Co$_3$O$_4$ nanoparticles embedded in electrospun carbon nanofibers as free-standing nanocomposite electrodes as highly sensitive enzyme-free glucose biosensors

Abstract: Numerous researches have been directed toward enzyme-free biosensors to alleviate the shortcomings encountered with enzymatic biosensors, in particular the intricate enzyme immobilization procedure. Herein, Co$_3$O$_4$/electrospun carbon nanofiber (ECNF) nanocomposites are successfully prepared to be employed as enzyme-free biosensors for diagnosis of glucose. Two parameters including the carbonization time and the amount of Cobalt(II) acetate tetrahydrate precursor are optimized, which are 5 h and 0.5 g, respectively. The 0.5 Co$_3$O$_4$/ECNF-5 h nanocomposite delivers superior sensitivity (475.72 $\mu$A·mM$^{-1}$·cm$^{-2}$), broad linear range (2–10 mM), and detection limit (LOD) less than 1 mM (0.82 Mm). In addition, the electrode shows excellent selectivity. The chronoamperometric analysis of 0.5 Co$_3$O$_4$/ECNF-5 h nanocomposite is performed by adding successively glucose analyte and interfering agents to the 0.1 M sodium hydroxide solution. No significant amperometric signal to the interfering agents including uric acid, ascorbic acid, and dopamine is delivered by this electrode, testifying the great selectivity of the electrode toward the diagnosis of target analyte (glucose) in spite of the existence of interfering species. Taking the aforementioned explanations into account, it can be concluded that the Co$_3$O$_4$/ECNF nanocomposite can be an appropriate free-stand electrode for high-performance enzyme-free glucose biosensor.

Keywords: electrospun carbon nanofibers, enzyme-free glucose biosensor, Co$_3$O$_4$ nanoparticles, electrochemical biosensor

1 Introduction

Diabetes as a metabolic disease is one of the main world health problems that can result in many health complications, including heart disease, amputation, renal failure, blindness, disability, and mortality [1–3]. Owing to their high sensitivity, simplicity in their construction and being cost-effective, electrochemical biosensors have been considered the reliable means to detect the dose of glucose in human blood [4–6]. Electrochemical biosensors are classified into two prime groups: enzymatic and nonenzymatic biosensors [7]. The former benefits from superior selectivity, rapid performance, and good catalytic activity. But, some handicaps, consisting of the complex immobilization procedure of the glucose oxidase (GOx) enzyme, the effect of temperature, pH, and humidity on the electrocatalytic performance of GOx and the high cost of this enzyme have hindered the further development of enzymatic biosensors [8,9]. Hence, the recent research has hugely been concentrated on enzyme-free biosensors to mitigate the shortcomings encountered with the enzymatic biosensors [10–12].

The studies on enzyme-free glucose sensors largely revolved around the direct response of glucose oxidation on the electrode [13]. Thus, the electrocatalytic activity of
the electrode material is one of the major parameters regarding the enzyme-free glucose sensors’ performance, which impacts their selectivity and sensitivity [3]. With the significant progress made in nanoscience and nanotechnology, new approaches have been introduced for the progression of enzyme-free biosensors for the diagnosis of glucose. In this regard, various nanostructured electrocatalysts have been developed to be adopted as enzyme-free glucose sensors, one of which is hybrid system made up of carbon-based nanomaterials and metal nanoparticles [1,8]. The latter not only are used as substrates for electrocatalysts but also provide them with large surface area, which simplify the charge transfer and fast electron transfer, giving rise to an increment in the biosensors’s sensitivity [14,15].

One of the most effective approaches to fabricate nanofibers holding diameters ranging from nanometers to microns is electrospinning [16,17]. In addition, this technique is facile, straightforward, and fast for the production of nanofibers [18,19]. Some features, including high specific surface area and high conductivity, have made electrospun nanofibers an appropriate candidate for an extensive range of applications [20,21].

The development of nanotechnology has resulted in the development of a number of nanomaterials with unique properties, such as optical, electrical, and mechanical [22]. In recent years, carbon nanomaterials such as graphene and its derivatives, carbon nanotubes, and carbon nanofibers have been used in a variety of applications due to their high electrical conductivity and high specific surface area [23–26]. Among the conductive carbon-based nanomaterials, electrospun carbon nanofibers (ECNFs) can be employed as a substrate to anchor electrocatalysts to be used as electrochemical biosensors [27]. One of the features distinguishing ECNFs from other types of carbon-based nanomaterials is that they can be utilized directly as free-stand electrodes without the need for any binder, which leads to the increasing of the conductivity of the electrodes [15,28]. Metal nanoparticles are considered electronic wires and electrocatalysts due to their nanosized structure and their role in providing fast electron transfer between the transducer and analyte molecules [29,30]. The electrocatalysts based on noble metals like Pt and Au show high performance toward glucose oxidation [31]. But these materials are expensive, and their electrocatalytic activity is adversely influenced by adsorption and accumulation of intermediates or chloride ions [32,33]. Hence, considerable efforts have been made to develop highly selective, sensitive, cost-effective, trustworthy, and fast electrocatalysts, which can be pointed out nanoscale metal oxides [34]. Some merits, including low cost, benignity to environment, relatively good conductivity, and electrocatalytic properties, have made Co3O4 nanoparticles as a suited material for enzyme-free glucose sensing [12,14,35].

In this research, an electrospinning technique combined with a heat treatment process was hired to synthesize Co3O4/ECNF nanocomposites to use as high-performance electrodes for biosensors. The nanocomposites were structurally studied using field emission scanning electron microscopy, X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, and thermogravimetric analysis (TGA). The electrochemical properties of the composite electrodes for glucose detection were also analyzed using cyclic voltammetry (CV), chronoamperometric (CA), and electrochemical impedance spectroscopy (EIS) tests.

2 Experiment

2.1 Chemicals and reagents

Polyacrylonitrile (PAN) with an average molecular weight of 80,000 g mol−1 as ECNF matrix source was purchased from Polycryle Company (Iran). Cobalt(II) acetate tetrahydrate (CoAc) as a Co source was provided from Sigma Aldrich Company. N,N-Dimethylformamide (DMF) as a solvent of electrospinning solution was purchased from Mojalaly Company. Isopropanol (IPA) and acetic acid were purchased from Mojalaly Company. Copper (II) chloride (CuCl₂) and sodium hydroxide (NaOH) were purchased from Sigma Aldrich Company. The solvent of electrospinning solution was prepared by mixing 8 wt% of PAN in DMF and 5 wt% of CoAc. The solution was stirred and sonicated to obtain a clear solution. The solution was used as the electrospinning solution.

2.2 Synthesis procedure of Co3O4/ECNF

The Co3O4/ECNF nanocomposites were synthesized based on the carbonization process of CoAc/PAN nanofibers as in our previous work [36]. In this regard, the dissolution of 12 wt% of PAN was first done in DMF at environment temperature under vigorous stirring for 2 h. Then, 5 wt% CoAc was included in the prepared solution and stirred overnight. A plastic syringe was filled with the solution, and it was then electrospun using an electrospinning device. 12 kV was applied during the electrospinning process. In addition, the distance between the collector and the spinneret was 10 cm, and the feeding rate was 0.5 mL h⁻¹. The resulting product was CoAc/PAN nanofiber. Afterward, CoAc/PAN nanofibers were stabilized at 250°C for 2 h in
2.3 Morphological and Structural Characterization

Field emission-scanning electron microscopy (FESEM) (Mira 3-XMU, TESCAN Co.) test was used to examine the morphology of the nanocomposites. The determination of atomic percentage and identification of elements were carried out by the EDS analysis. The crystal structure of the nanocomposites was assessed by the XRD test with a monochromatic Al Kα radiation using PHI5600, Physical Electronics. Diﬀerent carbonization times were selected to investigate the eﬀect of carbonization time on the morphological structure of nanofibers, including 1, 3, and 5 h, and the resulting products were, respectively, named as 0.5 Co3O4/ECNF-1 h, 0.5 Co3O4/ECNF-3 h, and 0.5 Co3O4/ECNF-5 h. In addition, to optimize the compositions, various amounts of CoAc precursor were added to the PAN/DMF solution, namely, 2, 3.5, 5, and 6.5 wt%. The viscosity of the electrospinning solution containing 6.5 wt% CoAc was very low, and no ﬁber was formed on the collector. Therefore, for other amounts of CoAc, consisting of 0.2, 0.35, and 0.5 g, the ﬁnal nanofibers were labeled as 0.2 Co3O4/ECNF-5 h, 0.35 Co3O4/ECNF-5 h, and 0.5 Co3O4/ECNF-5 h, respectively. As well, pure ECNF was synthesized using the same procedure from 12 wt% PAN without the inclusion of CoAc.

2.4 Electrochemical properties

The electrochemical analyses were conducted using a three-electrode setup and 0.1 M NaOH as the electrolyte at environment temperature. Platinum and Ag/AgCl electrodes were adopted as auxiliary and reference electrodes, respectively. A free-standing electrode was fabricated without the use of a binder in speciﬁc dimensions using Co3O4/ECNF nanocomposites. Electrochemical evaluation of the prepared electrodes was carried out with CV and CA methods on an electrochemical workstation (Autolab PG STAT302 N). The measurements were done using various scan rates in a potential range of 0.2–0.8 V. EIS was also used to examine the electrode’s resistance, which was performed in a frequency ranging from 10−2 to 105 Hz. Moreover, 5 mV was set as the alternating current (AC) voltage amplitude, and the measurement was performed in 0.1 M KCl electrolyte, including 6 mM [Fe (CN)6]3−/4−. Electrodes’ sensitivity, linear range (LR), and LOD were determined using linear regression. The LR was calculated in the range of glucose concentrations where the current response is linear. All obtained electrodes had a cross-section of 0.6 cm2. In addition, the LOD of the electrodes was calculated by equation (1) [37].

\[
\text{LOD} = 3.3S_b/S,
\]

where \(S_b\) is the standard deviation of the blank signal and \(S\) is the slope of the calibration curve.

3 Results and discussion

3.1 Morphological and structural characteristics of Co3O4/ECNF nanocomposites

The FESEM images of the samples are depicted in Figure 1. The synthesized nanofibers show nanometer diameters and micron lengths (see Figure 1a). In addition, according to Figure 1b–f, the Co3O4/ECNF nanofibers have been uniformly synthesized with diﬀerent amounts of CoAc salt and diﬀerent carbonization times without any beads. The analysis of image processing performed by Image J Software revealed that fiber diameters vary from 250 to 450 nm. Moreover, a number of small nanoparticles are found on the ECNF surface, which are cobalt oxide nanoparticles (Figure 1c). Electrospinning produced PAN nanofibers infused with the cobalt acetate salt. The main mechanism involved in the formation of cobalt oxide nanoparticles from primary cobalt acetate salt is that during the carbonation process at temperatures between 250 and 500°C, cobalt acetate decomposes, allowing the acetate ligand to be separated under heat stress [38].
Due to the concentration gradient of cobalt between the bulk and the ECNF surface, cobalt atoms migrated from the bulk to the ECNF surface [39]. After the carbonization step, the fibers underwent a postheating step, which converted the cobalt atoms on the ECNF surface to cobalt oxide. The number of cobalt oxide nanoparticles and their dispersion state depend on the amount of cobalt acetate precursor added to the electrospinning solution, as well as the temperature and the time of the carbonization process.

Figure 1b–d illustrate that the number of particles on the carbon surface increases as the amount of cobalt acetate increases. Also, by selecting different carbonization times of 1, 3, and 5 h, it is observed that the number of particles on the ECNF surface increases as carbonization time increases. Thus, the sample with 0.5 Co₃O₄/ECNF shows the best dispersion state, making it the optimal sample (Figure 1d–f). By processing the image, the diameter of particles of cobalt oxide on the ECNF surface was calculated, which was in the range of 60–85 nm.

The EDS diagram of the Co₃O₄/ECNF composite is shown in Figure 2a, which proves the presence of the elements of O, N, C, and Co. To verify the existence of Co₃O₄ and also to confirm the distribution of the Co element, the element mapping was conducted. Accordingly, the Co element was distributed uniformly (Figure 2b–d). Figure 2e manifests the XRD pattern of 0.5 Co₃O₄/ECNF-5h nanocomposite. The major peaks are located at 31.2, 36.8, 44.8, 59.3, and 65.2° ascribing, respectively, to the (220), (311), (400), (511), and (440) planes of Co₃O₄. Also, the peak situated at 2θ = 26.0° is imputed to the carbon (002) plane, implying that a graphite structure formed in the CO₃O₄/ECNF composite.

The formation of the graphitic structure in ECNF resulted in an increment in the structural order, which not only improves the electrical conductivity but also encapsulates Co₃O₄ nanoparticles within the ECNFs, thereby the separation of Co₃O₄ from the ECNF substrate is prevented [40].

Figure 3a shows the Raman spectra of Co₃O₄/ECNF nanocomposites containing different amounts of CoAc. Two main peaks situated at 1,319 and 1,589 cm⁻¹ commonly appear in carbon materials, which correspond to the D and G bands of carbon, respectively [41]. The \( I_D/I_G \) ratio is used to determine the degree of graphitization, so the lower the ratio, the higher the degree of graphitization [42]. According to Table 1, the \( I_D/I_G \) ratio changed from 1.02 for 0.2 Co₃O₄/ECNF to 0.94 and 0.81, respectively, for 0.35 Co₃O₄/ECNF and 0.5 Co₃O₄/ECNF, meaning that with increasing the content of cobalt in the nanocomposite, the carbon graphitic layer grew and...
Figure 2: (a) EDS diagram; element mapping of (b) O, (c) C, and (d) Co; and (e) XRD of Co$_3$O$_4$/ECNF nanocomposite.

Figure 3: (a) Raman spectra and (b) TGA of Co$_3$O$_4$/ECNF nanocomposites with different amounts of CoAc.
decrease occurred in the spacing between two graphite layers. The reason behind it is that the presence of Co$_3$O$_4$ nanoparticles induced the localized heating during the pyrolysis process, which subsequently gives rise to the production of highly graphitized carbon fiber.

To further examine the effect of cobalt nanoparticles on the ECNF properties, the electrical conductivity of the composites was measured. Table 1 shows the conductivity of composites. Electrical conductivity is highest for sample 0.5 Co$_3$O$_4$/ECNF containing the largest amount of cobalt. In other words, when the amount of cobalt in the composite system increases, the graphite layer and degree of graphitization in ECNF increase, which facilitates electron transfer within the composite and increases electrical conductivity. Therefore, the electrical conductivity data are consistent with the Raman spectroscopy results.

Figure 3b shows the TGA curves obtained under air atmosphere for determining the composition of the composites. Accordingly, all the samples have a sharp weight loss at 300–400°C, which is related to the carbon oxidation reaction released as CO$_2$ gas [36]. Starting at 550°C, all nanocomposites achieved a continual weight accompanied with the remaining weight corresponding to the amount of Co$_3$O$_4$ present in each composite. As a result, the weight values of Co$_3$O$_4$ in 0.2 Co$_3$O$_4$/ECNF, 0.35 Co$_3$O$_4$/ECNF, and 0.5 Co$_3$O$_4$/ECNF are 19, 22.9, and 26.7 wt%, respectively.

XPS measurements were performed on Co$_3$O$_4$/ECNF-5h composite to assess the surface chemistry and chemical elements, and the results are shown in Figure 4. The general XPS spectrum in Figure 4a clearly shows that the Co$_3$O$_4$/ECNF is composed of C, O, N, and Co elements. Owing to the usage of PANs as the initial polymer material having N atom in their structure and also the carbonization process performed in nitrogen atmospheres, nitrogen is present. C 1s spectrum deconvoluted in Figure 4b shows three binding energies at 284.5, 285.4, and 288.1 eV, imputing to carbon sp$^2$ and sp$^3$, as well as the C–O functional group, respectively [40]. In Figure 4c, two well-defined peaks are observed at 779.8 and 781.4 eV, which are imputed to the Co 2p$^{3/2}$ orbit of Co$_3$O$_4$. Furthermore, the presence of a satellite peak located around 787.6 eV further confirms the formation of the Co$_3$O$_4$ phase [43]. There are four peaks having the binding energies of 529.8, 531.3, 533.2, and 536.2 eV, which are ascribed to the O atoms in Co$_3$O$_4$, oxygen bands in C=O and C–O, and adsorbed surface water in the deconvoluted O 1s spectrum (Figure 4d) [40].

### 3.2 Electrocatalytic performance of glucose at the electrodes

The CV of the 0.5 Co$_3$O$_4$/ECNF-5h electrode with and without glucose is portrayed in Figure 5a. Two pairs of redox peaks can be detected in the CV curves arising from the redox reactions between Co$_3$O$_4$ and OH$^-$ ions [44]. One of them is located at lower potential, which originates from the reversible transition between the Co$_3$O$_4$ and CoOOH (equation (2)). The other pair found at the higher potential stems from the redox reaction occurring between CoOOH and CoO$_2$ (equation (3)) [45–47].

\[
\begin{align*}
\text{Co}_3\text{O}_4 + \text{OH}^- + \text{H}_2\text{O} & \rightarrow 3\text{CoOOH} + e^-, \\
\text{CoOOH} + \text{OH}^- & \rightarrow \text{CoO}_2 + \text{H}_2\text{O} + e^-.
\end{align*}
\]

(2)

It is worth mentioning that the addition of glucose resulted in an increment in the current density of the anodic peak, implying that the Co$_3$O$_4$ nanoparticles are capable of detecting of glucose. According to equation (4), oxidation of glucose leads to the production of gluconolactone as a result of a two-electron electrochemical reaction. During the oxidation reaction of glucose, the consumption and production of CoO$_2$ and CoOOH, respectively, take place, which is advantageous to the forward reaction of equation (2) and also the backward reaction of equation (3) because of the increment happening in the oxidation current at the higher potential and also in the reduction current at the lower potential, respectively [45,47]. The CV curves of the electrode in alkaline solutions containing various amounts of glucose varying from 2 to 10 mM are illustrated in Figure 5b.

| Samples        | $I_0/I_6$ ratio | Degree of graphitization (%) | Electrical conductivity (S·cm$^{-1}$) |
|----------------|----------------|-----------------------------|---------------------------------------|
| 0.2 Co$_3$O$_4$/ECNF | 1.02           | 49.47                        | 1.52                                  |
| 0.35 Co$_3$O$_4$/ECNF | 0.94           | 51.63                        | 5                                     |
| 0.5 Co$_3$O$_4$/ECNF | 0.81           | 55.08                        | 5.54                                  |

Table 1: $I_0/I_6$ ratio, degree of graphitization, and electrical conductivity of Co$_3$O$_4$/ECNF nanocomposites with different amounts of CoAc
Clearly, the increase in the glucose concentration led to the increase in the oxidation peak current, testifying the good sensitivity of the electrode toward the different concentrations of glucose. The CV curves of the electrode at various scan rates ranging from 20 to 200 mV·s⁻¹ are depicted in Figure 5c. With increasing the scan rate, the redox peak currents increase. Figure 5d shows a linear relationship between the anodic and cathodic peak currents and the square root of the scan rate, indicating that a diffusion-controlled reaction is taking place during the redox reaction of glucose on the 0.5 Co₃O₄/ECNF-5 h electrode [25–27].

The Nyquist plots of the Co₃O₄/ECNF nanocomposites synthesized using various amounts of CoAc precursor, including 0.2, 0.35, and 0.5 g, are shown in Figure 6a. Each of the Nyquist plots comprises a small semicircle and also a straight line located, respectively, in the high-frequency and low-frequency zones. The semicircle is attributed to the electron transfer resistance ($R_{ct}$) determining the transfer kinetics of the electron at the electrode interface [47–49]. Among the samples, 0.5 Co₃O₄/ECNF-5 h electrode shows the lowest $R_{ct}$, implying the easiest electron transfer and the highest electron transfer rate in this electrode during the redox process. The straight line situated in the low-frequency zone denotes the Warburg impedance, attesting that the electrochemical reaction on the 0.5 Co₃O₄/ECNF-5 h electrode is the diffusion-controlled process [48–50]. As a consequence, the EIS results are in cohesive with the CV analysis.

To opt an optimal potential applied during the amperometric experiments of glucose, the impacts of varied applied potentials in the range of 0.3–0.65 V were tested on the amperometric signal of the 0.5 Co₃O₄/ECNF-5 h electrode in an alkaline solution containing 4.0 mM glucose. As evidenced by Figure 6b, the maximum current was achieved at 0.55 V. To this end, this value was considered the optimal applied potential for amperometric measurements.
Figure 5: (a) and (b) Cyclic voltammogram of 0.5 Co$_3$O$_4$/ECNF-5h in the absence (Blank) and presence of analyte glucose with the scan rate as 50 mV s$^{-1}$ in a 0.1 M NaOH solution, (c) cyclic voltammogram of 4.0 mM glucose at 0.5 Co$_3$O$_4$/ECNF-5 h with different scan rates ranging from 20 to 200 mV s$^{-1}$ in a 0.1 M NaOH solution, and (d) the calibration curve of the current vs the square root of the scan rate.

Figure 6: (a) EIS patterns of Co$_3$O$_4$/ECNF nanocomposites, and (b) effects of applied potential on the amperometric response of the 0.5 Co$_3$O$_4$/ECNF-5h electrode in 0.1 M NaOH solution containing 4.0 mM glucose.
Figure 7a depicts the CA plots of Co$_3$O$_4$/ECNF electrodes with different concentrations of glucose in 0.1 M NaOH solution recorded at +0.55 V, and (b) calibration curves of the electrodes.

**Table 2:** Sensor parameters values of Co$_3$O$_4$/ECNF composite electrodes

| Sample          | Sensitivity (μA·mM$^{-1}$·cm$^{-2}$) | L.R. (mM) | LOD (mM) |
|-----------------|------------------------------------|-----------|----------|
| 0.2 Co$_3$O$_4$/CNF | 70.46                              | 2–10      | 0.93     |
| 0.35 Co$_3$O$_4$/CNF | 254.85                             | 2–10      | 0.61     |
| 0.5 Co$_3$O$_4$/CNF | 475.72                             | 2–10      | 0.82     |

Figure 7a depicts the CA plots of Co$_3$O$_4$/ECNF electrodes containing various amounts of metal salt precursor after sequential inclusion of glucose having a specified concentration of 2 mM at each time of addition. As it can be seen, an increment in the dose of CoAc resulted in the increase in the amperometric current response. In addition, based on the calibration plots of the samples presented in Figure 7b, with the increasing the amount of catalyst in the electrode, an increment took place in the slope of the calibration plot, resulting from the increased conductivity and also the surface area, which leads to the increase of the biosensor sensitivity respect to the glucose. Among the samples, 0.5 Co$_3$O$_4$/ECNF-5h electrode delivered the highest sensitivity (475.72 μA·mM$^{-1}$·cm$^{-2}$) (Table 2). Worth mentioning that in the range of 2–10 mM, all electrodes manifested a linear signal to the changes in the glucose concentration. Another merit of the electrodes is their LOD, which is less than 1.

Selectivity is considered a key parameter in the assessment of the free enzyme glucose biosensors’ performance. For doing the selectivity test, ascorbic acid, uric acid, and dopamine were selected as interferences since they exist in the real sample of blood. Blood glucose levels in humans are approximately determined by 3–8 mM, whereas the concentration of the aforementioned interfering species is roughly 0.1 mM [51]. The CA analysis of the samples was carried out through the addition of glucose analyte and interfering agents successively into the 0.1 M NaOH electrolyte. According to Figure 8, 0.5 Co$_3$O$_4$/ECNF-5h electrode to the addition of 0.1 mM of different interferent’s analytes in the presence of glucose analyte in 0.1 M NaOH solution.

Figure 8: Amperometric response of Co$_3$O$_4$/ECNF composite electrode to the addition of 0.1 mM of different interferent’s analytes in the presence of glucose analyte in 0.1 M NaOH solution.
composite electrode delivered no considerable current response toward the interfering agents, indicating that a superior selectivity toward the diagnosis of glucose is attained for the electrode in spite of the presence of interfering agents.

Moreover, our work has been compared to some recent glucose biosensors whose key parameters, including sensitivity, LOD, and LR are summarized in Table 3. Accordingly, the Co$_3$O$_4$/ECNF glucose biosensor possesses excellent electrochemical properties and can detect glucose analytes with superior sensitivity.

### 4 Conclusions

Co$_3$O$_4$/ECNF nanocomposite was synthesized through electrospinning the polymer and metal precursor, followed by the thermal treatment process for the reduction of metals and carbonization of PAN fibers. The carbonization process was performed at three different times including 1, 3, and 5 h, of which 5 h was selected as the optimum since, at this time, a superior dispersion of particles on the nanofibers was achieved. In addition, 5 g CoAc precursor was considered the optimal amount for the fabrication of Co$_3$O$_4$/ECNF electrode. CV measurements of the 0.5 Co$_3$O$_4$/ECNF-5 h electrode showed that by increasing the amount of glucose in the alkaline solution, the oxidation peak current increased, which is evidence of the good sensitivity of the electrode. Moreover, in accordance with the results obtained from the CV and EIS analyses, the redox reaction of glucose on the electrode is a diffusion-controlled process. Another merit making the 0.5 Co$_3$O$_4$/ECNF-5 h composite electrode, an appropriate candidate to be used as an enzyme-free glucose biosensor is its excellent selectivity. This electrode delivered no considerable current response toward the interfering agents, implying that the electrode showed a superior selectivity to the diagnosis of the target analyte in the presence of interfering species.

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