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An Ultra-Sensitive Electrochemical Sensor for the Detection of Carcinogen Oxidative Stress 4-Nitroquinoline N-Oxide in Biologic Matrices Based on Hierarchical Spinel Structured NiCo_2O_4 and NiCo_2S_4 ; A Comparative Study

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Abstract: Various factors leads to cancer; among them oxidative damage is believed to play an important role. Moreover, it is important to identify a method to detect the oxidative damage. Recently, electrochemical sensors have been considered as the one of the most important techniques to detect DNA damage, owing to its rapid detection. However, electrode materials play an important role in the properties of electrochemical sensor. Currently, researchers have aimed to develop novel electrode materials for low-level detection of biomarkers. Herein, we report the facile hydrothermal synthesis of NiCo_2O_4 micro flowers (MFs) and NiCo_2S_4 micro spheres (Ms) and evaluate their electrochemical properties for the detection of carcinogen-causing biomarker 4-nitroquinoline n-oxide (4-NQO) in human blood serum and saliva samples. Moreover, as-prepared composites were fabricated on a glass carbon electrode (GCE), and its electrochemical activities for the determination of 4-NQO were investigated by using various electrochemical techniques. Fascinatingly, the NiCo_2S_4 -Ms showed a very low detection limit of 2.29 nM and a wider range of 0.005 to 596.64 μM for detecting 4-NQO. Finally, the practical applicability of NiCo_2S_4 -Ms in the 4-NQO spiked human blood serum and saliva samples were also investigated.

Keywords: 4-NQO detection; NiCo_2O_4 ; NiCo_2S_4 ; biologic samples; hierarchical spinel structure; hydrothermal method

1. Introduction

Currently, bimetallic oxide and sulfide-based nanoparticles have been widely used in the field of electrochemical application [1–3]. A well-known electrode material with superior electrocatalytic property prepared based on nickel and cobalt due its higher surface area, structures and unique morphologies than monometallic oxides or sulfides [4–8]. Thus, we have synthesized hierarchical spinel NiCo_2O_4 -MFs and NiCo_2S_4 -Ms as an electrode material for the electrocatalytic reduction of 4-NQO detection. The better response occurs on the NiCo_2S_4 -Ms modified electrode because sulfides

provide the lower bandgap energy and less electronegativity than oxides [8–10]. The synthesis method for NiCo₂S₄ and NiCo₂O₄ with various structures such as, micro flowers, nanoprism [11], nanoplates [12] and nanorods [13] and their electrocatalytic properties were previously reported. However, the hydrothermal method are more convenient to synthesis nanomaterials with different morphology than other available methods [14–16]. The chemical reaction in hydrothermal process occurs due to the influential high-temperature and pressure, which results the formation of exceptional products [17].

The term oxidative stress is defined to be the imbalance or the excessive production of reactive oxygen species (ROS) in cellular metabolism [18,19]. It plays an important role in living organisms such as maintenance of cell growth and production of important biologic substances, etc. [20]. However, in living organisms free radicals such as nitric oxide, peroxy nitrite produce the ROS, which undergoes nitration in protein molecules, forms the 4-nitroquinoline *n*-oxide [21]. Thus, the excess level of 4-NQO leads to various types of disorders such as cancer, neurodegeneration and lung diseases, etc. [22]. Therefore, it is essential and important to develop analytical techniques to identify the oxidative stress biomarker 4-NQO in biologic matrices. The current analytical techniques that are used to determine 4-NQO ions are fluorescence-based screening assay, high performance liquid chromatography (HPLC) and electrochemical methods [22]. Among the aforementioned techniques, electrochemical techniques are easy to handle, require low consumption of samples, are rapid and most importantly, are low-cost [22,23]. Moreover, the fabrication of the electrode surface with the suitable materials will precisely improve the electrochemical parameters such as stability and sensitivity [24].

Herein, we reported that the facile synthesis of hierarchical spinel structure of NiCo₂S₄ microspheres and NiCo₂O₄ micro flowers for the effective detection of oxidative stress biomarker 4-NQO. The as-prepared NiCo₂S₄-Ms shows the excellent electrochemical activities for the determination of 4-NQO on comparing with NiCo₂O₄-MFs. Furthermore, the differential pulse voltammetry (DPV) results exhibited a low limit of detection and a wider linear range for the detection of biomarker 4-NQO. Moreover, the prepared NiCo₂S₄-Ms shows excellent long-term stability and selectivity. Finally, real samples of the sensor were tested in 4-NQO spiked human blood serum and saliva samples.

2. Results and Discussion

2.1. Microscopic and Elemental Analysis of Synthesized Nanomaterials

The morphologic structure of synthesis nanoparticles micro flowers and microspheres were obtained using FESEM. Figure 1A characteristic the morphology of NiCo₂O₄, which appears as a flower-like structure and Figure 1B, shows the magnified microscopic image of NiCo₂O₄ in which clearly demonstrated that the numerous nano-needles are shrinks together and forms a micro spiky structure. Moreover, the FESEM images of the NiCo₂S₄ Figure 1C,D show that obtained particles were micro structured spheres, which were regularly distributed with an average particle size of 1.64 μm. Figure 2A–D illustrates the elemental mapping of NiCo₂S₄ Ms (A), which confirmed the presence of Ni (B), Co (C) and S (D) elements. In addition, the EDX analysis were taken to investigate the elemental composition present in NiCo₂S₄ Ms. Figure 2E shows the EDX spectrum of NiCo₂S₄ Ms with the expected signal response of the elements Ni, Co and S. The inset image of Figure 2E presents the weight percent NiCo₂S₄ Ms. Moreover, the elemental mapping and EDX spectrum including the weight percentage of NiCo₂O₄ MFs are presented in supplementary Figure S1A–E.

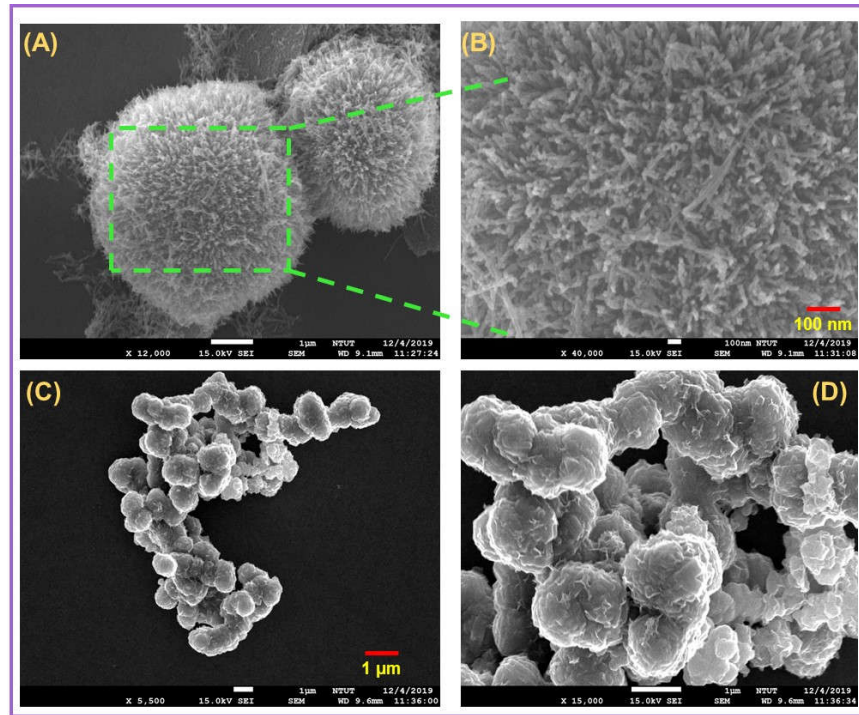


Figure 1. (A) FE-SEM of NiCo₂O₄-MFs, (B) magnified image of NiCo₂O₄-MFs and (C,D) NiCo₂S₄-Ms. Scale bar 100 nm.

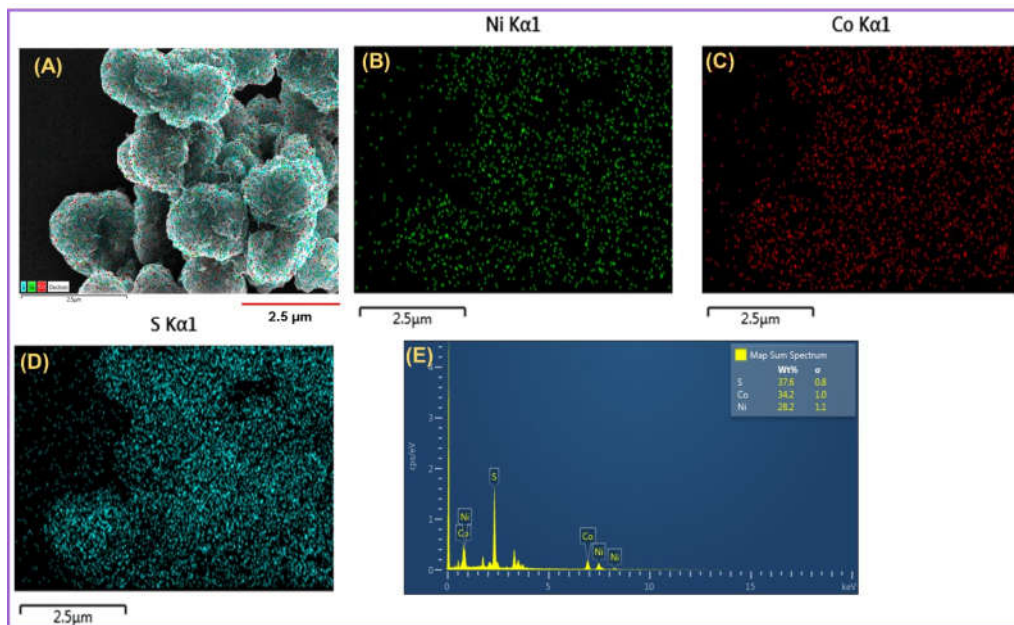


Figure 2. (A) Mapping image NiCo₂S₄-Ms mix, (B) Ni, (C) Co and (D) S. (E) EDX profile of NiCo₂S₄-Ms. Inset weight percentage of NiCo₂S₄-Ms. Scale bar 2.5 μm.

2.2. XRD and XPS Analysis of NiCo₂S₄-Ms and NiCo₂O₄-MFs

The XRD patterns of the as synthesized NiCo₂S₄-Ms and NiCo₂O₄-MFs are shown in Figure 3A. The XRD pattern of NiCo₂S₄-Ms Figure 3Aa shows the characteristic peaks at 16.3° (111), 26.8° (220), 31.6° (311), 38.3° (400), 50.4° (511), 55.3° (440). These peaks were reliable with the standard XRD pattern of NiCo₂S₄-Ms (JCPDS NO: 20-0782) [25]. Moreover, Figure 3Ab shows the signal response at 18.9° (111), 31.1° (220), 36.7° (311), 44.6° (400), 55.4° (422), 59.1° (511), 64.9° (440) shows the maximum

crystallization, which confirms the formation of NiCo₂O₄-MFs (JCPDS NO: 20-0781) [26]. In addition, the XPS were taken to conform the chemical composition and the oxidation state of NiCo₂S₄-Ms and NiCo₂O₄-MFs. Figure 3B,C shows the XPS survey spectra of NiCo₂S₄-Ms and NiCo₂O₄-MFs confirms that, the presence of Ni, Co, S and O [27,28]. Thus, the XRD and XPS studies clearly confirms that the excellent formation of NiCo₂S₄-Ms and NiCo₂O₄-MFs.

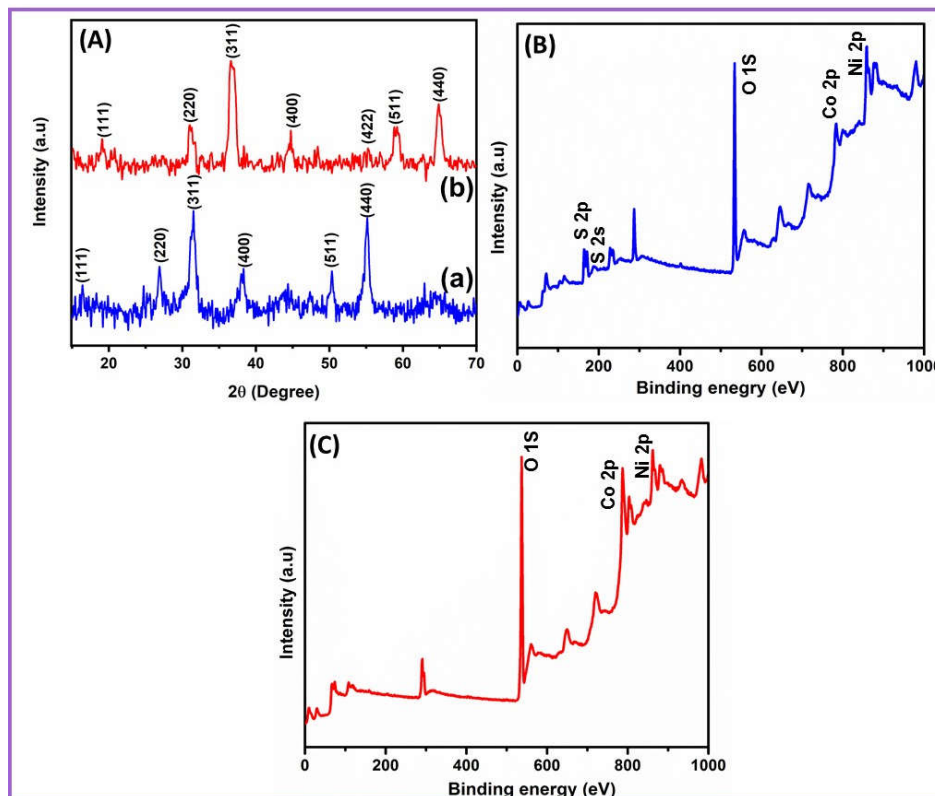


Figure 3. (A)—(a) XRD study of NiCo₂S₄-Ms and (b) NiCo₂O₄-MFs. (B) XPS survey spectrum of NiCo₂S₄-Ms and (C) NiCo₂O₄-MFs.

2.3. EIS and Electrochemical Investigation of Different Electrodes

Electrochemical impedance spectroscopy (EIS) is a method to investigate the interfacial effects between electrolyte and surface of the electrode. Figure 4A depicts the EIS curves of bare GCE (a), NiCo₂O₄-MFs/GCE (b) and NiCo₂S₄-Ms/GCE (c) in 0.1-M KCl containing 0.05 M of [Fe(CN)₆]^{3-/4-} solution and the frequency range was set to be of 100 MHz to 100 kHz. The obtained impedance data were fit according to the Randle's equivalent circuit model shows in the inset Figure 4A, where R_{ct} denotes the charge transfer resistance, Z_w , R_s and C_{dl} refer to Warburg impedance, ohmic resistance and the double layer electron-transfer resistance, respectively. Moreover, the R_{ct} value of bare GCE, NiCo₂O₄-MFs/GCE and NiCo₂S₄-Ms/GCE were measured to be 250.7 Ω , 214.2 Ω and 64.25 Ω , respectively. As can be seen that the lowest R_{ct} value were obtained for NiCo₂S₄-Ms electrode. This was due to the microsphere structure possessing the larger surface area; when it contacted the electrolyte, it may be more effective and efficient in capturing the active materials.

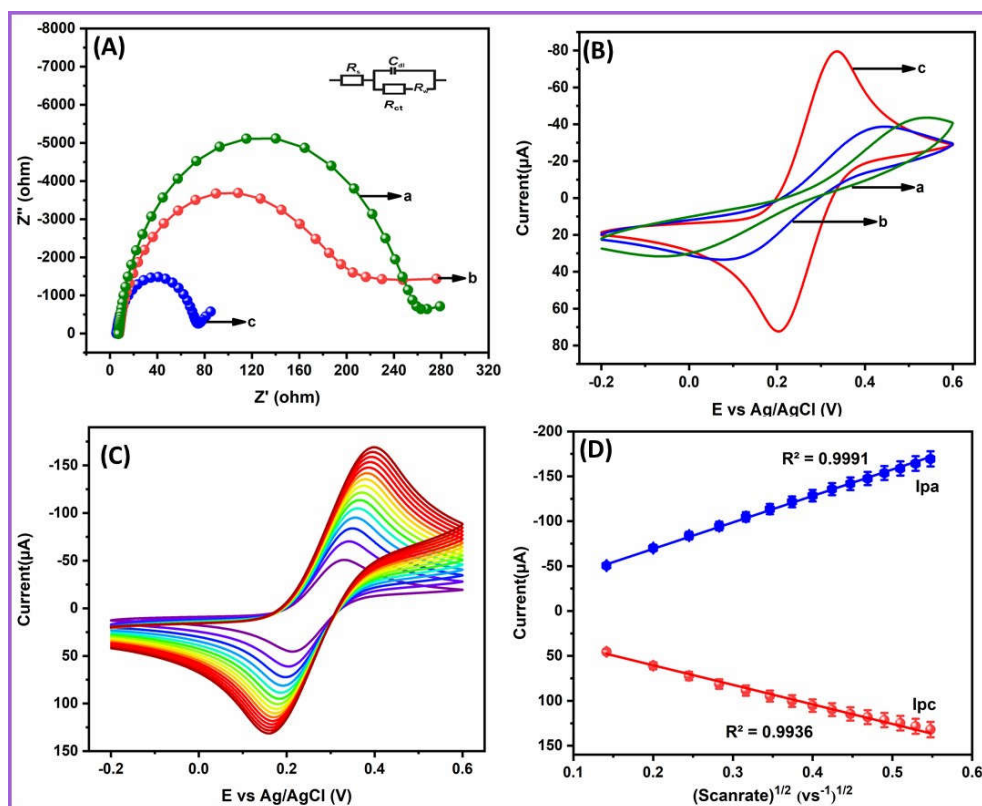


Figure 4. (A) EIS and (B) cyclic voltammetry (CV's) (a) of unmodified electrode, (b) NiCo₂O₄-MFs/GCE and (c) NiCo₂S₄-Ms/GCE in 0.1-M KCl containing 0.05 M of [Fe(CN)₆]^{3-/4-}. (C) Scan rate of the nanocomposite modified electrode. (D) Calibration plot of (i_p vs. Vs^{-1})^{1/2}.

In addition, the cyclic voltammetry (CV's) were performed to investigate the electrochemical behavior of bare GCE (a), NiCo₂O₄-MFs/GCE (b) and NiCo₂S₄-Ms/GCE (c) in 0.1-M KCl containing 0.05 M of [Fe(CN)₆]^{3-/4-} solution. In Figure 4B as can be seen that, the highest reduction and reduction peak current and lower peak-to-peak separation (ΔE_p) value of 89.24 mV were observed for the NiCo₂S₄-Ms/GCE. Moreover, the peak-to-peak separation (ΔE_p) of unmodified electrode (a) and NiCo₂O₄-MFs/GCE (b) were measured to be 189.51 and 145.36 mV, respectively. Figure 4C shows the different scan rates at NiCo₂S₄-Ms/GCE, in which the redox peak current increased consistently and their linear relationship with the square root of scan rates were plotted as shown in Figure 4D. Furthermore, the electrochemical active surface area value was measured based on the Randel's Sevcik equation (1). The electrochemical active surface area of bare GCE (a), NiCo₂O₄-MFs/GCE (b) and NiCo₂S₄-Ms/GCE were calculated to be 0.082, 0.102 and 0.161 cm² respectively. The results indicate the combination of bimetallic sulfides increased the surface area and showed excellent electrochemical contact between the surface of electrode and electrolyte solution.

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2} \quad (1)$$

2.4. Electrochemical Activity and Different pH at NiCo₂S₄-Ms/GCE

As shown in Figure 5A, the CV's with different electrodes, bare GCE (a), NiCo₂O₄-MFs/GCE (b) and NiCo₂S₄-Ms/GCE (c) were recorded in 0.1 M of phosphate buffer solution (PB) pH 7 containing 100- μ M 4-NQO with the fixed scan rate of 0.05 V/s. The lower reduction peak current exhibited an unmodified GCE, which suggest the poor conductivity on the electrode surface. Furthermore, when the electrode was modified with NiCo₂O₄-MFs/GCE there was a drastically increase in the current. The excellent peak current of -26μ A with the minimized reduction potential of -0.29 V was noted on NiCo₂S₄-Ms/GCE, which suggest that the larger surface area of NiCo₂S₄-Ms can considerably enhance the sensitivity of the electrode. Moreover, the probable electrochemical reduction mechanism of 4-NQO on NiCo₂S₄-Ms/GCE are shown in Figure 6. Typically, 4-nitroquinoline n-oxide is irreversibly reduced to form 4-hydroxyaminoquinoline n-oxide. After this, the oxidation of 4-hydroxyaminoquinoline n-oxide occurs, which is subsequently reduced to 4-nitrosoquinoline n-oxide at the increasing scan. The accumulation time is one of the important parameters in electrochemical sensors to improve the sensitivity. Therefore, the accumulation time of the proposed sensor was studied in 0.1-M pH 7 containing 100- μ M 4-NQO using the CV technique and the corresponding current response versus time were plotted as shown in Figure S2. The obtained results indicate that the reduction peak increased with the increasing accumulation time. However, the accumulation time exceeds 20 s, the reduction peak current of 4-NQO decreased. This was due to the adsorption taken at the surface of the electrode, which may have resisted the active site of the NiCo₂S₄-Ms and the surface reached its saturation point at 20 s. Thus, the optimal accumulation time of 20 s was preferred to achieve the high sensitivity for the proposed sensor.

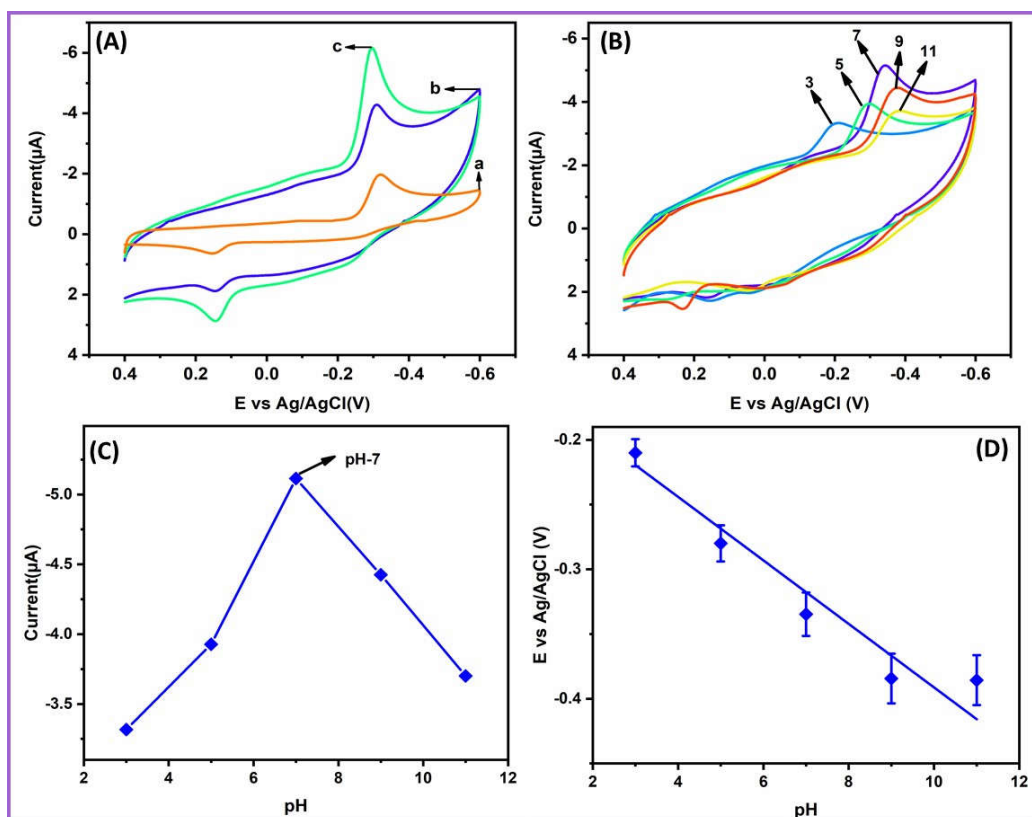


Figure 5. (A)—(a) CV's of unmodified glass carbon electrode (GCE), (b) NiCo₂O₄-MFs/GCE, and (c) NiCo₂S₄-Ms/GCE in 0.1-M pH 7 containing 100- μ M 4-NQO. (B) CV's obtained at NiCo₂S₄-Ms/GCE for the varied pH from (3 to 11) containing 100- μ M 4-NQO. (C) The plot current (μ A) versus pH and (D) potential versus pH.

Furthermore, the influence of pH at NiCo₂S₄-Ms/GCE for the detection of 4-NQO was investigated by varying the PB pH from 3 to 11 at a fixed scan rate of 0.05 Vs⁻¹. Figure 5B displays the CV's curves of different pH 3 to 11 containing 100-μM 4-NQO. The well-shaped peak with higher reduction peak current of -5.29 μA was noted at pH 7 Figure 5C. At some point, the peak current started to decrease, which indicates that the biomolecules reached its maximum pH value of 9–11. Hence, the pH 7 was chosen as the enhanced pH for the reduction of 4-NQO. In addition, the plot between the reduction peak potential and pH were plotted as shown in Figure 5D, which was linear, and its regression equation were represented as E_p (V) = -0.056 pH -0.624. The obtained slope value of -56 mV/pH indicates that the reaction was equal number of proton and electron transferred.

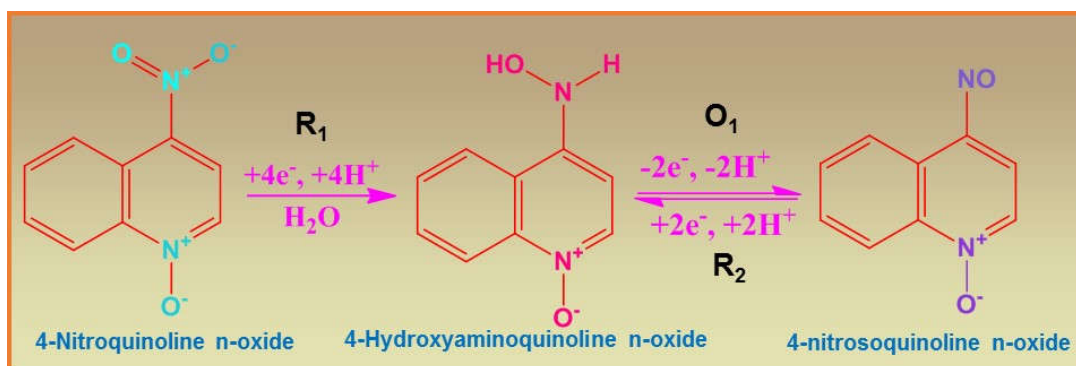


Figure 6. Possible reduction mechanism of 4-NQO at NiCo₂S₄-Ms/GCE.

2.5. Influence of Different Concentration and Scan Rate

In addition, the influence of various concentration of 4-NQO at as-prepared NiCo₂S₄-Ms/GCE were analyzed in 0.1-M pH 7 at fixed scan window of 0.05 Vs⁻¹. As shown in Figure 7A, the CV curve were recorded for the increasing concentration of 4-NQO. For every sequential addition of 4-NQO from 25 to 250 μM the reduction peak current also increased linearly. Finally, the linear relationship of reduction peak current versus the concentration of 4-NQO were plotted as Figure 7B. Moreover, the regression equation is written to be $y = 0.0253x + 2.2034$ with the correlation coefficient of $R^2 = 0.9938$. The obtained results confirm that, the as-prepared NiCo₂S₄-Ms is a promising electrode material for the rapid detection of 4-NQO without fouling.

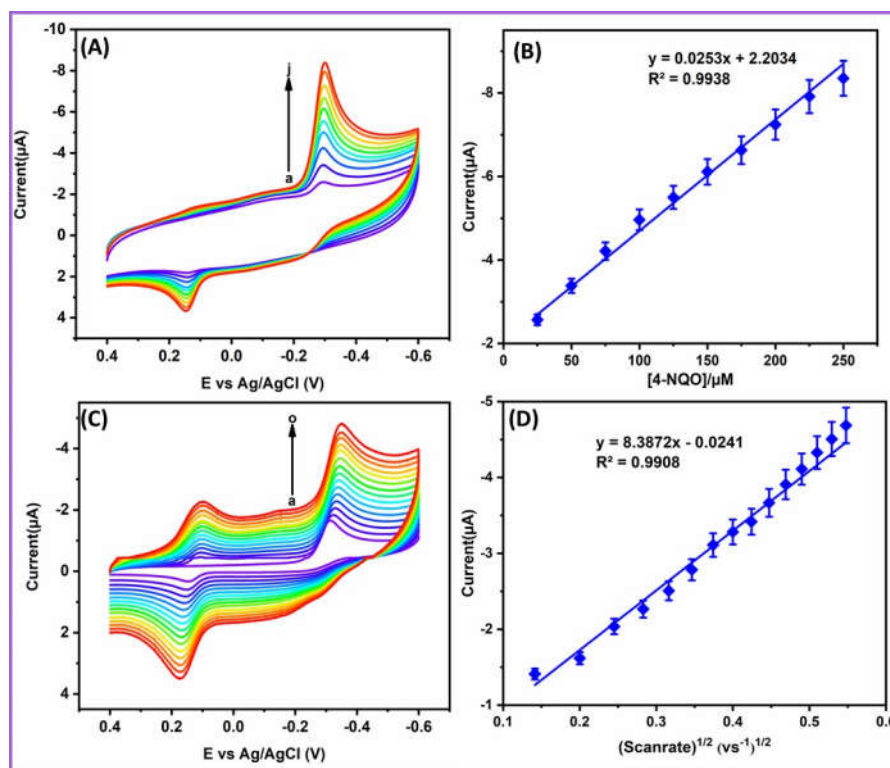


Figure 7. (A) CVs obtained at different concentration of 4-NQO (a–j 25–250 μM) and (B) Linear relationship between the current (μA) and 4-NQO concentration. (C) CVs attained for the different scan rate (a–o 20 to 300 mVs^{-1}) in 0.1-M pH 7 at $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$. (D) Calibration plot of the cathodic peak current and scan rates.

In order to study the effects of scan rates at $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ was investigated by CV techniques. Figure 7C, indicates the CVs of different scan rates from 20 to 300 mVs^{-1} in 0.1-M PB pH 7 contains 100- μM 4-NQO. With consistently increasing scan rates, there was a linear increase in the cathodic peak current. Figure 7D shows the linear relationship between the cathodic peak current of 4-NQO versus square root of the scan rates. In addition, the regression equation was calculated to be $y = 8.3872x - 0.0241$ with the correlation coefficient of $R^2 = 0.9908$, which indicates that reduction of 4-NQO at $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ was a diffusion-controlled process.

2.6. DPV Analysis of 4-NQO Ions at $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ Techniques

Differential pulse voltammetry common techniques were used to measure the essential electrochemical parameters such as linear range, sensitivity and limit of detection (LOD). Hence, the DPV technique was used to detect the 4-NQO at $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ in 0.1-M pH 7 at a fixed potential of 0.4 to -0.6 V. Figure 8A displays the DPV curve of increasing concentration of 4-NQO. The obtained results indicate the reduction peak current increased with increasing concentration of 4-NQO. The $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ modified electrode showed an excellent linear relationship between the reduction peak current and the concentration of 4-NQO, which were plotted as shown in Figure 8B. Moreover, the linear regression equation was calculated to be $i_{pa} (\mu\text{A}) = 0.4014 \mu\text{M} - 0.0153$ ($R^2 = 0.9985$). Wherein, the $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ shows the wider range of 0.005 to 596.64- μM 4-NQO. In addition, the LOD of the as-prepared sensor was measured using the standard formula of $\text{LOD} = 3\sigma/S$, where the σ and S , is the standard deviation and slope of the curve, respectively. Moreover, the LOD of $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ for the detection of 4-NQO were measured to be 2.29 nM. Moreover, the proposed sensor was compared with previously reported 4-NQO sensor as the results as-prepared $\text{NiCo}_2\text{S}_4\text{-Ms/GCE}$ shows the very low detection limit Table 1. Thus, it is a promising electrode material for the detection of 4-NQO.

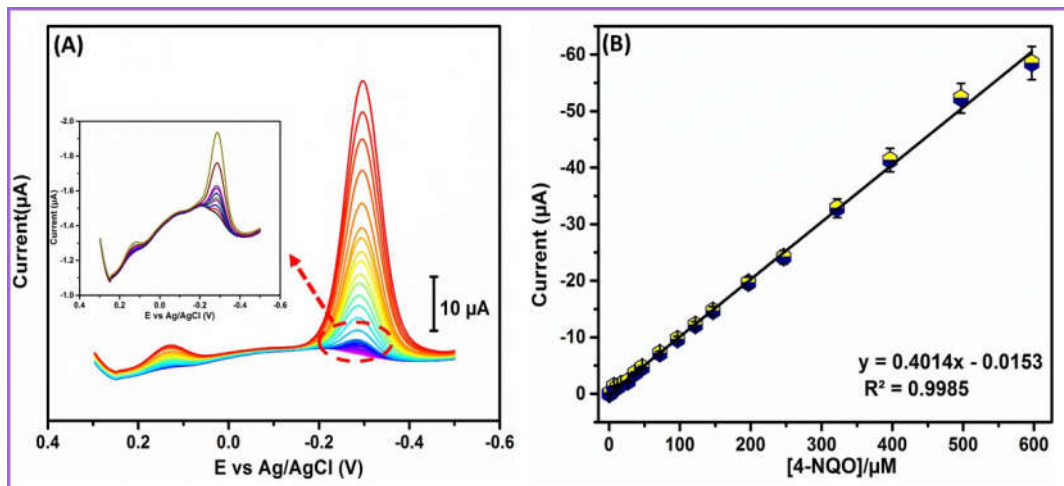


Figure 8. (A) DPV response of the different concentration of 4-NQO; (B) linear response of the reduction peak current versus concentration of 4-NQO.

Table 1. Comparison of as-prepared sensor with the other previously reported 4-NQO sensor.

| Method | Linear Range (μM) | LOD (nM) | Ref |
|---|-------------------|----------|-----------|
| Bi ₂ WO ₆ /rGOS-NC/ ^a GCE/ ^b it | 0.025–718 | 6.11 | [22] |
| Fe ₂ N·NPs@rGOS/ ^c SPCE/ ^d DPV | 0.05–574.2 | 9.24 | [16] |
| ZnFe ₂ O ₄ ·NCs/rGO/ ^a GCE/ ^d DPV | 0.025–534.12 | 8.27 | [19] |
| ^e HPLC | - | 0.157 | [29] |
| NiCo ₂ S ₄ -Ms/GCE/DPV | 0.005–596.64 | 2.29 | This work |

^a Glassy carbon electrode; ^b (i-t) amperometric; ^c screen printed carbon electrode; ^d differential pulse voltammetry; ^e high-performance liquid chromatography.

2.7. Interference and Stability Studies of NiCo₂S₄-Ms/GCE

Selectivity and stability are the most considered parameter in electrochemical studies. Hence, the selectivity of the sensor was examined using NiCo₂S₄-Ms/GCE with 4-NQO, as well as in the presence of possible bioactive and nitro compounds. The selectivity of the prepared sensor was studied using DPV techniques. The DPV results of the NiCo₂S₄-Ms/GCE in presence of 0.5 μM 4-NQO and 0.7 μM possible interferents such as 3-nitro-L-tryosine (3-NT), 4-nitrophenol (4-NP), chloramphenicol (CAP), nitro benzene (NB) and continued addition of 1.5 μM common biologic analytes glucose (Glu), ascorbic acid (AA), hydrogen peroxide (H₂O₂) and dopamine (DA) are shown in Figure S3. The related nitro compounds such as 3-NT, 4-NP, NB, CAP showed minor interference with the 4-NQO due to structural similarity. However, the corresponding relative error was found less than 7% as shown in Figure 9A. Notably, most of the other nitro compounds show their reduction peaks at high overpotential of -0.50 to -0.60 V, which is far from the reduction potential of 4-NQO. In other words, although all these compounds have nitro groups, they require different energy to be reduced, which provides the electrode a good selectivity. Approximately three-fold excess concentrations of Glu, AA, H₂O₂ and DA did not show any significant interference, indicating that the method is selective in biologic samples. Moreover, the working stability of NiCo₂S₄-Ms/GCE were examined using the DPV technique. Figure 9B shows the stability current response of the prepared sensor in 0.1 M pH 7 containing 10-μM 4-NQO. After 15 days usage, the sensor maintained a stability of 94.59%. Thus, the stability test of the NiCo₂S₄-Ms/GCE confirms the outstanding working stability for the detection of 4-NQO.

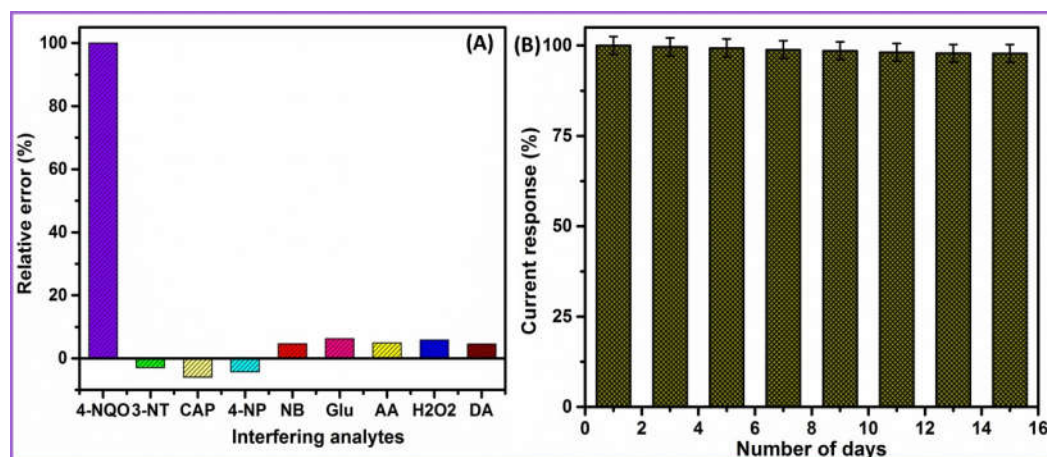


Figure 9. (A) Influence of interfering ions in 0.1-M pH 7 containing 0.5 μ M of 4-NQO and higher concentration of interfering analytes; (B) stability plot of NiCo₂S₄-Ms/GCE of its usage for 15 days.

2.8. Real Sample Analysis

The practical applicability of prepared sensor was examined in the biologic sample human blood serum and saliva samples. The preparation of biologic samples was diluted with buffer solution and the known concentration of 4-NQO were spiked. Finally, DPV techniques were carried out for the prepared real samples. Fascinatingly, the blood serum and saliva samples were showed outstanding found and recovery rates, which are tabulated in Table 2. At the end, the prepared NiCo₂S₄-Ms/GCE established as the effective electrode for the real time applicability.

Table 2. Determination of 4-NQO in biologic samples at NiCo₂S₄-Ms/GCE modified electrode.

| Real Samples | Added/nM | Found/nM | Recovery/% | * RSD/% |
|-------------------|----------|----------|------------|---------|
| Human blood serum | 50 | 47.58 | 95.16 | 2.45 |
| | 100 | 96.48 | 96.48 | 2.81 |
| Saliva | 50 | 49.12 | 98.24 | 2.56 |
| | 100 | 94.29 | 94.29 | 2.76 |

* Related standard deviation (RSD) of $n = 3$.

3. Experimental Section

3.1. Synthesis Method for NiCo₂S₄-Ms and NiCo₂O₄-MFs

All chemicals were purchased from Sigma-Aldrich in analytical grade and used without any further purification. The details of chemical purchases, preparation methods of buffer solutions and instrumentation techniques are detailed in the supplementary data (S1). The NiCo₂O₄-MFs and NiCo₂S₄-Ms were synthesized by the hydrothermal method. Briefly, a 1:2 molar ratio of Ni(CH₃COO)₂·4H₂O and Co(CH₃COO)₂·4H₂O were thoroughly dissolved in 50 mL of C₂H₆O₂ under magnetic stirring for 30 min. Then the mixture was carefully transferred into a 100 mL Teflon-lined stainless-steel autoclave and maintained for 180 °C for 12 h in a hydrothermal oven. After 12 h, the reaction mixture was allowed to cool room temperature and centrifuged with water and ethanol to collect the NiCo₂O₄-MFs precipitate. Finally, the collected precipitate was dried in air at 80 °C for 10 h [30]. To synthesis, the NiCo₂S₄-Ms, a 1:2 molar ratio of the NiSO₄·6H₂O and CoSO₄·7H₂O were dissolved in 0.5 M Na₂S·5H₂O. Then the same procedure was followed to obtain the NiCo₂S₄-Ms (Scheme 1).



Scheme 1. Synthesis methodology of NiCo₂S₄-Ms and its electrocatalytic properties for the detection of 4-nitroquinoline n-oxide.

3.2. Fabrication of NiCo₂S₄-Ms Modified GCE

The surface of GCE was cleaned by 0.5 mg of alum in a slurry. Then the GCE was dipped into an ethanol solution and sonicated for 10 min. After this, the GCE was pre-cleaned by cycling between -0.8 to 0.8 V in PB pH 7 for the 25 continuous cycle. Then, 1 mg of as-prepared NiCo₂S₄-Ms was dispersed in 1 mL of ethanol and sonicated for 20 min. Approximately 6 μ L of NiCo₂S₄-Ms suspension was drop-cast on the surface of GCE and dried in a 50 °C oven. Finally, the as-prepared NiCo₂S₄-Ms modified GCE was used for the electrochemical characterization.

4. Materials and Reagents

Nickel acetate Ni(CH₃COO)₂·4H₂O, cobalt acetate Co(CH₃COO)₂·4H₂O, ammonium hydroxide (NH₄OH), nickel sulfate NiSO₄·6H₂O, cobalt sulfate CoSO₄·7H₂O, 4-nitroquinoline n-oxide, uric acid (UA), ascorbic acid (AA), dopamine (DA), glucose (GLU), 3-nitrotyrosine (3-NT), L-cysteine and H₂O₂ and all other chemicals were purchased from Sigma-Aldrich and used as received. Double-distilled water was used for all the experiments. 0.1-M phosphate buffer (PB) (pH 7.0) prepared from sodium dihydrogen phosphate and disodium hydrogen phosphate was used as supporting electrolyte. Human blood serum and saliva samples were acquired from Chang Gung Medical Hospital, Taoyuan, Taiwan. The research protocols of human blood serum and saliva samples experiments were followed as per the laws and institutional guidelines of Chang Gung Medical Hospital (CGMH), Taiwan.

5. Methods

The surface modification of the as-formed composite was examined using field emission scanning electron microscope (FESEM-JEOL-7600F, Jeol instruments, Musashino, Akishima, Tokyo, Japan): Ingredients of the elemental composition and elemental mapping were analyzed by energy-dispersive X-ray spectroscopy (EDX) with HORIBA EMAX X-ACT (Horiba instruments, AkzoNobel House, Singapore). The quantitative analysis and defects and disordered nature of the as-prepared composite were investigated by PerkinElmer PHI-5702 (PerkinElmer Inc., Waltham, MA, USA).

The crystalline nature of the composite was examined by XRD, XPERT-PRO spectrometer (Malvern Panalytical B.V., Almelo, The Netherlands). The electrochemical properties and electrocatalytic activity were examined using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV); i-t amperometry was carried out on CHI 1205A (CH instruments, Inc. Austin, TX, USA). The CHI instrument consists of a three-electrode system, in which the platinum wire and Ag/AgCl (sat. KCl) were used as auxiliary and reference electrodes, and a pre-washed GCE (glassy carbon electrode) acted as a working electrode.

6. Conclusions

In summary, we successfully synthesized NiCo₂S₄-Ms and NiCo₂O₄-MFs through the hydrothermal method. The formation of NiCo₂S₄-Ms and NiCo₂O₄-MFs were confirmed through the several analytical techniques such as FESEM, EDX, XPS and XRD. Further, the as-prepared nanocomposites were used as electrode materials for the detection of 4-NQO. Fascinatingly, the NiCo₂S₄-Ms modified electrode showed excellent electrochemical performances such as a wider range, low limit detection, higher selectivity and outstanding stability for the detection of 4-NQO. In addition, the practical applicability of the as-prepared nanocomposites were scrutinized in the human blood serum and saliva samples, which showed good recovery for both samples. To our knowledge, the hydrothermally synthesized NiCo₂S₄-Ms is the one of the most effective electrocatalysts for the detection of 4-NQO.

Supplementary Materials: The following are available online at www.mdpi.com/1422-0067/21/9/3273/s1, Figure S1. Mapping image of NiCo₂O₄-MFs (A). (B-D) confirms the presence of Ni (B), Co (C), O (D). (E) EDX profile for NiCo₂O₄-MFs; Figure S2. Effect of accumulation time on the reduction peak current of 100 μM 4-NQO at NiCo₂S₄-MS/GCE; Figure S3. DPV curve of obtained at NiCo₂S₄-Ms/GCE in the 0.1M pH-7 containing 0.5 μM of 4-NQO with other bioactive and electro active nitro species.

Author Contributions: T.-W.C. and E.T. developed the theory and performed the computations and carried out the analytical techniques. S.-M.Chen and X.L. helped supervise the project, financial support and helped to written the draft manuscript. M.A. and S.M. performed the analytic calculations and performed the numerical simulations. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that there is no conflict of interest.

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