

Biosynthesis and potential application of silver and gold nanoparticles to the electroanalysis of hydrogen peroxide and nitrite

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Recently, nanotechnology has become one of the most active research fields in the areas of material science, chemistry and electrocatalysis. Metal nanoparticles (NPs) are of great scientific interest because of their unique properties. Due to their extremely small size and large specific surface area, metal NPs exhibit unusual physicochemical and electronic properties, compared to those of bulk metals. Nowadays, the development of NPs-based electrochemical sensor devices has drawn great attention in the field of industrial, environmental, clinical and food analysis owing to their attractive performances: fast response, convenient operation, high sensitivity and selectivity. The electrochemical sensors offer possibility of miniaturization and potential for development of portable hand-held devices for real time monitoring. Recently, extensive studies have been performed to explore the electrochemical behavior of biosynthesized metal NPs and their potential in sensor design. Current review article features recent advancements in the plant-mediated synthesis of silver and gold nanoparticles (AgNPs and AuNPs), applicable in the electrochemical sensing of hydrogen peroxide (H₂O₂) and nitrite (NO₂⁻). Additionally, perspectives and challenges for promoting the development of electrochemical sensors based on bio-mediated nanostructured materials are commented.

Keywords: electrochemical sensor, green synthesis, plant-mediated synthesis, metal nanoparticles, hydrogen peroxide, nitrite.

INTRODUCTION

Electroanalytical chemistry, also known as electroanalysis, lies at the interface between analytical science and electrochemistry. It is concerned with the development, characterisation and application of chemical analysis methods employing electrochemical phenomena [1]. The electrochemical sensor devices are rapid and precise analytical tools that play an essential role in the fields of industrial analysis, environmental monitoring, and clinical analysis [2, 3]. Electrochemical sensors have great impact because of their relatively simple design, low cost, simplicity of use, high sensitivity, low detection limits, fast response time, long-term stability, miniaturization potential for development into portable/handheld devices, and possibility of on-site monitoring.

Although electrochemical techniques are versatile, voltammetry and amperometry are favored in sensing application. Typical voltammetry methods used in electrochemical sensing include cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The current signal is produced by the oxidation/or reduction of electroactive species (analyte) at the surface of the working electrode and the value of current magnitude is proportional to the concentration of

the analyte present in the sample.

Carbon-based electrode materials (glassy carbon, graphites, etc.) have been widely explored in electroanalytical applications, especially in electrochemical sensor design because of their excellent properties such as chemical inertness, wide potential window and low cost. The unmodified carbon surfaces exhibit slow electron transfer and often lack the sensitivity and selectivity required for the electrochemical detection. In order to overcome this shortcoming, nanomaterials have been incorporated within the electrode modifiers. Thus, the current research is mainly focused on the electrode modifications in order to increase the electron transfer kinetics and to decrease high overpotentials required for redox reactions of the target analyte on unmodified carbon electrode materials. The use of nano-sized materials (metal NPs, metal oxide NPs, alloy NPs, etc.) provide an enhanced sensor response, lower detection limit, better selectivity and sensitivity toward the detection of specific analyte due to increased surface area, enhanced electrocatalytic activity and faster electron transfer kinetics. The most commonly used metal NPs as electron-transfer mediators are AgNPs and AuNPs due to their unique physicochemical properties, good conductivity and electrocatalytic activity.

Recently, development of novel eco-friendly and cost-effective methods for synthesis of metal

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NPs has remained in the focus of researchers. Plant-mediated green synthesis of metal NPs involves the use of extracts of plant parts (leaves, flowers, fruits, roots) for the bioreduction of metal ions into their zero-valent elemental form in the size range 1–100 nm. Plant-mediated synthesis of metal NPs is distinguished by ecologic effectiveness (uses aqueous solvents, easily available plant material, biocompatible plant extracts, normal temperature and pressure), simple one-step formation and stabilization of metal NPs, as well as a possibility for waste biomass valorization.

Considerable efforts have been devoted to developing green strategies for facile synthesis of AgNPs and AuNPs. There is convincing evidence that green synthesis of metal NPs has potential to provide a new direction in the fabrication of cheap and highly effective electrode-catalysts. This review is aimed to summarize recent development of plant-mediated strategies for the sustainable production of AgNPs and AuNPs as alternative efficient green methods. Then, the application of biosynthesized AgNPs and AuNPs as electrocatalysts in constructing non-enzymatic electrochemical sensors for H₂O₂ and nitrite is discussed in detail.

Green synthesis of metal nanoparticles by plant extracts

Using plant extracts for the production assembly of metal NPs has drawn attention because its rapid, eco-friendly, non-pathogenic, and economic protocol provides a single-step technique for the biosynthetic process [4, 5]. The data show that the synthesis of metallic NPs using plant extracts is simpler, easier to scale up, and less expensive than that using bacteria or fungi. A wide range of molecules present in the plant extracts, ranging from proteins to various low-molecular weight compounds as flavonoids, polyphenols, terpenoids, alkaloids, amino acids, organic acids have been reported to play a role in the bioreduction of metal salts. Generally, during the synthesis, the reducing agents donate electrons to the metal ions and convert them to NPs. These NPs exist at a high-surface energy state and tend to convert to their low-surface energy conformations by aggregating against each other. Thus, the presence of higher amounts of reducing and stabilizing agents prevents the aggregation of NPs and promotes production of smaller NPs. Additionally, proteins can trap metal ions on their surface and convert them to their corresponding nuclei, which could further aggregate and, consequently, form metal NPs [6].

A number of experiments have shown that the source of the plant extract affects the characteristics

of metal NPs because different extracts may contain different combinations and concentrations of organic reducing agents. Therefore, the size and shape of biosynthesized NPs can be controlled by modifying the nature and concentration of the plant extract, concentration of the precursor (metal salt), temperature, pH and reaction time. Fig. 1(A) shows the basic steps in the plant-mediated synthesis of metal NPs and the subsequent modification of electrode surface through a dropwise addition of the resulting colloidal solution.

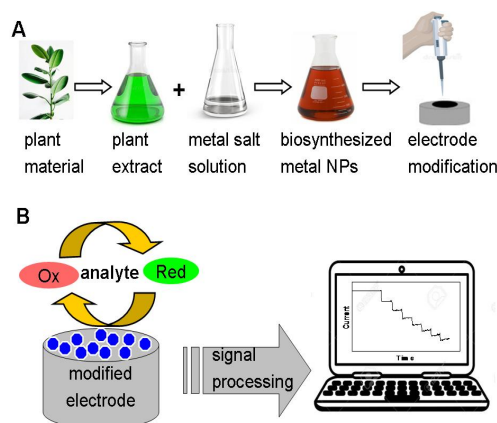


Fig. 1. Illustrative representation of: A) preparation of electrode modified with biosynthesized metal NPs; B) general principle of electrochemical detection.

The sensing system uses a three-electrode system connected to a potentiostat. The general principle of electrochemical detection is illustrated on Fig. 1(B). The electrochemical reactions take place at the electrode interface by means of a heterogeneous electron transfer. Briefly, at the surface of the working electrode the analyte participates in a redox reaction catalyzed by the electrode modifier (biosynthesized metal NPs acting as an electron transfer mediator between the electrode and a reaction substrate). The current generated in the process is converted into a signal that could be processed and displayed easily. Analyzing the signal magnitude we can obtain information about the concentration of the substance being analyzed.

Electrochemical H₂O₂ sensors based on biosynthesized silver nanoparticles

Fast, reliable and accurate detection of H₂O₂ is one of the topical problems in analytical chemistry, since H₂O₂ is an important analytical target in the field of environmental chemistry, industry, clinical analysis, food chemistry and biochemistry. In the past decades, enzyme-free electrochemical sensors have been studied increasingly because of their high performance for H₂O₂ detection. Today, the development of newly advanced greenly synthesized electrode materials for reliable H₂O₂

detection is essential. Considerable research interest has been focused on the subject how to modify the electrode surface using biosynthesized metal NPs for the purpose of obtaining a sensor with high sensitivity, selectivity and stability.

Salve *et al.* have used *Tagetes erecta* (Marigold) flowers extract for AgNPs synthesis by a green route [7]. The morphology and crystal structure of the prepared nanomaterial was characterized by field emission scanning electron microscopy (FESEM), ultraviolet–visible spectroscopy (UV–Vis), elemental dispersive X-Ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). The UV-Vis studies showed the occurrence of an absorption band at 430 nm which is specific for AgNPs. FESEM analysis indicated that the biosynthesized AgNPs have a homogenous size distribution and XRD patterns reflected that the particles are crystalline in nature with a face-centered cubic structure. Using the biosynthesized AgNPs and chitosan (CS), modified pencil graphite electrode (PGE) was fabricated by a drop-casting method and the as-prepared hybrid material AgNPs/CS/PGE was used for supercapacitor and electrochemical sensing applications. It has been shown that the modified electrode AgNPs/CS/PGE possesses remarkable catalytic activity towards electrochemical reduction of H_2O_2 . Cyclic voltammetry measurements in electrolyte 0.1 M HCl/KCl (pH 2.0) demonstrated that effective electroreduction of H_2O_2 was realized and the sensor based on biosynthesized AgNPs exhibited a high sensing performance for H_2O_2 detection in the concentration range 1.0 – 10.0 μ M with a detection limit of 0.52 μ M and sensitivity of 0.129 mA μ M⁻¹. Furthermore, the proposed sensor was used to detect H_2O_2 in cosmetic as well as in medical samples with high accuracy and selectivity, making it a good choice in the development of a disposable, low-cost device for H_2O_2 detection. The result obtained gave appreciable recovery value suggesting high sensitivity of the electrode towards H_2O_2 .

The essential oil industry wastes are rich in non-volatile polar metabolites (flavonoids, organic acids, carbohydrates, amino acids, etc.) that have reduction properties and could influence the synthesis and stabilization of AgNPs. Dodevska *et al.* have reported for the first time utilization of *Rosa damascena* waste for synthesis of AgNPs and demonstrated the applicability of the biosynthesized AgNPs for development of electrochemical sensors for H_2O_2 and vanillin [8]. TEM micrographs

showed that using an aqueous extract of *Rosa damascena* AgNPs were obtained as sphere-like particles with an average size calculated to be 25.8 ± 11.5 nm. Biosynthesized AgNPs were deposited onto a spectroscopic graphite (Gr) electrode and the electroactive layer was stabilized by applying a thin film of chitosan onto the modified electrode surface. Chitosan is a preferable low-cost material in designing electrochemical sensors and biosensors. It is a natural linear amine-rich polysaccharide, biocompatible polymer distinguished by its membrane-forming ability, good adhesion and mechanical strength. Chitosan adsorption on the surfaces of metal NPs can stabilize and protect the nanoparticles. In the present case, the coverage of chitosan on the newly generated AgNPs will prevent it from growing further, and also stabilize the size and surface property of the AgNPs in the cluster during its applications. The electrochemical behaviour of the modified electrode AgNPs/CS/Gr was studied by means of CV, DPV and chronoamperometry in neutral medium and its applicability for quantitative detection of H_2O_2 and vanillin was investigated. Vanillin (4-hydroxy-3-methoxybenzaldehyde) has a specific aroma and it is one of the most commonly used food supplements. For adults the permissible daily intake of vanillin is less than 10 mg kg⁻¹; the addition of vanillin in baby formula and infant food is not permitted. The overweight content of vanillin in food products, as the excessive ingestion via the dietary intake has potential toxic effect. Therefore, the reliable and accurate detection of vanillin has become an important research topic in food safety control. Electrochemical studies suggested that graphite electrode modified with AgNPs, biosynthesized using *Rosa damascena* waste, possesses a stable response to vanillin up to 0.5 mM with a detection limit of 8.4 μ M at an applied potential of 0.58 V (vs. Ag/AgCl, 3 M KCl). Additionally, the developed electrode exhibited a sensitive and reproducible response for quantitative determination of H_2O_2 . Amperometric measurements at a constant potential of -0.3 V showed a highly sensitive response to H_2O_2 up to 6.6 mM.

Electrochemical studies with AgNPs biosynthesized using flower aqueous extracts of *Achillea millefolium* and *Lavandula angustifolia* wastes as reducing agents, which is a novel simple approach, inexpensive and eco-friendly in nature, also were reported [9]. The representative electron micrographs of AgNPs showed that the nanoparticles grew very tiny with spherical shape. From the presented histograms it can be seen the

size distribution of AgNPs and their mean sizes were 2.8 nm for AgNPs/*Achillea millefolium* and 3.1 nm for AgNPs/*Lavandula angustifolia*, respectively. Selected area electron diffraction (SAED) pattern represents the (111), (220) and (222) crystal planes of the cubic structure of AgNPs in both samples. Biosynthesized AgNPs were deposited onto a spectroscopic graphite surface, applying two different procedures, and stabilized using chitosan to build new electrocatalysts. The electrochemical performance of the modified electrodes was studied by means of CV and chronoamperometry and their applicability for amperometric detection of H₂O₂ was demonstrated. The modified electrodes showed a remarkable activity at applied potentials of -0.3 V and -0.2 V (vs. Ag/AgCl, 3 M KCl), rapid, stable and reproducible amperometric response. It was stated that amperometry at a constant potential of -0.3 V is distinguished by extremely high sensitivity of 533.5 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ up to 4.3 mM H₂O₂. In order to study the selectivity, the amperometric response was examined in the presence of common interfering species (nitrate, glucose, uric acid, ascorbic acid, citric acid). The presented authentic record of the electrode signal clearly shows that the tested species have no effect on the H₂O₂ detection – no response was observed in the presence of the above mentioned substances and the current response for H₂O₂, registered after adding the substances, corresponds to the one determined in the calibration study. The results prove that the modified electrode has good selectivity for H₂O₂ and reveal the application potential of the so-biosynthesized AgNPs for electrochemical sensing of H₂O₂ in real samples.

Integrating graphene materials with metal NPs can offer synergistic effects, which could effectively enhance the catalytic performance of sensor systems [10]. Salazar *et al.* reported a one-step green strategy to obtain nanocomposite rGO/AgNPs using green tea extract for reducing both silver ions (Ag⁺) and graphene oxide (GO) sheets [11]. TEM analysis showed that the biosynthesized AgNPs have a quasi-spherical shape and an average size of 25 nm. Glassy carbon (GC) electrode was conveniently modified with rGO/AgNPs nanohybrid and electrochemical tests were carried out to study the properties of rGO/AgNPs/GC towards H₂O₂ detection. Amperometric studies at an applied potential of -0.4 V (vs. Ag/AgCl) in 0.1 M PBS (pH 8.0), revealed that the rGO/AgNPs/GC sensor possesses high sensitivity (236 $\mu\text{A mM}^{-1} \text{cm}^{-2}$, R² = 0.999) in a wide linear range of 0.002 – 20 mM, rapid

response (~ 2 s) and low detection limit of 0.73 μM H₂O₂ (signal-to-noise S/N = 3). The selectivity of the modified electrode was tested against different biological interferences including dopamine, glutamate, glucose and ascorbic acid with promising results. In the presented study, rGO introduces interfacial phenomena that greatly improve sensor sensitivity, selectivity, response time and limit of detection. The improved electrical conductivity, as well as the high density of edge-plane defect sites on rGO are valuable for accelerating electron transfer between the electrode and H₂O₂ molecules, leading to the superior electrocatalytic activity and selectivity towards H₂O₂ detection. The practical applicability of the developed rGO/AgNPs/GC sensor to detect selectively H₂O₂ in real samples (antiseptic solutions, commercial milk and urine) was tested and satisfactory results were obtained. After 7-month storage it was established that rGO/AgNPs/GC has no significant loss of sensitivity.

Other researchers have also shown that the biomediated approach provides a promising platform for the application of rGO-based composites in the non-enzymatic amperometric sensor field [12-14]. Kumar *et al.* have developed an Ag-Au-rGO composite through a facile and green reduction process using *Azadirachta indica* extract [12]. The morphological and structural characterization revealed that the prepared composite exhibited the unique features of uniformly distributed nanoparticles with alloy structure over the rGO sheets. The authors have proven that the electron transfer kinetics were enhanced for Ag-Au-rGO/GC over the GO/GC, rGO/GC and Ag-rGO/GC. The rGO supported Ag-Au bimetallic nanoparticle based non-enzymatic H₂O₂ sensor exhibited good selectivity at an applied potential of -0.4 V (vs. Ag/AgCl). Compared to the work [11] cited above, Kumar *et al.* have reported a shorter linear range (0.1 – 5 mM) and a higher detection limit (1 μM) towards the amperometric detection of H₂O₂. Unfortunately, the authors did not provide data on the sensitivity and stability of the Ag-Au-rGO/GC electrode, as well as on the sensing ability of the prepared Ag-Au-rGO composite towards H₂O₂ in real samples.

A facile synthesis of reduced graphene oxide-silver nanocomposite (rGO-Ag) was carried out from *Plectranthus amboinicus* leaf extract [13]. TEM observation revealed that the AgNPs formed are in spherical shape with diameter of 22.5 nm and uniformly decorated themselves on the rGO surface. In contrast to both articles [11, 12]

commented above, Zheng *et al.* did not use the hazardous reagent dimethyl formamide (DMF) during the electrode surface modification. At an applied potential of -0.32 V (vs. Ag/AgCl) the modified rGO-Ag/GC attains a steady-state current within 5 s, suggesting that the fabricated sensor has a rapid response towards H_2O_2 . A linear relationship between the current response and H_2O_2 concentration ($0.1804 \mu A \mu M^{-1}$) was observed in the range of $1 - 800 \mu M$; the detection limit was calculated to be $0.312 \mu M$. The amperometric records presented in both commented articles [12, 13] clearly show that organic species such as ascorbic acid, dopamine, glucose, uric acid, etc., have no effect on the quantitative H_2O_2 determination. The results reveal the application potential of the modified electrodes Ag–Au–rGO/GC [12] and rGO-Ag/GC [13] for precise sensing of H_2O_2 in real samples.

The electrochemical enzyme-free H_2O_2 sensors based on biosynthesized AgNPs and the corresponding sensing performances are summarized in Table 1.

Electrochemical nitrite sensors based on biosynthesized silver and gold nanoparticles

Electrochemical detection of nitrite ion (NO_2^-) is significant due to its environmental and biological-related issues. Nitrite is frequently found in industrial waste water and fertilizers. Potassium nitrite (KNO_2) and sodium nitrite ($NaNO_2$) are listed as permitted food additives (E249 and E250, respectively). KNO_2 and $NaNO_2$ are widely used as preservatives in the preparation of cured meat products – nitrite has a pronounced antimicrobial activity, acts as a color fixative and inhibits lipid

oxidation, thereby slowing meat spoiling. Nitrite intake is associated with a higher relative risk of gastric cancer and colorectal cancer, since NO_2^- is a precursor of carcinogenic nitrosamine. Nitrite is toxic and mutagenic to both humans and animals. Therefore, NO_2^- detection is important for environmental security, public health and food quality control. Owing to the rapid response and simple use, electrochemical techniques are favorable for nitrite detection [16]. To date, very few data on the nitrite sensors based on biosynthesized AgNPs and AuNPs, are available. Shivakumar *et al.* reported on a facile, cost effective, green synthesis method of silver nanospheres (AgNS) by using pre-hydrolyzed liquor (PHL) from the *Nilgiri* wood generated by pulp industry [17]. XRD pattern of AgNS evidences face-centered cubic crystalline structure of metallic silver; the average crystallite size of AgNS calculated from Scherrer equation was found to be ~ 30 nm. Authors suggested that hemicelluloses present in PHL were responsible for the reduction of Ag^+ and the subsequent stabilization of biosynthesized AgNS. Repeated synthesis and characterization of AgNS from different batches of the PHL demonstrated the reproducibility with the current material and method. Glassy carbon electrode modified with the so-produced AgNS exhibits excellent electrocatalytic activity towards nitrite oxidation – low detection limit of 31 nM and high electrode sensitivity of $580 \mu A mM^{-1} cm^{-2}$ in the concentration range $0.1 - 8.0 \mu M$. The modified electrode is selective for NO_2^- and most of the common interferences do not affect the quantitative nitrite determination.

Table 1. Operational characteristics of non-enzymatic H_2O_2 electrochemical sensors based on biosynthesized AgNPs.

Modified electrode	Reducing agent	Sensitivity, ($\mu A mM^{-1} cm^{-2}$)	Linear range, (mM)	LOD, (μM)	Ref.
AgNPs/CS/Gr	<i>Achillea millefolium</i>	533.5	up to 4.3	–	9
AgNPs/CS/Gr	<i>Lavandula angustifolia</i>	374.7	up to 3.5	–	9
AgNPs/CS/PGE	<i>Tagetes erecta</i>	$0.129 mA \mu M^{-1}$	0.001 – 0.01	0.52	7
AgNPs/rGO/GC	<i>Green tea</i>	236	0.002 – 20	0.73	11
AgNPs/CS/Gr	<i>Rosa damascena</i>	115.2	up to 6.6	–	8
AgNPs/Gr	<i>Rosa damascena</i>	214.7	up to 3.9	–	8
AgAu/rGO/GC	<i>Azadirachta indica</i>	–	0.1 – 5	1	12
AgNPs/rGO/GC	<i>Plectranthus amboinicus</i>	–	0.001 – 0.8	0.312	13
AgNPs/GO/GC	<i>Callicarpa maingayi</i>	–	0.005 – 0.7	0.6	14
AgNPs/rGO/GC	<i>Rumex roseus</i>	$64 mA mM^{-1}$	0.035 – 1.95	1.1	15

LOD (limit of detection); CS (chitosan); GC (glassy carbon); Gr (graphite); GO (graphene oxide); rGO (reduced graphene oxide) PGE (pencil graphite electrode).

The here presented electrode retains up to 98 % of its initial activity after a period of 1 month. The sensor demonstrates successful nitrite detection in real samples with good stability and reproducibility. The feasibility of employing the sensing system for real sample (tap water) analysis was explored by the standard-additions method. Satisfactory recoveries (average recovery ranging from 98 – 106 %, $n = 3$) of nitrite concentrations were obtained indicating the viability of employing the sensor for real sample analysis. Reproducibility of the catalytic activity of AgNS/GC was tested by carrying out the nitrite oxidation with four different GC electrodes loaded with the same amount of AgNS. The oxidation currents for nitrite at 0.86 V at these four electrodes show a very small change with a RSD of 3.6 % (three repeated experiments for each electrode). The authors concluded that AgNS/GCE can serve as a reliable platform for long-term application towards electrochemical nitrite sensing.

A facile and environmentally benign method exploiting *Piper betle* biomass as a reducing and stabilizing agent was proposed for the green preparation of AgNPs by Ramachandran *et al.* [18]. AgNPs biosynthesized from dried *Piper betle* leaves extract exhibited face-centered cubic structure with preferred (111) orientation and average particle size of 20 nm associated with homogeneous distribution. The fabricated AgNPs/GC sensor possesses electrocatalytic activity toward nitrite oxidation with a response time of 10 s, high sensitivity of $1642.27 \mu\text{A mM}^{-1} \text{cm}^{-2}$ and detection limit of $0.046 \mu\text{M}$. The amperometric current response exhibited linearity over a wide range of nitrite concentrations from $1 \mu\text{M}$ to 6mM . However, in that paper, a significantly higher applied potential (1.0 V vs. Ag/AgCl) was necessary for achieving effective detection of NO_2^- . The operational stability of the fabricated AgNPs/GC was evaluated for 30 days with an interval of a day (the electrode was stored in normal atmospheric conditions, when not in use) and was found to be 91.2 % of its original response after one month. Although the constructed sensor exhibited long-term stability and good anti-interference properties, quantitative determination of nitrite in real samples was not reported.

AuNPs have attracted much attention due to their remarkable properties including high mechanical stability, unique tunable optical and electronic properties, high electrical conductivity, and catalytic activity. Mohd Taib *et al.* have

described a new, reliable, environmentally friendly and cost-effective green procedure for synthesis of AuNPs using an aqueous extract of *Hibiscus sabdariffa* leaves [19]. The authors suggested that chlorogenic acid (an ester of caffeic acid and quinic acid) in *H. sabdariffa* extract is the major compound involved in the reduction of Au^{3+} to Au^0 . TEM analysis confirmed that the biosynthesized AuNPs were formed with a narrow distribution and an average particle size of $7 \pm 2 \text{nm}$. In order to fabricate a modified electrode-catalyst, the dispersed solution containing AuNPs was sonicated for 30 min before immobilizing the NPs on the surface of the GC electrode by a casting method. The so-prepared electrode AuNPs/GC was left in an oven at $55 \text{ }^\circ\text{C}$ for 6 h and then was tested as a catalyst in the electrooxidation of nitrite. At a voltage of 0.8 V, the electrode detects nitrite in the range of 0.37 to 10mM (LOD = 0.11mM) and the sensitivity is $917 \pm 30 \mu\text{A mM}^{-1} \text{cm}^{-2}$. The obtained results confirmed that the prepared AuNPs/GC has adequate stability, repeatability and reproducibility and could be used for determination of nitrite. After 3-week storage the prepared electrode possesses around 80 % of its initial response. Analysis of ten sequentially prepared electrodes showed an RSD of 4.27 % which confirmed the repeatability of AuNPs/GC. The sensor-to-sensor reproducibility was investigated by measuring the current responses of five diverse electrodes prepared independently by the same procedure. The results showed that the response produced by different electrodes had a good reproducibility with RSD of 4.21 % and the authors concluded that the sensor fabrication methodology was reliable. The sensor performance towards real samples was studied through recovery studies by adding known concentrations of nitrite to tap water samples and mineral water. According to the data presented and considering the RSD values, as well as calculated recoveries, it can be concluded that the AuNPs/GC holds possible applications for evaluating specific concentration range of nitrite ions.

Table 2 provides an overview of the nitrite electrochemical sensors based on biosynthesized AgNPs and AuNPs. Unpublished results of our work in progress were also included. Extensive studies have been performed to explore the electrochemical behavior of biosynthesized AgNPs and AuNPs (*Rosa damascena* – mediated synthesis) and their potential applications for electrochemical quantitative detection of nitrite.

Table 2. Operational characteristics of enzyme-free NO₂⁻ electrochemical sensors based on biosynthesized AgNPs and AuNPs.

Modified electrode	Reducing agent	Sensitivity, (μA mM ⁻¹ cm ⁻²)	Linear range, (mM)	LOD, (μM)	Ref.
AgNS/GC	<i>Nilgiri wood</i>	580	0.0001 – 0.008	0.031	17
AgNPs/GC	<i>Piper betle</i>	1642	0.001 – 6	0.046	18
AuNPs/GC	<i>Hibiscus sabdariffa</i>	917	0.37 – 10	110	19
AgNPs/Ch/Gr	<i>Rosa damascena</i>	224.4	0.02 – 1.7	10	work in
AuNPs/Ch/Gr	<i>Rosa damascena</i>	156.8	0.05 – 3.7	20	progress

CLOSING REMARKS

Green development of metallic nanoparticles, particularly AgNPs and AuNPs, is important to avoid the adverse effects on the environment that are commonly associated with chemical synthesis of metallic nanoparticles. Adhering to the principles of green chemistry, a number of research groups have used plant extracts for safe and fast synthesis of stable metal NPs. This approach completely avoids the hazardous solvents/ surfactants, does not require external stabilizing agents because biogenic components of plants themselves act as stabilising, as well as capping agents; thus enables a clean and sustainable synthesis. Biomediated metal NPs are receiving increasing interest for sensor construction in recent years. In this review article we highlighted the application of biosynthesized AgNPs and AuNPs as electrocatalysts for non-enzymatic electrochemical quantitative detection of H₂O₂ and nitrite. According to Web of Science database (by end-November, 2021) the number of original research articles reporting on AgNPs-based non-enzymatic electrochemical sensors for H₂O₂ is 41 (publication years 2015-2021). Only 8 studies on electrochemical sensing platforms based on biosynthesized AgNPs were identified by conducting a search of current literature. Although relatively few studies have appeared on these issues to date, the initial results are promising. The experimental data reveal that the sensors exhibit fast amperometric sensing, low detection limit, wide linear range, high sensitivity and good selectivity. Therefore, we can conclude that bio-mediated nanostructured electrode materials offer encouraging electrochemical results paving new dimensions for future research in this field.

However, there are still some obstacles to overcome compared to the conventional synthesis methods. Generally, the main challenges encountered in the development of electrochemical sensors based on biosynthesized metal NPs can be listed as follows:

1/ Optimization of plant-mediated synthesis protocols. Every plant extract varies in its

capabilities to supply metallic NPs. It is well known that some properties of metal NPs that are critical to their specific applications may vary significantly, depending on the green production method used. For research works on the specific applications of NPs in electroanalysis, it is important to select a reproducible synthesis technique based on the properties required for the targeted electrochemical reaction. A main limitation has been the control over crystal phase, sizes and shapes of greenly produced metal NPs that are mostly predetermined by different phytochemical compositions in the plant. Therefore, researchers should refine the plant-mediated synthesis protocols.

2/ Optimization of electrode modification protocols to give an improved electrochemical response. For electrochemical sensing purposes, electrode modification procedure should provide stable, reproducible, sensitive and selective analytical response. Also sensor-to-sensor reproducibility is extremely important. However, using various plant extracts it is difficult to control the bio-mediated synthesis and immobilization of the resulting metal NPs with varying populations of size and shape. Hence, the surface morphology of these nanomaterials might differ between each modified electrode. Moreover, when using nanomaterials, operational and long-term stability can become a major concern due to issues related to aggregation and flaking of NPs-modified layers on the electrode surface.

Furthermore, efforts should be devoted to optimization of electrode modification procedures to improve the electron transfer rate and to enhance the anti-interference ability of the sensors, suppressing the non-specific adsorption of interfering species. It is important for researchers to keep on developing innovative solutions in order to create more reliable sensors.

Further studies should be performed to investigate the potential of biogenic AgNPs and AuNPs in design of electrochemical sensors with advanced properties and emerging practical applications.

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