for comprehending the intrinsic thermodynamic and kinetic characteristics of SODs. According to Iyer, who used a bare Au electrode in a phosphate buffer solution (pH 4.0) to directly and irreversibly oxidise Cu and Zn-SOD, a conformational shift takes place at the active sites due to its adsorption on the electrode surface, allowing for direct electron transfer. With the use of so-called promoters for direct electron transfer, researchers Borsari and Azab and Wu et al. were able to examine the reversible redox response of bovine and human Cu, Zn-SOD at an Au electrode. Research by Ohsaka et al. shown that methyl viologen, when used as a redox mediator, effectively facilitated electron transport between a glassy carbon electrode and polyethylene oxide-modified Cu, Zn-SOD. The direct electron transfer of SODs via self-assembled monolayers (SAMs) confined onto Au electrodes has recently been the focus of a lot of research. As an example, Ohsaka et al. investigated the electron transport characteristics of SODs by growing several types of alkanethiol SAMs onto an Au electrode [17-19]. In order to show how the electron transfer of the SODs stimulated by the SAMs of alkanethiols works, we will use the SAM of cysteine as an example. In the same conditions, the CV at a bare Au electrode (curve c) was also provided for comparison. In the phosphate buffer containing SOD, the cysteine-modified electrode displays two distinct voltammetric peaks, as demonstrated (curve a). The naked Au electrode (curve c) did not produce these redox peaks. Based on these findings, it appears that the bare electrode is not the best place for direct electron transfer between Cu, Zn-SOD, and Au, but that an Au electrode modified with SAM of cysteine can greatly enhance this process. The redox activity of Cu (II) and Zn (II) is

imparted to each monomer subunit of Cu, Zn-SOD, as previously mentioned. The voltametric peaks at _0.642 V and _0.98 V (vs SCE) were attributed to the redox reactions of Cu (II) and Zn (II), respectively, according to Wang et al., who discovered two redox waves for the Cu, Zn-SOD at a mercury electrode. The absorption spectra of the reconstituted SOD differ noticeably from those of Cu2_ aqua ion and Cu-free SOD (EZnSOD) as reported by Cocco, but they are in excellent accord with those of native Cu and Zn-SOD with Λmax _ 680 nm. The above-mentioned procedure—which involves adding Cu2_ to a solution of Cu-free SOD and then incubating the combination at room temperature for 1 hour—can be used to manufacture the reconstituted SOD. This suggests that the electrochemical redox reaction of the Cu moiety, rather than the Zn moiety, in the potential range of _0.5 to 0.5 V is responsible for the cysteine-promoted direct electron transfer of Cu, Zn-SOD. Additional confirmation of this can be found in the fact that the reconstituted SOD exhibits the same formal potentials as the native Cu, Zn-SOD.

Amperometric Sensors:

One common and highly sensitive analytical technique is amperometric measurements, which work by using an applied voltage to drive electrocatalytic redox processes that produce electrical currents that are directly proportional to the analyte concentration. The electrochemical cell consists of two electrodes immersed in an electrolyte of the right composition, and the basic apparatus necessitates a controlled-potential system. A more complex and prevalent form utilises a three-electrode cell, where one of the electrodes serves as a reference electrode. On the other hand, a reference electrode (e.g., Ag/AgCl or Hg/Hg2Cl2) is one that keeps a constant potential in comparison to a working electrode, while the former is the one at which the reaction of interest takes place. It is common practice to use a non-conductive material (such as graphite or platinum) as an auxiliary electrode [19]. To avoid electromigration effects, which lower the solution resistance and keep the ionic strength constant, a supporting electrolyte is required for controlled-potential research. Both the theoretical and practical aspects have been thoroughly discussed.

Impedimetric Sensors:

One approach is to induce a steady-state response by stimulating the cell with an alternating potential of tiny size. This approach offers numerous advantages. The most important ones are the following: the capacity to treat the response theoretically using generalized linear current-potential characteristics; measurement over a broad time or frequency range; and the ability to conduct sensitive measurements using an experiment, which is possible due to the response being permanently steady and thus averaging over a long period of time. Another common material is polymer, which can be used alone or in conjunction with a conductor. To illustrate the point, polypyrrole can be utilised as an NH3 sensor after being doped with ClO4- and tosylate; it can also detect volatile amines.

Electrochemical Sensor Applications:

The examination of biological, environmental, industrial, and pharmaceutical species has long made use of electrochemical sensors, which are highly prized for their reliability, precision, speed, low cost, and ease of downsizing, in addition to their high sensitivity and accuracy. Electrochemical assays have been incorporating a wide variety of nanomaterials with unique properties—including metals, conductive polymers, metal oxides, and frameworks of metal-organic and carbon-based nanomaterials—to improve analytical performance for over twenty years [20-22]. Because of this change, bioinspired receptors can increase the specificity of electrochemical sensors by capturing targets efficiently and using recognition molecules like aptamers, enzymes, and antibodies to increase the loading capacity. Providing significant electrocatalytic activity for specific electrochemical processes is strongly tied to this. These experiments should be more sensitive if we can increase the electrical conductivity and surface area by changing the shape and structure of the surface. New uses for electrochemical

sensors, such as in vivo analysis, wearable technology, point-of-care diagnostics, and single-molecule sensing, have contributed to their recent surge in popularity. The many benefits of electrochemical sensors include: low limits of detection (LODs) and levels of quantification (LOQs); fast analytical response (great for flow analysis and alert systems); simplicity (nearly infinite variety of geometries, electrode materials, and configurations are possible); and ease of use (simple and inexpensive equipment, the ability to be integrated as a detection module in a variety of analytical systems).

Biomolecule Electrochemical Detection:

The physiological and biological functions of tiny biomolecules (e.g., enzymes, nucleic acids, and hormones) as they transfer genetic information, regulate biological activity, and catalyse cellular processes allow for their detection. It is still a challenging undertaking to create biomolecule-sensing technology [23-25]. The examination of biomolecules has led to the development of biomolecular methods like Western blotting, polymerase chain reaction (PCR), and gel electrophoresis. They are precise, but they have limitations like a lot of reagents, a lot of work, and a long time need. Several publications discuss electrochemical methods of biomolecule identification as a potential early diagnostic tool. In order to identify biomarkers in urine, Mohan et al. created an integrated electrochemical biosensor.

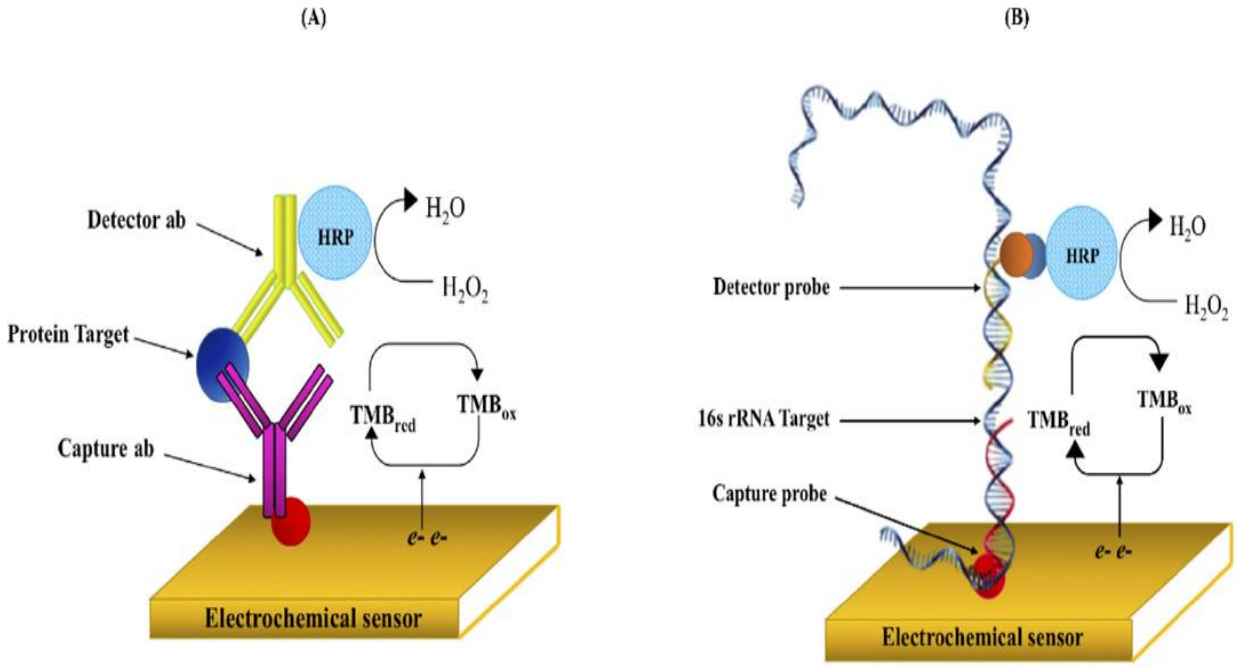

Figure 3. Nucleic acid and protein electrochemical biosensor detection using urine as a diagnostic tool: Part A: A method for identifying pathogens by hybridising bacterial 16S rRNA with probes that use capture and detection oligonucleotides; Part B: A method for detecting pathogens by utilising antibodies that use capture and detection.

Applications of Enzyme-Based Electrosensors:

Enzymes are organic catalytic molecules that are naturally occurring in living organisms. They can speed up biological processes and boost cellular metabolism's substrate-to-product conversion rate by a factor of 10 million or more by lowering the activation energy. Enzymes mediate a highly specific substrate conversion. Some enzymes catalyse reactions with only certain substrates, while others can work with substrates that are structurally similar. In order for an enzyme-catalyzed reaction to begin, the substrate and enzyme must first form a complex. Enzymes are reusable and effective down to extremely low concentrations because the processes they catalyse do not alter them in any manner [26-29]. Any

way you look at it, the enzyme can catalyse a reaction. The need to track enzyme activity is great. Enzyme activity has been studied using a variety of analytical techniques, including mass spectrometry spectrophotometry, Raman spectroscopy, and electrochemical methods. The rapidity, cheap cost, and ease of use of electrochemical treatments make them the method of choice among analytical techniques. A highly trained operator and elaborate pretreatment and filtration processes may be necessary for other methods. Enzymatic sensors, created by immobilising an enzyme on an electrode, may measure the concentration of a matching substrate. The key differences among enzyme-based biosensors are the immobilisation process and mediator used. Using RuO2 as a mediator and Gox immobilised on MWCNTs as a biorecognition element, the authors of a recent work created an amperometric Glc biosensor. To make the sensor more stable, the enzyme was covered with a Nafion® membrane. The suggested sensor was used to test the amounts of hydrogen peroxide and glycol. The developed sensor was utilised as an electroanalytical tool to study how heavy metal cations (Cd2+, Hg2+, and Ag+) affected the activity of the Gox enzyme.

The Role of Nanomaterials in Electricity Detectors:

The field of sensors has seen significant growth in the use of nanotechnology in recent years. One school of thought holds that using nanoscale materials and such technologies improves sensor performance. Researchers have found that nanomaterials have several interesting and unusual physical and chemical properties. A new subfield of condensed-matter physics has emerged in the last several decades devoted to materials and systems with dimensions on the order of nanometres. The materials listed above are only a few examples of the many types of materials that might be utilised to construct nanosensors. Carbon is considered a unique element due to its wide range of applications. There are many interesting forms of carbon, and some of these forms include graphene, diamond, fullerenes, and graphite. According to earlier research, carbon-based nanostructures have enabled several recent and major advancements in nanotechnology, including the development of chemical and biological sensors and their application in the pharmaceutical and biomedical industries.

Novel electrochemical sensors for the detection of oxidative stress:

When oxygen or nitrogen molecules undergo redox reactions, they produce reactive oxygen and nitrogen species, abbreviated as ROS and RNS, respectively. Hyperchlorous acid/hypochlorite (HOCl/-OCl), superoxide (O2.-), hydroxyl (HO.), peroxyl radical (ROO.), hydrogen peroxide (H2O2), and singlet oxygen (1O2) are all examples of reactive oxygen species. Ribonitrite (ONOO−), nitric oxide (•NO), and nitrogen dioxide (•NO2) are all examples of reactive nitrogen species. Various cellular activities, such as oxidative phosphorylation, fatty acid catabolism, phagocytosis, macromolecular complex breakdown, and protein folding, continuously generate reactive oxygen and nitrogen species. The reactivity rate, biological activity, and cellular significance of ROS can be classified as radical (e.g., O2.-, NO•, and OH•) or non-radical (e.g., HOCl and H2O2). The oxidative stress that cells experience is caused by an overabundance of reactive oxygen species (ROS) and nonradical ROS, both of which add to the cellular oxidative load. Despite widespread agreement on ROS's importance, questions remain about how exactly these molecules differentiate between normal and pathological activities. In spite of ROS/RNS's significance, there are currently no adequate analytical methods for tracking their evolution selectively in order to resolve the oxidative state of the cell where it occurs. Electrochemistry offers one-of-a-kind chances to detect reactive species in living tissues using custom-designed microelectrodes, giving high-resolution, real-time proof of ROS levels [30-32]. Here, we go over some of the ways that ROS/RNS can be detected electrochemically, as well as certain microsensors that can detect them, with an emphasis on H2O2, NO, ONOO−, and

O2. Lastly, we wrap off by looking ahead to how this technology could be improved in the future to create electrochemical sensors that can monitor these species in biological systems in real-time and have medical implications.

Conclusion:

modern electrochemical sensors have been developed to detect a wide range of inorganic and organic contaminants, as well as to detect and identify extremely small biomolecules (e.g., DNA, enzymes, hormones, etc.). New sensors constructed from a wide variety of chemical and biological detecting materials are constantly being developed and introduced. Additionally, mass production technology is perfect for the microelectronics industry since it enables the development of extremely tiny, reproducible, and inexpensive (disposable) sensor devices. Instrumentation based on microprocessors is used in conjunction with these devices to make them lightweight and easy to use. New developments in stable and selective identification elements, like nanotechnology, molecular devices, micromachining, "smart" sensors and remote electrodes, and multiparameter sensor arrays will surely impact pollution management. The use of electrochemical sensors is a recent innovation in the field of biology. Furthermore, the future industry offers benefits in processing speed, selectivity, and sensitivity. Electrochemical techniques are useful for the nondestructive, rapid, and precise analysis of many different types of objects. Nanomaterials (such as carbon nanotubes, graphene, graphene derivatives, metal nanoparticles, and gold nanoparticles) and functional peptides (aptamers) have been employed to enhance sensitivity. During an electrochemical measurement, a measurable read signal is produced when the target interacts with a certain probe or composite.

References:

1. Walter, E.C.; Penner, R.M.; Liu, H.; Ng, K.H.; Zach, M.P.; Favier, F. Sensors from electrodeposited metal nanowires. Surf. Interface Anal. 2002, 34, 409-412; DOI 10.1002/sia.1328.
2. Lu, Y.; Yang, M.; Qu, F. Enzyme-functionalized gold nanowires for the fabrication of biosensors. Bioelectrochem. 2007, 71, 211-216; DOI 10.1016/j.bioelechem.2007.05.003.
3. Qu, F.; Yang, M.; Shen, G.; Yu, R. Electrochemical biosensing utilizing synergic action of carbon nanotubes and platinum nanowires prepared by template synthesis. Biosen. Bioelectron. 2007, 22, 1749-1755; DOI 10.1016/j.bios.2006.08.003.
4. Cusmà, A.; Curulli, A.; Zane, D.; Kaciulis, S.; Padeletti, G. Feasibility of enzyme biosensors based on gold nanowires. Mat. Sci. Eng. C. 2007, 27, 1158-1161; DOI 10.1016/j.msec.2006.09.035.
5. Aravamudhan, S.; Kumar, A.; Mohapatra, S.; Bhansali, S. Sensitive estimation of total cholesterol in blood using Au nanowires based micro-fluidic platform. Biosen. Bioelectron. 2007, 22, 2289-2294; DOI 10.1016/j.bios.2006.11.027.
6. Aravamudhan, S.; Ramgir, N.S.; Bhansali, S. Electrochemical biosensor for targeted detection in blood using aligned Au nanowires. Sensors and Actuators B. 2007, 127, 29-35; DOI 10.1016/j.snb.2007.07.008.
7. Gao, Z.; Agarwal, A.; Trigg, A.D.; Singh, N.; Fang, C.; Tung, C.H.; Fan, Y.; Buddharaju, K.D.; Kong, J. Silicon nanowire arrays for label-free detection of DNA. Anal. Chem. 2007, 79, 3291-3297; DOI 10.1021/ac061808q; PubMed 17407259.
8. Lapierre-Devlin, M.A.; Asher, C.L.; Taft, B.J.; Gasparac, R.; Roberts, M.A.; Kelley, S.O. Amplified electrocatalysis at DNA-modified nanowires. Nano Lett. 2005, 5, 1051-1055; DOI 10.1021/nl050483a; PubMed 15943441.
9. Liu, L.; Song, J.F.; Yu, P.F.; Cui, B. A novel electrochemical sensing system for inosine and its application for inosine determination in pharmaceuticals and human serum. Electrochem. Comm. 2006,

8, 1521-1526; DOI 10.1016/j.elecom.2006.07.013.

10. Basu, M.; Seggerson, S.; Henshaw, J.; Jiang, J.; Cordona, R.D.A.; Lefave, C.; Boyle, P.J.; Miller, A.; Pugia, M.; Basu, S. Nano-biosensor development for bacterial detection during human kidney infection: Use of glycoconjugate-specific antibody-bound gold NanoWire arrays (GNWA). Glycoconjugate Journal. 2004, 21, 487-496; DOI 10.1007/s10719-004-5539-1; PubMed 15750790.
11. Liu, Z.; Searson, P.C. Single nanoporous gold nanowire sensors. J. Phys. Chem. B. 2006, 110, 4318-4322; DOI 10.1021/jp056940t; PubMed 16509729.
12. Ramgir, N.S.; Zajac, A.; Sekhar, P.K.; Lee, L.; Zhukov, T.A.; Bhansali, S. Voltammetric detection of cancer biomarkers exemplified by interleukin-10 and osteopontin with silica nanowires. J. Phys. Chem. C. 2007, 111, 13981-13987; DOI 10.1021/jp073371b.
13. Murray, B.J.; Walter, E.C.; Penner, R.M. Amine vapor sensing with silver mesowires. Nano Lett. 2004, 4, 665-670; DOI 10.1021/nl049841k
14. Sakaguchi, T.; Morioka, Y.; Yamasaki, M.; Iwanaga, J.; Beppu, K.; Maeda, H.; Morita, Y.; Tamiya, E. Rapid and onsite BOD sensing system using luminous bacterial cells-immobilized chip. Biosens. Bioelectron. 2007, 22, 1345–1350. [Google Scholar] [CrossRef]
15. Chen, S.M.; Chzo, W.Y. Simultaneous voltammetric detection of dopamine and ascorbic acid using didodecyldimethylammonium bromide (DDAB) film-modified electrodes. J. Electroanal. Chem. 2006, 587, 226–234. [Google Scholar] [CrossRef]
16. Vasantha, V.S.; Chen, S.M. Electrocatalysis and simultaneous detection of dopamine and ascorbic acid using poly(3,4-ethylenedioxy)thiophene film modified electrodes. J. Electroanal. Chem. 2006, 592, 77–87. [Google Scholar] [CrossRef
17. Simoyi, M.F.; Falkenstein, E.; Dyke, K.V.; Blemings, K.P.; Klandorf, H. Allantoin the oxidation product of uric acid is present in chicken and turkey plasma. Comparative Biochemistry and Physiology Part B 2003, 135, 325–335. [Google Scholar] [CrossRef]
18. Balasubramanian, K.; Burghard, M. Biosensors based on carbon nanotubes. Anal. Bioanal. Chem. 2006, 385, 452–468. [Google Scholar] [CrossRef]
19. Zhang, S.; Wang, N.; Niu, Y.; Sun, C. Immobilization of glucose oxidase on gold nanoparticles modified Au electrode for the construction of biosensor. Sens. Act. B. 2005, 109, 367–374. [Google Scholar] [CrossRef]
20. Wang, J.; Musameh, M.; Lin, Y. Solubilization of carbon nanotubes by nafion toward the preparation of amperometric biosensors. J. Am. Chem. Soc. 2003, 125, 2408–2409. [Google Scholar] [CrossRef]
21. Yogeswaran, U.; Thiagarajan, S.; Chen, S.M. Nanocomposite of functionalized multiwall carbon nanotubes with nafion, nano platinum, and nano gold biosensing film for simultaneous determination of ascorbic acid, epinephrine, and uric acid. Anal. Biochem. 2007, 365, 122–131. [Google Scholar] [CrossRef]
22. Yogeswaran, U.; Chen, S.M. Separation and concentration effect of f-MWCNTs on electrocatalytic responses of ascorbic acid, dopamine and uric acid at f-MWCNTs incorporated with poly (neutral red) composite films. Electrochim. Acta. 2007, 52, 5985–5996. [Google Scholar] [CrossRef]
23. Hubalek, J.; Hradecky, J.; Adam, V.; Krystofova, O.; Huska, D.; Masarik, M.; Trnkova, L.; Horna, A.; Klosova, K.; Adamek, M.; Zehnalek, J.; Kizek, R. Spectrometric and voltammetric analysis

of urease – nickel nanoelectrode as an electrochemical sensor. Sensors 2007, 7, 1238–1255. [Google Scholar

24. Yogeswaran, U.; Thiagarajan, S.; Chen, S.M. Pinecone shape hydroxypropyl-β-cyclodextrin on a film of multi-walled carbon nanotubes coated with gold particles for the simultaneous determination of tyrosine, guanine, adenine and thymine. Carbon 2007, 45, 2783–2796. [Google Scholar] [CrossRef]
25. Yogeswaran, U.; Chen, S.M. Electrocatalytic properties of electrodes which are functionalized with composite films of f-MWCNTs incorporated with poly(neutral red). J. Electrochem. Soc. 2007, 154, E178–E186. [Google Scholar]
26. Kim, I.J.; Han, S.D.; Han, C.H.; Gwak, J.; Lee, H.D.; Wang, J.S. Micro semiconductor CO sensors based on indium-doped tin dioxide nanocrystalline powders. Sensors 2006, 6, 526–535. [Google Scholar]
27. Lyons, M.E.G.; Keeley, G.P. The redox behaviour of randomly dispersed single walled carbon nanotubes both in the absence and in the presence of adsorbed glucose oxidase. Sensors 2006, 6, 1791–1826. [Google Scholar]
28. Liang, W.; Zhuobin, Y. Direct electrochemistry of glucose oxidase at a gold electrode modified with single-wall carbon nanotubes. Sensors 2003, 3, 544–554. [Google Scholar]
29. Hernandez-Velez, M. Nanowires and 1D arrays fabrication: An overview. Thin Solid Films. 2006, 495, 51–63. [Google Scholar] [CrossRef]
30. Yogeswaran, U.; Chen, S.M. Multi-walled carbon nanotubes with poly(methylene blue) composite film for the enhancement and separation of electroanalytical responses of catecholamine and ascorbic acid. Sens. Act. B 2008, 128. in press. [Google Scholar] [CrossRef]
31. Shie, J.W.; Yogeswaran, U.; Chen, S.M. Electroanalytical properties of cytochrome c by direct electrochemistry on multi-walled carbon nanotubes incorporated with DNA biocomposite film. Talanta 2008, 74, 1659–1669. [Google Scholar]
32. Li, S.; He, P.; Dong, J.; Guo, Z.; Dai, L. DNA-directed self-assembling of carbon nanotubes. J. Am. Chem. Soc. 2005, 127, 14–15. [Google Scholar] [CrossRef]