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Electrochemical Study of Nitrobenzene Reduction Using Potentiostatic Preparation of *nephrolepis* Leaf Like Silver Microstructure

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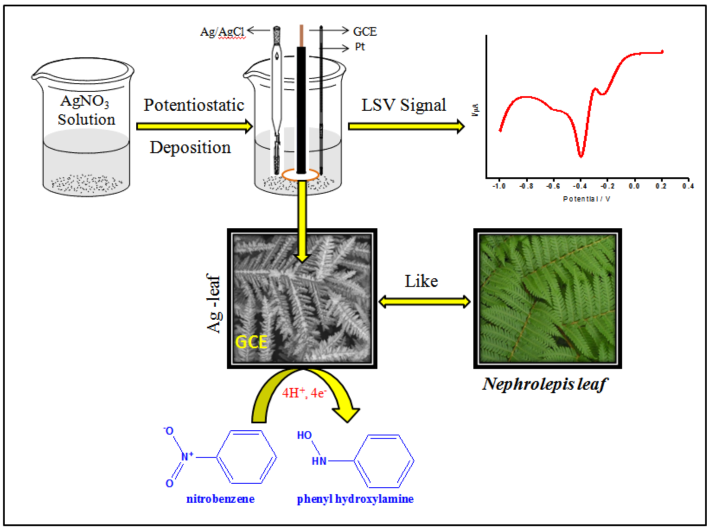
Nephrolepis leaf-like silver microstructures (NLLS-Ag) were prepared through a single step potentiostatic electrodeposition strategy and employed for the electrochemical reduction of nitrobenzene (NB). First, the NLLS-Ag microstructures were prepared on glassy carbon electrode (GCE) from aqueous solution of silver nitrate. The prepared NLLS-Ag microstructures were characterized by scanning electron microscopy, Energy-dispersive X-ray spectroscopy studies, X-ray diffraction and electrochemical methods. At NLLS-Ag/GCE, well-defined reduction peak corresponding to the reduction of NB was observed at lower overpotential with highly enhanced peak currents. The influences of different scan rates and different pH were investigated. An amperometric sensor is fabricated which exhibited excellent electroanalytical parameters towards NB, such as wide linear range of 0.05 – 38.8 μM and low detection limit of 0.01 μM. Moreover, the sensor exhibits good repeatability, reproducibility and stability. Besides, Practical applicability has been addressed in biological sample which presents appreciable recovery results.

Keywords: Metal Nanoparticles, Nanotechnology, Nitrobenzene, Electrochemical sensor, Electrocatalysis, Modified electrode

1. INTRODUCTION

Silver nanoparticles (AgNPs) are one of the most common metal ions widely known for their acclaimed medicinal and pharmaceutical applications [1-3]. They are extensively studied in nanotechnology and they are electrochemically active nanomaterials and hence they are active electrocatalysts to accelerate various important chemical reactions [4, 5]. The chemical methods used to produce AgNPs are require toxic reducing agents, time-consuming protocols, involves washing steps and encounter aggregation issues [6, 7]. In contrary, electrochemical methods are fast, do not require reducing agents and also avoids washing steps, yet they are feasible to prepare highly stable and electrocatalytically active metal nanoparticles [8-10]. Developing toxic chemicals free green routes for the preparation of metal nanoparticles is our lab's continuous research interest [11-13]. In the present work, we have developed a simple and fast one step potentiostatic electrodeposition method for the preparation of AgNPs (Scheme 1). The prepared AgNPs have shown excellent electrocatalytic ability to nitrobenzene (NB). Nitrobenzene (NB) is a significant solvent and raw material largely produced for its widespread industrial applications in the manufacturing of numerous important chemicals including aniline, dyes, plastics, pesticides, explosives, and pharmaceuticals [14, 15]. As a result, large quantity of NB is released in soil and water which cause environmental concern. NB is toxic even at low concentrations, carcinogenic, highly persistent, and difficulty in biodegradation due to the strong electron withdrawing groups [16, 17]. Notably, NB is listed as one of the 129 priority pollutants by United States Environmental Protection Agency (USEPA), while its maximum permissible concentration in wastewater is 1 mg/L [18]. Electrochemical methods are most preferable methods for the determination of NB over conventional analytical methods due to their simplicity, sensitivity, portability and low-cost [19]. Nevertheless, unmodified electrodes encountered important drawbacks such as large overpotential, surface fouling issues and interferences [15]. Therefore, numerous specifically designed chemically modified electrodes were reported for the sensitive determination of NB, such as palladium nanoparticles incorporated polymer-silica nanocomposite [20], cobalt-based metal–organic framework with incorporated macroporous carbon composite [21], Nitrogen-doped diamond electrode [22], nitrogen-doped porous carbon [23], electrochemically activated graphite modified screen printed carbon electrode [24] and gold nanoparticles [12].



Scheme 1 *Nephrolepis leaf* like silver microstructure: Potentiostatic preparation and its electrochemical application in determination of nitrobenzene

2. EXPERIMENTAL

2.1 Chemicals and Apparatus

Silver nitrate $Ag(NO)_3$, NB and all other reagents were purchased from Sigma-Aldrich. 0.1 M acetate buffer (acetic acid and sodium acetate) was used to prepare pH 3 and 5, 0.1 M phosphate buffer (sodium dihydrogen phosphate and disodium hydrogen phosphate) was used to prepare pH 7 and 0.1 M Tris-buffered saline (tris and sodium chloride) was used to prepare pH 9. Previous to each electrochemical testing, the electrolyte solutions were deoxygenated with pre-purified nitrogen gas for 5 min unless otherwise specified. The electrochemical experiments were carried out using electrochemical work stations CHI405a and CHI 900 at ambient temperature. A conventional three electrode system consisting of glassy carbon electrode (GCE) as a working electrode, Ag/AgCl (saturated KCl) as a reference electrode and Pt wire as a counter electrode. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectra were carried out using Hitachi S-3000H and HORIBA EMAX X-ACT, respectively. X-ray diffraction studies (XRD) were performed using XPERT-PRO diffractometer.

2.2 Preparation of nephrolepis leaf-like Ag microstructures

A single-step potentiostatic electrochemical deposition approach was established in order to prepare *nephrolepis leaf-like structured* Ag (NLLS-Ag) microstructures. First, 10 mM of $Ag(NO)_3$ aqueous solution was prepared by dissolving it in 10 mL of double-distilled water. Prior to performing the potentiostatic deposition, working, reference and counter electrodes were dipped for 2 min in the $Ag(NO)_3$ aqueous solution to attain equilibrium condition. Next, electrochemical potentiostatic deposition was carried out at a fixed applied potential of –0.30 V (*vs*. Ag/AgCl) on GCE for deposition time of 1000s. After completion of electrodeposition, the NLLS-Ag decorated GCE was gently washed with water and dried in air oven at $40^{o}C$ for 1 h.

3. RESULTS AND DISCUSSION

3.1 Morphological, elemental and crystal structure studies of NL-Ag

In order to investigate morphology of the electrodeposited Ag microstructures, SEM images were taken at two different magnifications (20 µm and 5 µm) and presented as Fig. 1. Interestingly, *nephrolepis leaf-like* Ag microstructures were formed on the electrode surface. The NLLS-Ag microstructures are uniformly grown and branched in all possible directions with large (boughs) and small (twigs) branches. The leaf-life structure possesses high surface area, porosity, active sites for catalysis. Furthermore, EDX analysis (Fig. 1C) confirmed the presents of Ag in the electrodeposited film. The crystal structure, grain size and phase purity of the as-synthesized NLLS-Ag were investigated by using X-ray diffraction analysis as documented in Fig. 1D. The characteristic distinctive Braggs peaks occur at 2θ of 38.02°, 44.30°, 64.35°, and 77.21° which agreed to the miller indices planes of (111), (200), (220) and (311) respectively. All the peaks can be clearly confirmed the face centered cubic structure of crystalline elemental silver with space group of Fm-3m (JCPDS No: 04-0783) [25]. The peaks occur at 2θ of 30°, 35° and 50° denotes the ITO plate peaks.

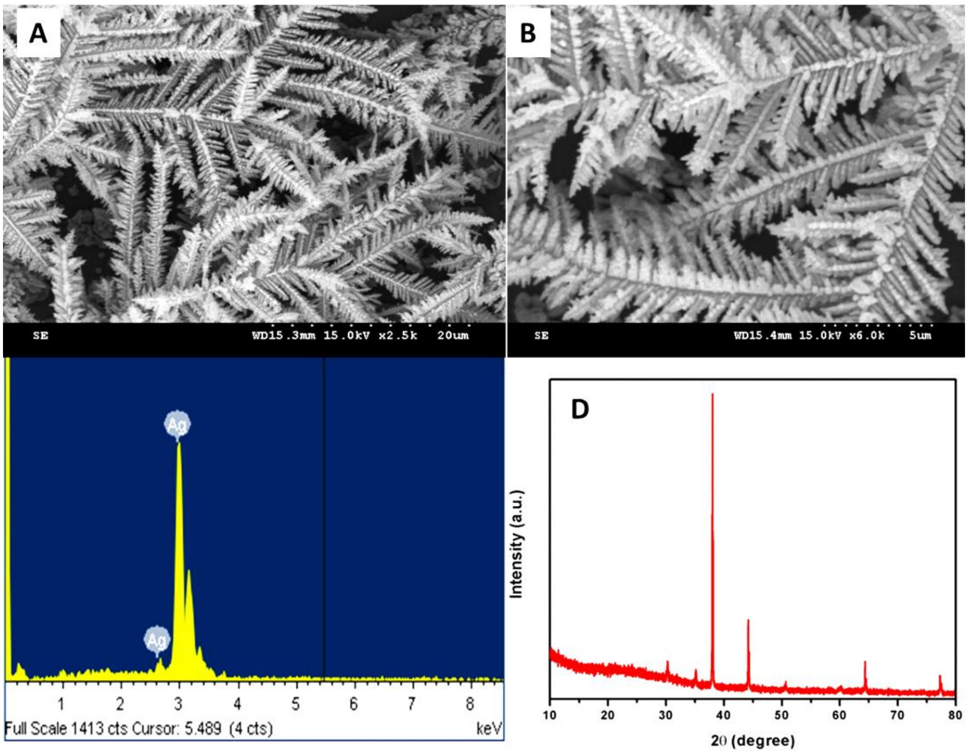


Figure 1. SEM images (magnification of 20 µm (A) and 5 µm (B), EDX profile (C) and XRD pattern (D) of *nephrolepis leaf-like* Ag microstructures.

3.2 Electrochemical studies

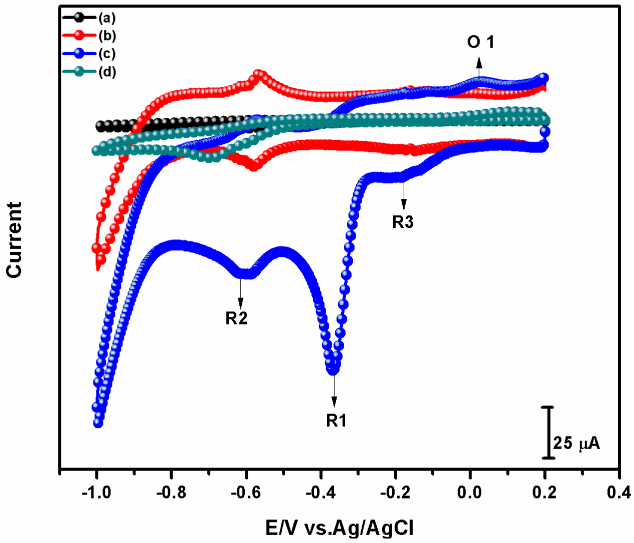


Figure 2. Cyclic voltammograms of unmodified GCE (a) in phosphate buffer (pH 7) containing 200 µM NB (c). Cyclic voltammograms of NLLS-Ag/GCE (b) in phosphate buffer (pH 7) containing 0.2 mM NB (d). Scan rate = 50 mV s^{-1}.

The electrochemical behavior of NB was investigated by using cyclic voltammetry. Fig. 2 presents the cyclic voltammograms (CVs) obtained at unmodified GCE and NLLS-Ag/GCE in phosphate buffer in the absence (a, b) and presence (c, d) of 0.2 mM NB. The scan rate was hold at 50 mV s^{-1}. In comparison with unmodified GCE, NLLS-Ag/GCE has shown significantly improved capacitance current indicating good conductivity of the as-prepared Ag microstructures. In the absence of NB, no noteworthy peak was observed at NLLS-Ag/GCE, however, highly enhanced reduction peak (R1) was observed at – 0.365 V in presence of 200 µM NB (curve d, Fig. 2). The reduction peak R1 is due to the reduction of NB to phenyl hydroxylamine. The entire reduction mechanism is given as **Fig. 3.** Additionally, three more peaks were observed in the voltammogram of NLLS-Ag/GCE which were assigned as R2, R3 and O1. The peak R2 (– 0.615 V) stands for the formation of aniline from phenyl hydroxylamine, while the reversible redox couple R3/O1 (R3: –0.179 V, O1: +0.026 V) is due to the reversible conversion of phenyl hydroxylamine to nitrosobenzene [19]. The reduction peak currents are predominant over oxidation peak currents, which confirmed that the reduction of NB is more preferential at NLLS-Ag/GCE. At unmodified GCE, the redox couple is not observed, while other reduction peak is appeared at high negative potential (–0.698 V). The voltammogram studies clearly revealed that the enhanced current response and lower overpotential for NB reduction at NLLS-Ag/GCE is due to the presence of more surface defects, which allows fast diffusion of NB on the electrode surface [19]. Therefore, NLLS-Ag/GCE is a suitable modified electrode for the fabrication of sensitive NB sensor.

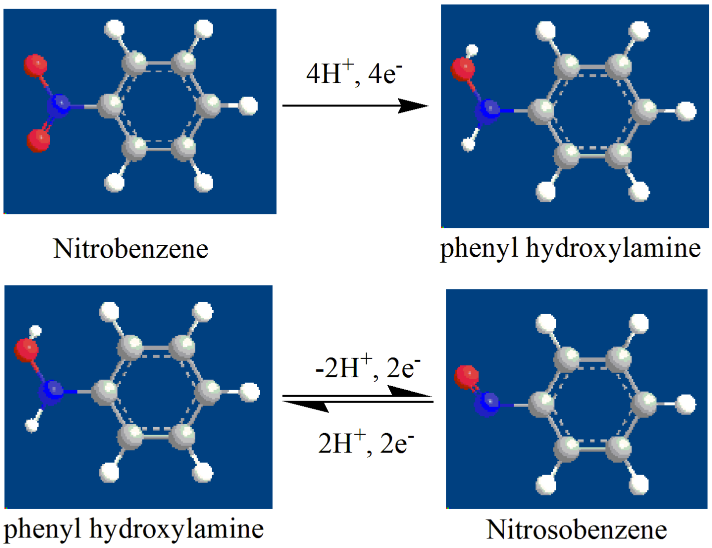


Figure 3. Scheme for the electron transfer processes involved in the NB reduction process at NLLS-Ag.

3.3 Different scan rates study

The effect of scan rate on the electrocatalytic behavior of NLLS-Ag/GCE was investigated at different scan rates ranging from 20 to 280 mV s^{-1} in phosphate buffer containing 0.2 mM NB (Fig. 4A). It can be seen that the reduction and oxidation peak currents were increased linearly as the scan rate increases. The cathodic peak currents have a linear dependence against the scan rates (Fig. 4B) which indicated that the reduction of NB at NLLS-Ag/GCE is a typical surface controlled adsorption process. The linear regression equation was I_{pc} (R1) = 19.2608 – 0.3694 ν (mV s^{-1}) (R^2 = 0.996).

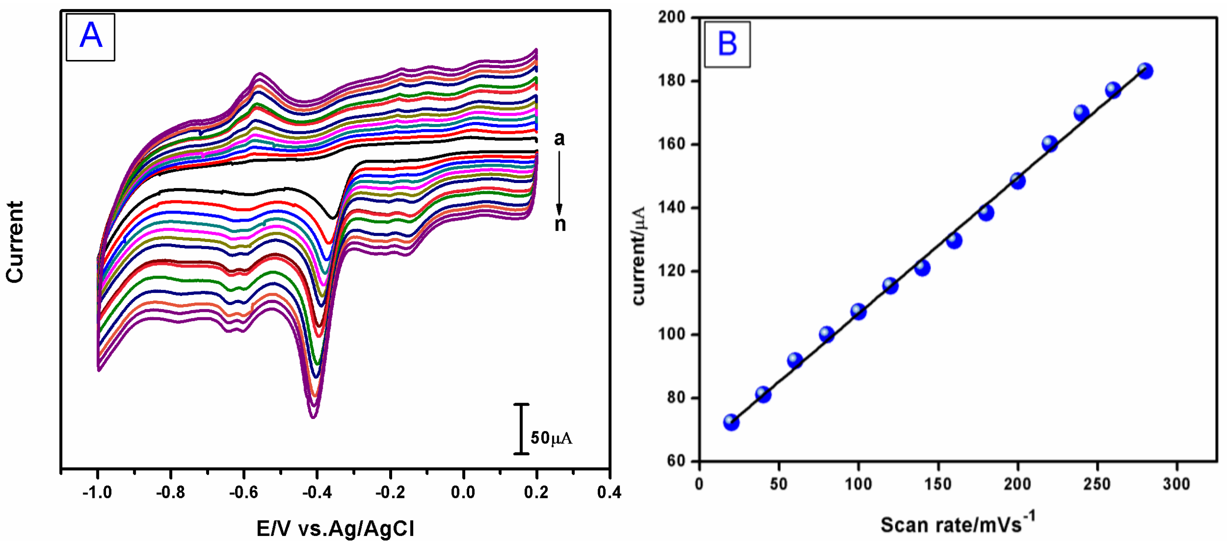


Figure 4. (A) Cyclic voltammograms obtained at NLLS-Ag/GCE in phosphate buffer containing 0.2 mM NB at different scan rates (a=20; b=40; c=60; d=80; e=100, f=120; g=140; h=160; i=180; j=200; k=220; l=240; m=260; n=280). (B) Calibration plot between current vs. scan rate

3.4 Different pH study

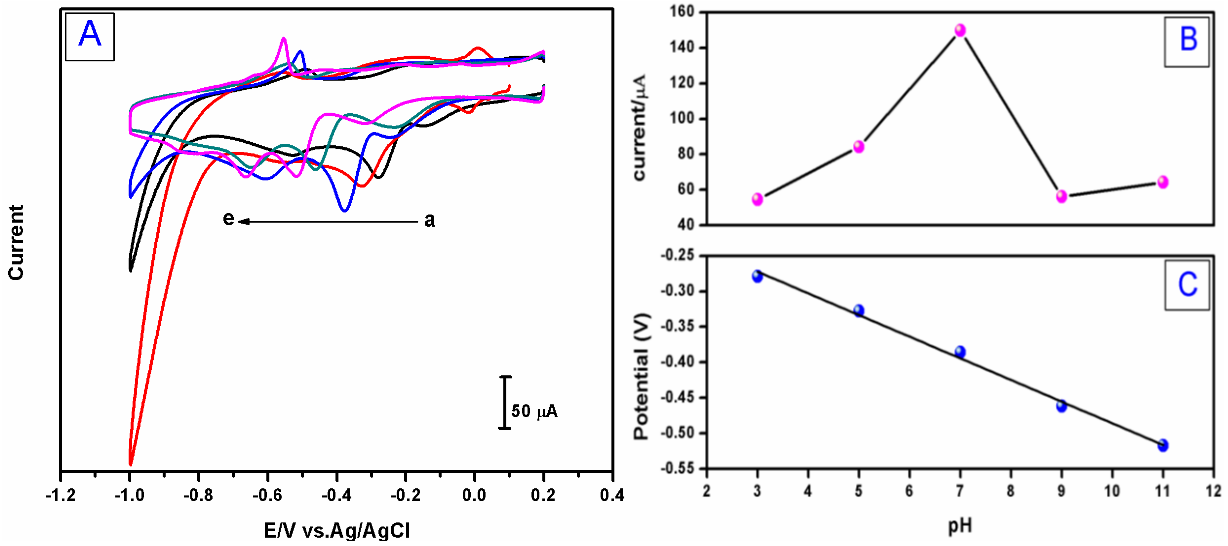


Figure 5. A) CVs obtained at NLLS-Ag/GCE in phosphate buffer containing 0.2 mM NB containing different pH (a=3; b=5; c=7; d=9; e=11) at NLLS-Ag/GCE. B) Calibration plot of I_{pc} *vs.* pH. C) Plot of E_{pc} *vs.* pH.

The electrocatalytic reduction of NB at NLLS-Ag/GCE at different pH was investigated. Cyclic voltammetry experiments were performed at different pH (3, 5, 7, 9 and 11) containing 0.2 mM NB and resulting voltammograms were compared (Fig. 5A). The plots between pH vs. current (Fig. 5B) and pH vs. peak potential (Fig. 5C) also given. As shown in figure, the NB reduction peak current increases as the pH increases from 3 to 7 with negative potential shift and then gradually decreases. This result proposes that the electrochemical reduction behavior of NB is pH dependent, while the concentration of hydrogen ion affects the rate of reduction reaction. Moreover, the reduction of NB to phenyl hydroxylamine is more favorable and active in neutral medium [26]. Therefore, the pH 7 was selected as the working pH condition for further electrochemical studies.

3.5 Amperometric determination of NB

Fig. 6A displays the amperometric i-t curves obtained at NLLS-Ag film modified rotating disc GCE for sequential addition of 100 nM NB into phosphate buffer (pH 7). The electrode rotation speed is 1500 RPM and the applied potential (E_{app}) is – 0.38 V. The NLLS-Ag film delivered quick amperometric responses to each addition of NB. The response current attained 95% steady-state current within 4s of NB injection. The concentration dependent calibration plot is prepared which showed good linearity. The linear regression equation is obtained as [Current] (μM) = 0.1397 [NB] (μM) + 0.2213 (inset to Fig. 6B). The working concentration range is 0.05 – 38.8 μM and the sensitivity is 0.665 $\mu A \mu M^{-1}$ cm^{-2}. The limit of detection (LOD) is calculated as 0.01 μM. The LOD was calculated using the formula, LOD= 3 s_b/S where, s_b is the standard deviation of ten blank measurements and S is the sensitivity. The important parameters such as LOD, linear range and sensitivity were comparable with previous reports [14, 23, 24, 27]. The operational stability of the NLLS-Ag/GCE towards NB (1 μM) was examined. 91.43 % of initial amperometric response current was retained after 2500 s in NB containing constantly stirred phosphate buffer. Therefore, the electrode has good operational stability.

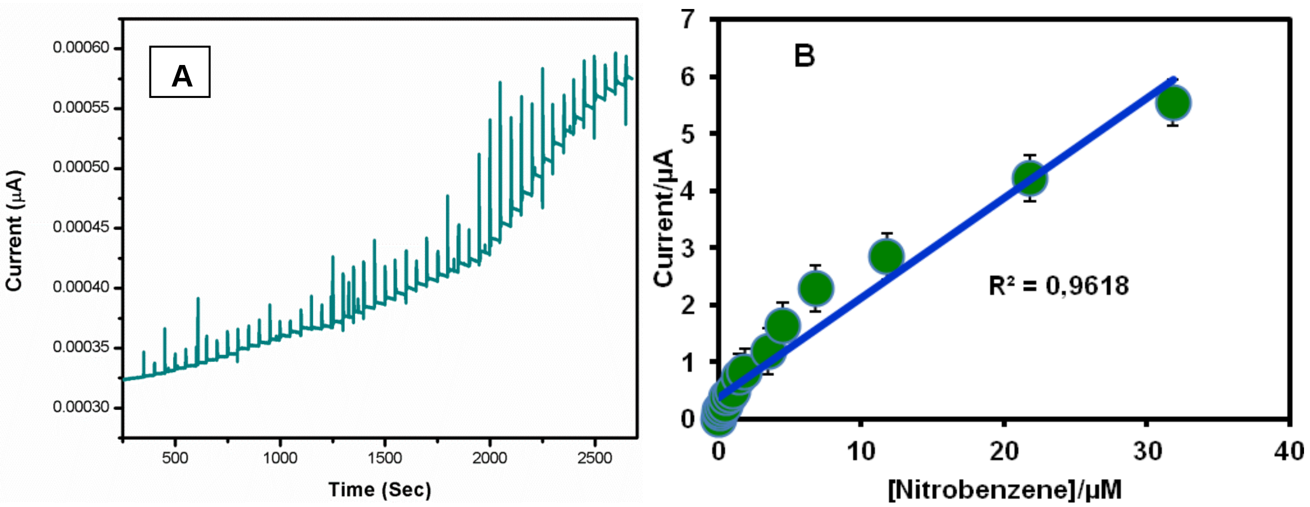


Figure 6. (A) Amperometric response of NLLS-Ag film modified rotating disc GCE for each sequential addition of 100 nM NB into continuously stirred phosphate buffer at rotation speed of 1500 RPM. (B) Current (μA) vs. [Nitrobenzene] (μM)

3.6 Repeatability, reproducibility and stability of the modified electrode

The repeatability and reproducibility of the modified electrode were evaluated in phosphate buffer (pH 7) containing 0.2 mM NB. The electrode possess appreciable repeatability with relative standard deviation (RSD) of 4.26% for 6 repetitive measurements carried out using single electrode. Besides, the modified electrode exhibits good reproducibility with RSD of 4.11% for 6 independent measurments carried out in five electrodes. Storage stability of our NLLS-Ag/GCE was investigated by monitoring its electrocatalytic response towards 0.2 mM NB for 15 consecutive days. The modified electrode was stored at 4°C when not in use. During one month storage period, the electrode delivered well defined catalytic responses. About 90.52% of initial catalytic response current was retained over one month of its continuous use validating good storage stability.

Table 1. Determination of NB at NLLS-Ag/GCE in water samples

Sample	Added (μM)	Found (μM)	Recovery (%)	* RSD (%)
Tap water	10	9.68	96.8	3.54
	20	19.25	96.25	3.66
Drinking water	10	9.56	95.6	2.40
	20	19.29	96.45	3.84
River water	10	9.85	98.5	3.73
	20	19.73	98.65	3.70

* Relative Standard Deviation of three individual measurements.

3.7 Real sample analysis

Practical feasibility of our newly developed electrode is demonstrated in water samples. The water samples were collected from different sources and known concentrations of NB were spiked and analyzed using our modified electrode. The standard addition method was adopted to calculate found and recovery values and the results were summarized in Table 1. The developed NLLS-Ag/GCE displayed satisfactory recoveries in water samples which validates the good practicality of the modified electrode.

4. CONCLUSIONS

In summary, *nephrolepis* leaf-like silver microstructures were successfully prepared through a single step potentiostatic electrodeposition strategy. The SEM, EDX, XRD and electrochemical methods were confirmed the successful formation of NLLS-Ag microstructures. The NLLS-Ag microstructures modified GCE exhibited significantly improved electrocatalytic ability to the reduction of NB. The scan rates and pH have great influences on the NB reduction. NLLS-Ag/GCE based amperometric sensor was fabricated which exhibited excellent sensing performances with linear range of (0.05 – 38.8 μM) and detection limit of 0.01 μM. Moreover, the sensor has shown good repeatability, reproducibility and stability. Besides, NLLS-Ag/GCE exhibited good practical feasibility to detect NB present in various water samples. The excellent electrocatalytic ability of NLLS-Ag microstructure revealed its great potential in electrochemical sensing applications.

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