Co₃O₄ nanocubes decorated single-walled carbon nanotubes for efficient electrochemical non-enzymatic glucose sensing

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Abstract

In this study, we are presenting a novel hybrid nanostructure, composed of Co₃O₄ nanocubes adorned on single-walled carbon nanotubes (SWCNT) for electrochemical and non-enzymatic detection of glucose. The Co₃O₄/SWCNT nanocomposite was fabricated by simple hydrothermal method, followed by drop casting onto a glassy carbon electrode (GCE). The composition and grain size of as-fabricated nanocomposite was verified by X-ray diffraction, the morphology was studied by scanning electron microscopy and the functional groups were examined by Fourier transform infrared spectroscopy. The electrochemical and electrocatalytic behaviors of Co₃O₄/SWCNT based GC electrodes toward glucose were estimated by cyclic voltammograms, electrochemical impedance spectroscopy, and amperometric methods, respectively. This Co₃O₄/SWCNT/GCE biosensing device demonstrated higher electrocatalytic activity for the electro-oxidation of glucose in the alkaline media, which furthermore proved to be strongly dependent on NaOH concentration. Our Co₃O₄/SWCNT/GCE biosensor showed a linear response from 1 to 5 mM with a sensitivity of 96.92 $\mu\text{A mM}^{-1} \text{cm}^{-1}$ and limit of detection of 0.25 μM . Besides, it showed higher selectivity (7–14 times) for glucose against various interfering species including dopamine, ascorbic acid, NaCl, urea and uric acid. Based on these findings, we can foretell potential field applications of this Co₃O₄/SWCNT/GCE fabricated biosensor for sensitive and selective non-enzymatic estimation of glucose.

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Graphic abstract

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1 Introduction

Maintaining an ideal blood sugar/glucose level is vital for good health. Divergence from normal blood glucose level causes serious problems and complications which can only be controlled and prevented with great care. Therefore, it is imperative to develop accurate, precise, reliable and rapid methods of blood glucose estimation with enhanced sensitivity and superior selectivity. Such efforts, will not only provide useful information for diabetic patients but also situate significant impact in non-medical areas including the food industry, medicine, and biotechnology, etc. [1]. In recent years, numerous efforts have been made to formulate glucose-sensing techniques including electrochemical [2], chemiluminescence, surface-enhanced Raman scattering and many other [3]. Among these, the electrochemical strategies have proved to be the most realistic and effective protocol for the detection of blood glucose levels. This is owing to the high sensitivity, selectivity, and simplicity

in the operation of the electrochemical devices. These devices could be categorized into following two types of glucose biosensors: (1) *enzyme-based glucose biosensors* and (2) *non-enzymatic glucose biosensors* [4]. Enzyme based biosensors use immobilized enzymes [5] which corroborate good selectivity, great sensitivity, and low detection limit. However, these biosensors are sensitive and their performance can easily be affected by external environmental factors such as variation in pH, temperature, and interaction with toxic chemicals and humidity [5]. These shortcomings restricted the progression and application of enzymatic biosensor. Besides, enzymatic biosensor also suffers from drawbacks such as high cost, complex immobilization process, and chemical instability. Thus, recent studies have been mainly focused on the fabrication of non-enzymatic biosensors, which showed pronounced selectivity, faster response, and lower detection limits. Furthermore, the non-enzymatic biosensing devices

Scheme 1 Steps in the fabrication of non-enzymatic glucose sensor based on $\text{Co}_3\text{O}_4/\text{SWCNT}$ modified glassy carbon electrode (GCE)

have eradicated all the aforementioned disadvantages of the enzymatic biosensors [6] (Scheme 1).

In recent years, carbon-based nanomaterials due to their intrinsic structural and physical properties have been extensively used for the fabrication of non-enzymatic based electrochemical biosensors [7]. In this context carbon nanotubes (CNTs) have gained substantial importance and plenty of efforts have been exerted to use CNTs in the fabrication of carbon-based nanocomposite materials. CNTs owe several unique properties such as high surface area for maximum signal transduction and amplification, excellent mechanical strength, excellent electrical conductivity i.e., has fast electrons transfer capacity, electrochemically stable in aqueous and nonaqueous solutions and high thermal conductivity, etc. Besides, remarkable surface sensitivity of CNTs ensures the transformation of a biological occasion into a measurable electrical signal. Thus, augmenting that CNTs can serve as an ideal material for the fabrication of highly sensitive biosensing devices. CNTs are rich in hydrophobic sites that restrict solubility in common polar solvents. Based on the required use, the functionalization of CNTs becomes vital to enhance the solubility and other functional properties.

The physicochemical and electrical properties of CNTs can be tuned by using covalent and non-covalent functionalization techniques. In non-covalent functionalization, the structure–property relation of CNTs remains intact and their original properties persist. Alternatively, covalent functionalization considerably improves CNTs solubility and biocompatibility [8] but one may have to compromise on electrical and mechanical properties of CNTs due to loss in original structural homogeneity [9, 10]. Different strategies have been adopted to use and modify CNTs e.g., electrodeposition, and sol–gel methodology are few of them. CNTs due to large surface area offer ease of interactions with other electrically connected structures. It corroborates strength and specific ordering into these systems, which ultimately constructs stable electroactive surfaces even in the absence of additional binding agents. [11] Besides, CNTs have a greater rate of electron transfer under their 3D nanostructure along with the presence of overweening active sites [12]. Moreover, several new types of CNTs based nanocomposites have been fabricated via direct surface modification or blending techniques to further enhance the usage spectrum of CNTs [13].

On similar lines, transition metal-oxide nanoparticles such as CoO , Cu_3O_4 , Fe_3O_4 , etc. have shown extraordinary intrinsic properties such as biocompatibility, semi-conductivity, electrocatalytic and above all cost-effectiveness. Therefore, they have been enormously used in the fabrication of biosensors for biological applications. Among different types of transition metal oxides, cobalt oxide nanoparticles have demonstrated superior qualities towards non-enzymatic glucose biosensors [14]. Owing to its distinctive polymorphic forms, namely cobaltous oxide (CoO), cobaltic oxide (Co_2O_3) and cobaltous oxide (Co_3O_4) [15]. Particularly the Co_3O_4 poses a unique electronic structure, advantageous for transfer of electrons among Co^{2+} and Co^{+3} ions [16], and demonstrates extraordinary electronic and electrocatalytic properties [17, 19]. It eventually broadens the possibility for its usage in various research areas including heterogeneous catalysts, energy storage batteries and electrochemical sensing and biosensing devices [18, 20, 21].

Fabrication of a highly sensitive and selective non-enzymatic glucose sensor entails strong interaction of glucose with nanostructured material of good conductive and electrocatalytic properties. Herein, we report a facile one-step superficial growth of cubical cobalt oxide nanoparticles onto the pristine SWCNTs for non-enzymatic detection of glucose. The $\text{Co}_3\text{O}_4/\text{CNT}$ nanocomposite was further dropped cast onto glassy carbon electrode (GCE). This modified electrode was used for further analytical characterization. When CNTs are combined with nanoparticles of metals or metal oxides, a unique host–guest interaction is created which produces excellent nanocomposite material due to greater electronic property of CNTs and good catalytic property of cobalt oxide. This fabricated sensor showed a good response towards glucose in alkaline medium through electrocatalytic oxidation with excellent selectivity and sensitivity, low detection limit. The same was demonstrated in spiked serum samples and in presence of various possible interfering species without using any enzyme to access the potential applicability of this fabricated biosensor.

2 Experimental methodology

2.1 Chemicals and reagents

Cobalt chloride hexahydrate was purchased from VWR chemicals. Potassium Ferro/ferricyanide was purchased from Uni-Chem Chemicals and Reagents. Sodium hydroxide was purchased from Fischer chemicals and used to prepare the alkaline solution as supporting electrolyte. Daejung chemicals supplied Ascorbic acid and urea whereas fructose, NaCl, dopamine were obtained from

Sigma Aldrich. BDH chemicals supplied glucose and uric acid. Black powder of single walled carbon nanotubes synthesized by electric arc discharged method was purchased from Sigma Aldrich having diameter of 1.2–1.7 nm and 100 nm to 4 μm in length. Doubly distilled water was used in all experiments. All the other solvents and chemicals were of analytical grade and were used as received unless otherwise stated.

2.2 Co_3O_4 nanocubes growth on CNT surface

Firstly, single-walled carbon nanotubes SWCNT (50 mg) were added in 20 mL of distilled water and ultra-sonicated for 15–20 min. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (1.0 mmol) and urea (1.0 mmol) were then added to the above dispersion and stirred for 10–15 min. The mixture was then transferred to a Teflon-lined stainless-steel autoclave of 30 mL capacity. This was followed by autoclaving the mixture at 120 °C for 14 h. After the heat treatment the autoclave was slowly cooled down to room temperature, and $\text{Co}_3\text{O}_4/\text{SWCNTs}$ mixture was centrifuged in deionized water/ethanol mixture for three times in order to remove the unreacted chemicals and impurities. The obtained greyish precipitates were then dried at 50 °C for 3–4 h in drying oven. Finally, the grey powder was calcined at 450 °C for 3 h in an air atmosphere to achieve better characterization and to further remove the impurities.

2.3 Preparation of $\text{Co}_3\text{O}_4/\text{SWCNT}$ modified electrodes

To prepare the modified electrodes, a glassy carbon electrode (GCE) was polished carefully with alumina slurry and rinsed with ethanol and water, respectively. The working solution of nanocomposite was prepared by mixing 3 mg of $\text{Co}_3\text{O}_4/\text{SWCNT}$ in 0.5 mL water and 0.5 mL ethanol followed by ultra-sonication of the solution for 1 h. 10 μL of this solution was coated on a polished glassy carbon electrode via drop-casting. Finally, the modified electrode was allowed to dry at room temperature [22]. $\text{Co}_3\text{O}_4/\text{SWCNT}$ nanocubes modified GC electrode was considered as $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$. For comparison, GCE was also modified with pristine SWCNTs and the corresponding electrode was represented as SWCNT/GCE [13].

2.4 Characterization

The elemental analysis and surface morphology of modified electrodes were examined using scanning electron microscope (SEM, Vega 3, LMU, and Tescan) and EDS spectra were recorded at 15 eV. X-ray diffraction (XRD) pattern was collected in the range of 10–110° 2 θ values, using Cu K α radiation (PANalytical Xpert Powder Diffractometer)

Fig. 1 FT-IR spectrum of $\text{Co}_3\text{O}_4/\text{SWCNT}$ showing the functional groups of Co_3^{2-} at 937 cm^{-1} , C–O at 1096 cm^{-1} , C=C at 1438 cm^{-1} , C=O at 1636 cm^{-1} , C–H at 2921 cm^{-1} and O–H at 3491 cm^{-1}

with a diffractive beam operated at 100 mA and 40 kV. For the determination of functional groups present in

the sample, Fourier transform infrared (FTIR) absorbance spectrum was recorded using Thermo spectrometer (Nicolet 6700, USA) with a resolution of 8 cm^{-1} and scan range 4000–600 at the attenuated total reflectance (ATR) mode. Cyclic voltammetry (CV) and amperometric measurement were carried out by using Amel-2553 and electrochemical impedance spectroscopy (EIS) was performed with Amel Zpulse-2700. All electrochemical studies were performed using a standard three-electrode system with $\text{Co}_3\text{O}_4/\text{SWCNT}$ s modified GCE as a working electrode, while a platinum wire and Ag/AgCl electrodes were used as counter and reference electrodes, respectively. The cyclic voltammetry measurements were carried out by using 0.1 M NaOH at a scan rate of 50 mV/s in the absence and presence of glucose.

3 Results and discussion

Fourier transform infrared (FTIR) spectrum was recorded at the attenuated total reflectance (ATR) absorption mode to evaluate the functionalization of SWCNT, as shown in Fig. 1. The spectrum of $\text{Co}_3\text{O}_4/\text{SWCNT}$ shows the appearance of a broad absorption band in the region

Fig. 2 SEM micrographs of **a** pristine single-walled CNTs, **b** Co_3O_4 decorated single-walled CNTs and **c** field emission scanning electron microscopic image of $\text{Co}_3\text{O}_4/\text{SWCNT}$. The aqueous suspension

of each sample was drop cast onto freshly cleaned silica substrate, **d** energy-dispersive X-ray spectroscopy of $\text{Co}_3\text{O}_4/\text{SWCNT}$ s and **e** XRD pattern of Co_3O_4 on CNTs composite

of 3491 cm^{-1} confirming the presence of O–H functional group. Moreover, the peak at 1096 cm^{-1} is attributed to the stretching vibration of C–O band indicating the presence of Co_3O_4 oxide on CNTs [23]. Similarly, a slight stretch at 1438 cm^{-1} was observed which indicated the presence of double-bonded carbon confirming the hexagonal structure of CNTs and stretching band at 1636 cm^{-1} showed the presence of C=O functional group. Likewise, conservancy of starting materials' intrinsic functionalities can be evidenced with the presence of a weak sp^2 C–H stretching band at 2921 cm^{-1} corroborated by CNTs presence [24].

Scanning electron microscopy (SEM) was used to observe the morphological changes in the CNTs after Co_3O_4 nanocubes were decorated on the lattice carbon surface of CNTs. Figure 2a shows the SEM micrographs of pristine SWCNTs and Fig. 2b reveals that the deposition of cobalt oxide nanocubes on the carbon surface of SWCNTs through non-covalent interaction resulted in a prominent increase in the diameter of the CNTs. The detailed morphology was further investigated by field emission scanning electron microscopy (FE-SEM) as shown in Fig. 2c. At higher resolution, uniformly distributed square shape nanocubes of Co_3O_4 with an average diameter of about 322 nm were observed on the surface of CNTs. This conformal distribution of Co_3O_4 nanocubes on sp^2 lattice carbons of SWCNTs is attributed to the larger surface area, availability of more active sites and fast electron transfer in CNTs [25] through non-covalent interactions [26]. Also,

the energy dispersive spectrum (EDS) was measured at the accelerating voltage of 15 eV for $\text{Co}_3\text{O}_4/\text{SWCNT}$ to investigate its atomic composition as shown in Fig. 2d. The EDS spectrum demonstrated peaks of cobalt (0.77 La and 6.90 ka), oxygen (0.52 Ka), and carbon (0.27 ka) which corroborates the presence of cobalt oxide nanocubes on the surface of CNTs. Two more peaks at 1.7 keV and 2.6 keV were also observed which could be attributed to the substrate (silicon $\text{ka} + \beta$) and residual Cl (2.6 ka), respectively. Similarly, Fig. 2e shows the XRD pattern of prepared $\text{Co}_3\text{O}_4/\text{SWCNT}$ composite. The XRD pattern showed two diffraction peaks at 44° and 54° along 2θ axis, which are well indexed to (400) and (511) for Co_3O_4 nanocubes, indicating the oxidation of cobalt precursor resulting into its conversion into cobalt oxide [27]. The one significant peak appearing at 26.6° (2θ) indexed to (003) plane which can be attributed to the reflection of CNTs. These results indicated the successful fabrication of Co_3O_4 on SWCNT [28].

4 Electrochemical analysis

4.1 Cyclic voltammetry and electrochemical impedance spectroscopy of $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$

To investigate the electrocatalytic behavior of $\text{Co}_3\text{O}_4/\text{SWCNT}$, Cyclic voltammetry was performed for different modified electrodes in alkaline solution with a potential

Fig. 3 Cyclic voltammetric response (A) and electrochemical impedance spectroscopy (B) of bare GCE (a) electrode modified with pristine SWCNTs (b) and electrode modified with $\text{Co}_3\text{O}_4/\text{SWCNT}$ (c) in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ (1:1) at scan rate of 50 mV/s. (C) CV curves of $\text{Co}_3\text{O}_4/\text{GCE}$ (a) SWCNT/GCE (b) $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ (c) in glucose solution at a scan rate of 50 mV/s. (D) CV curves of $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ measured in the absence (a) and the presence (b) of glucose

range from 0 to 0.8 V at a scan rate of 50 mV/s. Figure 3A shows the CV curves and (B) electrochemical impedance spectroscopy (EIS) in frequency range of 0.1 kHz to 10 kHz, performed in 5 mM potassium Ferro/ferricyanide solution with bare/GCE, SWCNT/GCE and $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$, respectively. In case of bare/GCE and SWCNT/GCE, weak redox peaks were observed while a well-defined redox peak was observed in case of electrode modified with $\text{Co}_3\text{O}_4/\text{SWCNT}$. The formal potential of $\text{Co}_3\text{O}_4/\text{SWCNT}$ was calculated to be 0.45 V, and peak to peak separation was about 0.135 V. This pronounced electrochemical response can be attributed to the reversible electron transfer ability of Co_3O_4 nanocubes of the prepared $\text{Co}_3\text{O}_4/\text{SWCNT}$ nanocomposite. It eventually promotes the electron transfer rate between $\text{Co}_3\text{O}_4/\text{SWCNT}$ GC electrode and electrolyte solution which could be envisioned from both the CV and EIS comparison spectra of electrodes modified with pristine SWCNT and $\text{Co}_3\text{O}_4/\text{SWCNT}$. These findings further confirmed that the higher conductivity of $\text{Co}_3\text{O}_4/\text{SWCNT}$ could play a significant role in the direct transfer of electron equipped with potential electrocatalytic properties. Likewise, EIS represented the charge transfer resistance which showed the enhanced capability of electron transfer through modified GC electrode. A broad linear spectrum showed a higher value of charge electron resistance. Figure 3B showed lower value of electrical resistance of SWCNT/GCE as compared to that of bare/GCE. However, in case of $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$, the diameter of Nyquist plot decreased dramatically which indicate the increased value of electron transfer rate [29]. It is important to note that nanocubes of cobalt oxide attached with CNTs increased surface area and helped in the measurement of electrons by changing the oxidation of Co^{+3} to Co^{+4} which eventually oxidized the glucose into gluconolactone. The electrochemical surface area of $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ was calculated from peak current obtained by the cyclic voltammograms in ferro/ferricyanide $[\text{Fe}(\text{CN})_6]^{3-/4-}$ using the following Randels–Sevick equation [2].

$$i_p = 2.69 \times 10^5 A n^{3/2} D_{\text{red}}^{1/2} C^* \nu^{1/2} \quad (1)$$

where C^* is the concentration of ferricyanide solution (5×10^{-3} M), D_{red} is the diffusion coefficient of potassium ferricyanide ($6.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) and ν is the scan rate (50×10^{-3} V). The electrochemically active surface area of the $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ was found to be 0.3011 cm^2 . Furthermore, the redox value of 5 mM potassium Ferro-ferricyanide solution was shifted to higher potential indicating the fabrication of Co_3O_4 on SWCNT. On the base of the electrochemical response of $\text{Co}_3\text{O}_4/\text{SWCNT}$ by direct electron transfer between composite and electrode surface, a non-enzymatic glucose sensor was fabricated.

To investigate the sensing ability of composite $\text{Co}_3\text{O}_4/\text{GCE}$, SWCNT/GCE and $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ towards glucose, CV was performed in the presence of 2 mM glucose in basic medium. Pronounced electrochemical peaks were observed in the case of $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ as compared to that of $\text{Co}_3\text{O}_4/\text{GCE}$ and SWCNT/GCE , as shown in Fig. 3C. Figure 3D displays the CV curves of composite $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ in the absence and presence of glucose in an alkaline solution of 0.1 M NaOH at a scan rate of 50 mV/s. It is noteworthy that no significant peak was observed at $\text{Co}_3\text{O}_4/\text{SWCNT}$ modified electrode in absence of glucose. A sharp oxidation peak was observed on addition of 2 mM glucose solution which confirms the electrocatalytic ability of composite $\text{Co}_3\text{O}_4/\text{SWCNT}$ towards glucose. The forward reaction would be greatly favored with the consumption of CoO_2 and production of CoOOH and OH^- from NaOH play an important role in oxidation of glucose from Co (III) to Co (IV). As a result, value of oxidation current increased upon addition of glucose. At the same time, value of reduction peak decreased indicating the following process was carried out on electrode. Based on these results, the mechanism of electrochemical reaction of $\text{Co}_3\text{O}_4/\text{SWCNT}$ on GCE can be explained by following mechanisms:

With the formation of CoOOH and the depletion of CoO_2 , the reaction would move towards the forward reaction, thus increased oxidation peak observed upon addition of glucose. Furthermore, when the $\text{Co}_3\text{O}_4\text{-SWCNT}/\text{GCE}$ was used to observe the response in neutral buffer solution to glucose addition, no peak was observed showing the critical role of OH^- ions in oxidation of glucose. [30].

Figure 4A shows the CV response of $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ at different scan rates from 10 to 120 mV/s in 0.1 M NaOH (correlation coefficient $R^2 = 0.9271$). With increasing scan rate, the value of oxidation peak current and peak to peak separation increased which represented the surface controlled electrochemical process. It was evidenced that peak potential shifted towards positive direction with increasing scan rate while the value of peak current colligated to glucose oxidation increased gradually. Moreover, the amount of glucose oxidation on the electroactive area was calculated by the Laviron's equation [31];

$$I_p = \frac{n^2 F^2 A \Gamma \nu}{4RT} \quad (2)$$

where n is the number of electrons transferred, F is the Faraday constant ($F = 96,485 \text{ C mol}^{-1}$), r is the electroactive glucose oxidative amount, A is the working area

Fig. 4 **a** Detection of glucose by using the $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ in 0.1 M NaOH at different scan rates of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 mV/s. **b** Linear dependence of peak current with scan rate. **c** Detection of glucose at various glucose concentrations of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5.0 mM at scan rate of 50 mV/s.

d Linear dependence of peak current with different concentrations of glucose. **e** Detection of glucose at different NaOH concentrations of 10, 30, 50, 70, 90 and 150 mM at a scan rate of 50 mV/s. **f** Linear dependence of peak current with different concentrations of NaOH

of a glassy carbon electrode and R and T represent gas constant and temperature of the electrochemical cell, respectively ($R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$, $T = 298 \text{ K}$). The amount

of glucose oxidized at the surface of GCE modified with $\text{Co}_3\text{O}_4/\text{SWCNT}$ was calculated to be $0.116 \cdot 10^{-6}$. As shown in Fig. 4C oxidation value of peak current increased upon

Fig. 5 **A** Amperometric response of composite electrode holding at 0.45 V upon different concentrations of glucose. **B** Linear dependence of peak current with different concentration of glucose. **C**

Amperometric response of real four sample analysis of human serum. **D** Current response of glucose in comparison to other interfering species

increasing concentration of glucose which indicate excellent response of modified electrodes towards different concentration of glucose (correlation coefficient $R^2 = 0.9826$). The presence of glucose triggered a non-enzymatic reaction in which glucose oxidation occurs. The above discussion confers that pH is an important parameter to examine the redox chemistry of $\text{Co}_3\text{O}_4/\text{SWCNT}$ on the surface of the glassy carbon electrode. Consequently, the effect of pH was studied using different concentrations of NaOH by $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ at a scan rate of 50 mV/s

for non-enzymatic detection of glucose (correlation coefficient $R^2 = 0.9725$) as shown in Fig. 4E. It can be evinced that a well-defined and stable oxidation peak was observed in each concentration of NaOH. These curves eventually endorsed the greater sensitivity and stability of $\text{Co}_3\text{O}_4/\text{SWCNT}/\text{GCE}$ at 0.1 M concentration of NaOH.

Table 1 Comparison of glucose sensing characteristics of various nanomaterial modified electrodes by different methods

Materials	Linear range (mM)	LOD (μM)	Sensitivity ($\mu\text{A mM}^{-1} \text{cm}^{-1}$)	References
r-GO with electrodeposited cobalt oxide NPs	0.04–4.0	1.4	1.21	[34]
3D Co_3O_4 -HPND/CNTs	—	0.35	22.21	[35]
Co_3O_4 Nanofibers-Nafion/GCE	Up to 2.04	0.97	36.25	[30]
$\text{CoOx.nH}_2\text{O}$ -MWCNT	Up to 4.5	2	162.8	[36]
$\text{Co}_3\text{O}_4/\text{SWCNTs}/\text{GCE}$	0.1–5	0.25	96.92	This work

4.2 Amperometric Analysis of Co₃O₄/SWCNT/GCE

The sensitivity of Co₃O₄/SWCNT/GCE was calculated based on the amperometric response upon different addition of glucose into well stirred 0.1 M NaOH solution as shown in Fig. 5A. In order to achieve a fine balanced calibration curve, in each volumetric addition the volume of glucose solution was kept less than 50 µL by diluting 20 mM stock solution of glucose.

A stable and discrete amperometric response with an interval of 50 s under magnetic stirring can be envisioned for the Co₃O₄/SWCNT/GCE at an applied potential of 0.45 V. After each glucose addition, less than 10 s were required to achieve a fine state of current response for the electrode showing sensitive and fast glucose detection [32]. With the help of Cottrell equation, the diffusion coefficient of glucose can be calculated by using the slope of line [33]:

$$I = nFAD^{1/2}C_g\pi^{-1/2}t^{-1/2} \quad (3)$$

where D is the diffusion coefficient, the mean value of the diffusion coefficient of glucose was calculated to be $1.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (Table 1).

To show the validity of the fabricated sensor, it is essential to demonstrate its functionality in complex biological environments. Hence, the fabricated biosensor was employed for glucose sensing for four different human serum samples which were preserved in the refrigerator, as shown in Fig. 5C. The Co₃O₄/SWCNTs modified GC electrode was insensitive to glucose-free serum samples as serum contains proteins and other biomolecules and sensitive to glucose-containing serum samples. This result signified the selectivity of nanocomposite Co₃O₄/SWCNTs for sensing glucose in the presence of various interfering species [30]. This excellent response of enzyme-free glucose biosensor in real sample analysis suggested that Co₃O₄/SWCNTs modified GC electrode has great potential in practical applications [37]. The selectivity of Co₃O₄/SWCNTs was also studied by injecting urea, uric acid, dopamine, NaCl, fructose and ascorbic acid which co-exist with glucose in the blood to examine the effect of these interfering species as presented in Fig. 5D. This amperometric response was carried out at a potential of about 0.45 V in the 0.1 M NaOH. This interfering test can be carried out by injecting 100 µM of interfering species as the concentration of glucose in the blood is higher than that of other interfering species. As shown in Fig. 5d, it can be observed that good response of current is observed in case of glucose where the interfering species do not show such response at Co₃O₄/SWCNT composite electrode. The tubular structure of CNTs provides larger surface area for diffusion of glucose towards active sites without any restriction and improved the contact between analyte

and electrode surface. The unpaired p orbital and vacant d orbital of cobalt (III) increased the electrocatalytic effect of composite and increased electrooxidation of glucose at active sites of composites in comparison of other interfering species. In other words, cobalt oxide nanoparticles are likely to adsorb hydroxide ion OH[−] which help in electrooxidation of glucose. It can be observed that greater value of current is observed in the case of glucose than that of other interfering species indicating the reliable and sensitive use of our fabricated Co₃O₄/SWCNT/GCE biosensor for the detection of blood glucose levels in human blood and serum.

5 Conclusion

In summary, Co₃O₄ nanocubes were anchored on the sp² lattice carbons of SWCNTs via hydrothermal approach and the composite was coated over a glassy carbon electrode by drop-casting. The Co₃O₄/SWCNT nanocomposite was used to engineer an easily operative and cost-effective sensor for non-enzymatic measurement of glucose in blood and serum. This novel Co₃O₄/SWCNT nanocomposite showed enhanced electrochemical properties towards glucose in alkaline medium within a standard three-electrode cell. Moreover, the results showed a 7–14 folds higher selectivity and sensitivity of Co₃O₄/SWCNT based sensor for glucose in comparison to possible interfering species (dopamine, ascorbic acid, NaCl, urea and uric acid). This excellent sensing recital of Co₃O₄/SWCNT based sensor was achieved by integrating the physicochemical and electrical properties of both Co₃O₄ and SWCNTs. The high surface area offered by Co₃O₄/SWCNT, semi-conductivity, electrocatalytic activity, and fast electron transfer, all played a vital role. Based on these findings, we can predict the potential future applications of Co₃O₄/SWCNTs nanocomposite in specific and many other transition-metal nanoparticles/CNTs based nanomaterials in general. Hence, our research will open new horizons for the economical fabrication of sensors and biosensors with elevated sensitivity, selectivity and non-enzymatic estimation of numerous biomolecules.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

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