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Square Wave Voltammetric Detection of Nitrite on Platinum Electrode Modified with *Moringa oleifera* Mediated Biosynthesized Nickel Nanoparticles

Solomon A. Mamuru[1,*], Nelson Saki[1], Dahiru M. Bello[1], Moorey B. Dalen[2]

[1]*Electrochemical NanoLab, Department of Chemistry, Adamawa State University, Mubi, Nigeria*
[2]*Department of Chemistry, University of Jos, Jos, Nigeria.*

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ABSTRACT

In this study nickel chloride was reduced to its "nanoform" through a one-step synthesis protocol using the leaves extract of *Moringa oleifera*. Microscopic and spectroscopic techniques such as UV-vis., FTIR and SEM were used to confirm the formation of nickel nanoparticles. Electrochemical characterization using cyclic voltammetry and square wave voltammetry further reveals the electrochemical activity of synthesised nickel nanoparticles; as preliminary study on nitrite electro-oxidation shows that nickel nanoparticles can catalyse this process better than some other nanoparticles.

1. Introduction

The use of nitrite (NO_2^-) as a preservative because of its anti-microbial activity has been known for ages [1]. It is a colour enhancing agent to cured meat products and also provides indirect beneficial effect on flavour [2]. When ingested it react with haemoglobin, leading to reduction in the blood oxygen-carrying capacity, and with amines converting them into nitrosamines, which are well known carcinogenic substances [3]. The build – up of nitrite concentrations in the environment, owing to their use in agricultural processes (as fertilizers), resulting in contamination of water sources for human consumption, has been of major concern. Hence, it is almost impossible not to encounter nitrite ions or the products of their use in our everyday domestic activities. Various researchers have suggested strategies to facilitate the detection, determination and monitoring of nitrite [4]. Such techniques include chromatography [5], spectroscopy [6], capillary electrophoresis [7] and spectrofluorimetry [8, 9]. However, these techniques have their shortcomings, which include exposure to toxic reagents, tedious extraction procedures and inherent interference. Electrochemical methods are often favoured over others due to their simplicity, rapidity, measurement precision in turbid solutions, and low cost [10, 11]. In principle, electrochemical determination of nitrite may involve both oxidation and reduction, but in actual practice, oxidation is usually preferred over reduction because cathodic nitrite measurement is prone to interference from other readily reducible species such as nitrate ion and molecular oxygen [12]. Nitrite is electroactive at traditional electrodes made up of glassy carbon, platinum, diamond, gold, copper, and transition metal oxides [13-15]. The electrochemical oxidation of nitrite on bare electrodes may be adversely affected by several species (such as nitrite oxidation products and intermediates) which can inhibit electrode processes via irreversible adsorption on the GCE surface and, thereby, decrease both sensitivity and accuracy [16, 17]. In addition, the direct electro-reduction/oxidation of nitrite ions requires high overpotentials at bare electrode surfaces. As a remedy to overcome these limitations, various modified electrodes have been exploited for nitrite determination [18-20]. For the purpose of sensing nitrite, electrodes have been chemically modified with facile electron-transfer materials, such as metallophthalocyanines and metalloporphyrins [21-24]. In recent years, an increasing variety of nano-materials has been employed for electrochemical studies, combining the advantages of electrochemistry and nanotechnology [25-27].

Stable nickel nanoparticles can be synthesized by chemical methods such as chemical reduction, electrochemical techniques, and photochemical reduction [28-30]. Meanwhile, the use of biosynthetic methods has attracted much attention because of their safe conditions in synthesis procedures, good distribution of synthesized nanoparticles, use of nontoxic solvents, etc., [31]. Many biotechnological applications such as remediation of toxic metals use microorganisms such as bacteria and yeast for the synthesis of nanoparticles [32]. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Moreover, plant extracts could be advantageous over microorganism synthesis because there is no need to expand the process of culturing and maintaining cell lines, and they are of low cost, fast, efficient, and generally lead to the formation of crystalline nanoparticles with a variety of shapes and sizes [33].

In this study plant mediated synthesized nickel nanoparticles is used to modify platinum bare electrode in order to investigate its possible application in the detection of nitrite. The electrochemical detection of nitrite is best performed in neutral pH phosphate buffer solution, (pH 7.4), this is because nitrite exists as nitrite ion (NO_2^-) in neutral phosphate buffer solution but disproportionate to produce neutral nitric oxide (NO) in slightly acidic conditions [34], hence this study was carried out in phosphate buffer solution pH 7.4. This is a follow up to a previous work carried out by Mamuru et al. [35] in which the electrochemical behavior of nickel nanoparticles synthesized using *Moringa oleifera* leaves extract was investigated.

2. Experimental Methods

2.1 Materials

Moringa oleifera leaves, nickel chloride, potassium ferrocyanide 98.5%, Guangdong Guanghua Chemical Factory Co. Ltd and potassium ferricyanide 97%, KEM LIGHT Laboratories Ltd; sodium nitrite, BDH Chemicals Ltd, Poole England; Autolab PGSTAT 302N; Perkin Elmer Frontier Fourier Transform Infra-red Spectroscopy; Jenway 6400 UV-visible spectrophotometer; Hanna Instrument 2211 pH meter; Disodium hydrogen phosphate dihydrate and sodium dihydrogen phosphate hydrate were used to synthesis and characterizations for this study.

2.2 Preparation of the Extract

Moringa oleifera fresh leaves were cut into small pieces, 10 g weighed and boiled in 200 mL distilled water and then filtered with Whatman filter paper No.1 to obtain the extract. The pH of the extract was increased by

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*Corresponding Author:mamuru19@adsu.edu.ng(Solomon A. Mamuru)

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adding few drops of NaOH solution. This is because formation of nanoparticles is favoured in alkaline medium.

2.3 Preparation of Nickel Nanoparticles

Exactly 0.0597 g of the nickel chloride was weighed with a top load balance and dissolved in 250 mL doubly distilled water to prepare 0.001 M nickel chloride solution; a light green colour was observed. This was stirred using magnetic stirrer for 1 hour 45 minutes with drop wise addition of 10 mL *Moringa oleifera* leaf extract until colour changed to dark brown as indicated in (Fig. 1C). The product was then centrifuged at 3000 rpm for 10 minute, washed with ethanol and distilled water until neutral and allowed to dry at room temperature.

2.4 Preparation of 0.005 M of Potassium Ferricyanide and Potassium Ferrocyanide in 0.2 M Sodium Hydroxide Solutions

About 0.4 g of potassium ferricyanide and 0.5 g of potassium ferrocyanide was placed in 250 mL volumetric flask, 2 g of sodium hydroxide was also added in the volumetric flask and make up to the mark with distilled water to prepare the potassium ferricyanide/ferrocyanide solution in 0.2 M sodium hydroxide.

2.5 Electrochemical Characterization

All electrochemical measurement was performed with an Autolab PGSTAT 302N Potentiostat driven by NOVA software version 1.9. A three–electrode system was employed for this study. The counter electrode is a platinum wire, and Ag|AgCl 3 M KCl was used as reference electrode, and working electrode is nickel nanoparticles modified platinum electrode.

2.6 Electrode Preparation

A 10 μL nickel nanoparticles solution was dissolved in distilled water and placed on the surface of an already polished and ultrasonically rinsed platinum electrode (3 mm diameter) using a micropipette and allowed to dry at room temperature to prepare platinum–nickel nanoparticles electrode (Pt/NiNPs). Electrochemical properties of the modified electrode were studied with an outer sphere redox probe 0.005 M potassium ferricyanide and potassium ferrocyanide in 0.2 M sodium hydroxide solution using cyclic voltammetry. Nitrite oxidation was studied using square wave voltammetry. 0.001 M sodium nitrite solution was prepared in phosphate buffer solution (pH 7.4) and was sweep scan from 0.1 V to 1.1 V.

3. Results and Discussion

3.1 UV-Visible Spectroscopy

During the course of adding 10 mL *Moringa oleifera* leaf extract to the flask containing 0.001 M nickel chloride there was no immediate colour change, however a change in colour was observed after 30 minute and the colour was slightly yellow/brown, at 45 minute it changed to light brown, and after 1 hour 20 minute the colour was completely brown, and finally turned dark brown (inset Fig. 1C) indicating the formation of the nickel nanoparticles [36]. The difference in shade of colour is mostly likely due to surface Plasmon resonance effect in nickel nanoparticles.

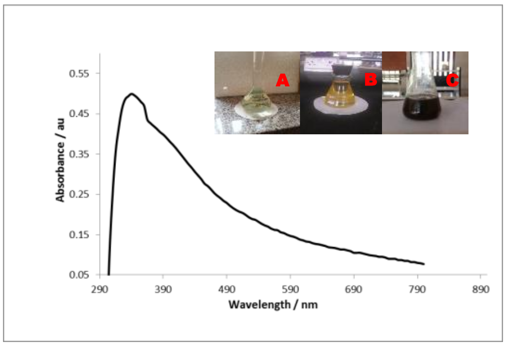

Fig. 1 UV-visible absorption spectrum of nickel nanoparticles synthesized using *Moringa oleifera* leaf (Inset images for reaction colour)

More information on nickel nanoparticles by reduction of aqueous metal ions during exposure with *Moringa oleifera* leaf extract was observed by UV-visible spectroscopy, a powerful technique in ascertaining the formation, size, purity and stability of metal nanoparticles in solution. The UV-vis. absorption spectrum of nickel nanoparticles is shown in Fig. 1. The spectra shows the characteristic surface plasmon resonance (SPR) with absorbance at approximately 295-350 nm with peak maxima at 340 nm, which can be attributed to the formation of nickel nanoparticles. The shape of the plasmon band was almost symmetrical, suggesting that the nanoparticle sizes are well-disperse and uniform. If the particles are not uniform, then it leads to a broad absorption peak at higher wavelength [37, 38].

3.2 Scanning Electron Microscopy Analysis

Scanning electron micrograph was obtained using a VEGA3 TESCAN scanning electron microscope operating at an accelerating voltage of 20 keV. From Fig. 2, it can be observed that the morphology of the nickel chloride changed from that of small "pebbles" to tiny "flakes" of the nickel nanoparticles.

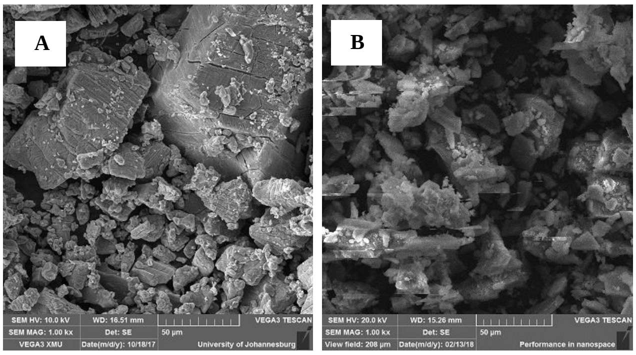

Fig. 2 Comparative SEM micrograph of A) nickel chloride and B) nickel nanoparticles

3.3 Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy analysis was performed to identify the possible biomolecules responsible for the reduction of $NiCl_2$ ions and capping of the reduced NiNPs synthesized using *Moringa oleifera* leaf extract.

Fig. 3 present the comparative IR absorption bands that were observed for *Moringa oleifera* leaves extract and nickel nanoparticles. Bands for the nickel chloride were observed at 3282.78 cm^{-1}, 2918.05 cm^{-1}, 2849.80 cm^{-1}, 1630.98 cm^{-1}, 1542.26 cm^{-1}, 1411.63 cm^{-1}, 1315.90 cm^{-1}, 1238.28 cm^{-1} and 1023.00 cm^{-1}. The bands which appeared at 3282.78 cm^{-1} is due to antisymmetric stretching, while the bands at 2918.05 cm^{-1} and 2849.80 cm^{-1} are due to asymmetric stretch, and symmetric stretch respectively. The bands at 1630.98 cm^{-1}and 1542.26 cm^{-1} are due to C=C stretching and C-N-H bend, respectively. The IR-bands observed at 1411.63 cm^{-1} and 1315.90 cm^{-1} may be ascribed to CH_2 scissoring deformation and CH symmetric rock, 1238.82 cm^{-1} is out of phase C-C-O stretch and 1023.00 cm^{-1} is due to the presence of aliphatic compound. All other bands observed for the *Moringa oleifera* leaf extract were observed in the spectra of the nickel nanoparticles except for the bands at 1542.26 cm^{-1} 1411.63 cm^{-1} and 1238.28 cm^{-1} which were absent and assigned to C-N-H bend which belong to amide -C(=O)-NHR functional group; CH_2 scissoring deformation as a functional group belonging to $R_2C=CH_2$ vinylidene and out of phase C-C-O stretch belonging to phenol may be responsible for the reduction of $NiCl_2$ to NiNPs. Inferentially, biomolecules of amide and phenolic constitution may be responsible for the reduction and capping process.

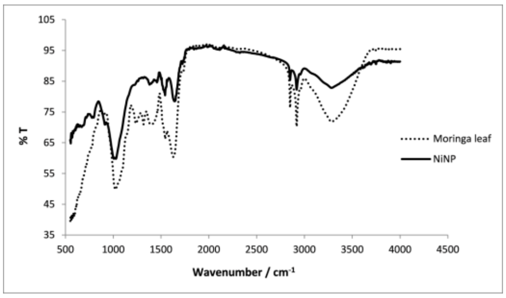

Fig. 3 FTIR absorption spectra of *Moringa oleifera* leaf extract and nickel nanoparticles synthesized using *Moringa oleifera* leaf

3.4 Electrochemical Characterization

It should be noted that $K_3Fe(CN)_6/K_4Fe(CN)_6$ is an important redox probe known to exhibit one-electron reversible process. The choice of this redox probe in this work allows us to know how fast one-electron of the $K_3Fe(CN)_6/K_4Fe(CN)_6$ species can be transferred when the bare electrode

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is modified. As presented in Fig. 4, the bare Pt electrode (A) shows a pair of oxidation (E_{ox}) and reduction (E_{red}) peak potentials at 250 and 190 mV ascribed to Fe^{3+}/Fe^{2+}. The Pt/NiNPs modified electrode (B) shows two pair of oxidation and reduction peaks at 254 and 191 mV which can be ascribe to Fe^{3+}/Fe^{2+} and 471 and 417 mV which is ascribe to the redox process [39]:

$$Ni + 2OH^- \rightleftharpoons Ni(OH)_2 + 2e^- \qquad (1)$$

The presence of the nickel redox process is an indication of the electro active nature of the nickel nanoparticles by reacting with the hydroxyl ion from the NaOH solution to form nickel hydroxide. Both electrodes gave formal potential ($E_{1/2} = E_{ox} + E_{red}/2$) of ~ 225 mV which is the standard electrode potential of $[Fe(CN_6)]^{3-} + e^- \rightleftharpoons [Fe(CN)_6]^{4-}$ redox process measured against Ag|AgCl 3 M KCl reference electrode. Analysis of the potential difference (ΔE), which is used to interrogate the electron transfer behavior of the electrodes, gave values of 80 mV and 60 mV for Pt and Pt/NiNPs electrodes, respectively. Using the expression ΔE = 60 mV/n where n is the number of electrons transferred; the number of electron involve for both electrodes are 0.75 and 1 for Pt and Pt/NiNPs electrodes, respectively indicating that the Pt/NiNPs permit better electron transfer of the $K_3Fe(CN)_6/K_4Fe(CN)_6$ species than bare Pt.

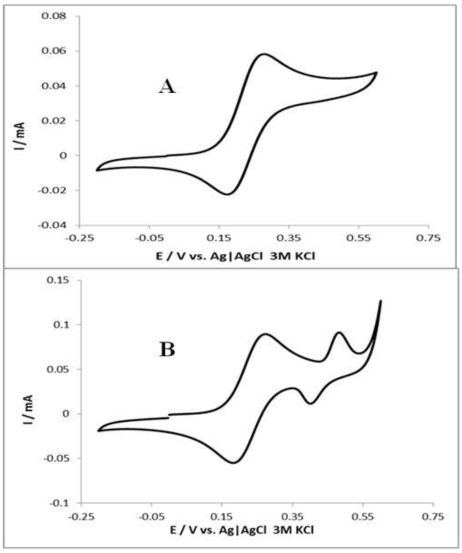

Fig. 4 Cyclic voltammetric evolutions of bare Pt (A) and Pt/NiNPs (B) electrodes recorded in 0.005 M $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution containing 0.2 M NaOH

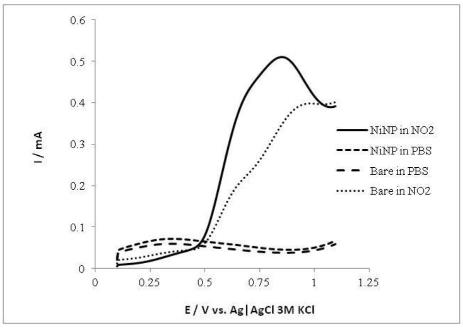

Fig. 5 Comparative square wave voltammetric evolutions of the various electrodes studied in 0.001 M sodium nitrite in phosphate buffer solution (pH 7)

3.5 Electrocatalytic Behaviour towards Nitrite Oxidation

Square wave voltammetry is more sensitive towards detection of analytes than cyclic voltammetry and linear sweep voltammetry, Fig. 5 shows the comparative square voltammetric evolutions of the various electrodes studied. The nickel nanoparticles modified electrode exhibited better catalytic behaviour towards nitrite oxidation compared to the other electrodes in terms of higher current response of ~ 0.5 mA to 0.4 mA for the bare Pt ; low onset potential of ~ 0.4 V to 0.5 V of bare Pt and less positive peak potential ~ 0.8 V to 0.9 V for the bare Pt. Furthermore, it is important to note that a 100 mV difference was observed for the onset potential and peak potential of a previously reported work [30, 40], suggesting that the bioreduced nickel nanoparticles exhibit better electrocatalytic behaviour than nanostructured iron (II) phthalocyanine and the chemically synthesized NiO nanoparticles.

4. Conclusion

Nickel nanoparticles were successfully synthesized using *Moringa oleifera* leaves extract, the formation of the nanoparticles was confirmed using UV-visible spectroscopy and scanning electron microscopy; the possible biomolecules responsible for the reduction of the nickel chloride to nickel nanoparticles may be of amide and phenolic constitution in the extract. Electrochemical characterization shows that the nickel nanoparticles are electroactive and can catalyse the oxidation of nitrite at low potential as against other reported nanoparticles.

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