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Coupled graphene oxide with hybrid metallic nanoparticles as potential electrochemical biosensors for precise detection of ascorbic acid within blood

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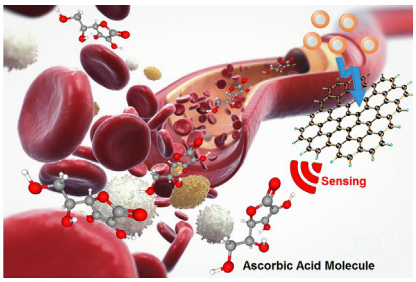
HIGHLIGHTS

- Coupled well-exfoliated graphene oxide with Ag or Ag–Fe_3O_4 used as sensitive biosensor.
- The developed platform used for accurate and real-time detection of ascorbic acid within blood.
- The developed platform significantly improved the sensitivity of glassy carbon electrode.
- The final platform detected the ascorbic acid till nano molar level with superior sensitivity.
- The modified electrode showed ideal sensitivity for detection of ascorbic acid in real samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Ascorbic acid (AA) as an essential biological molecule for proper performance of body can act as a biological metric for precise detection of various kinds of disease through measuring the level of oxidative stress; thus its precise/dividable detection is an urgent requirement for development of advanced biosensors. To address this requirement, we decorated well-exfoliated graphene oxide (GO) with Ag and hybrid Ag–Fe_3O_4 metallic nanoparticles toward precise, real-time and repeatable detection of AA within the blood plasma samples via electrochemical approaches that led to the development of a retrievable biosensor. Outcome of performed evaluations showed that modification of glassy carbon electrode (GCE) with selected additives significantly improved its sensitivity/selectivity. In this matter, the modified GCE with GO-Ag-Fe_3O_4 showed limit of detection and sensitivity of 74 nM and 1146.8 μA mM^{-1} cm^{-2}, respectively, within the concentration range of 0.2–60 μM. Additionally, the modified electrode kept 91.23% of its total performance after 15 days of performance and detected the oxidation peak of AA even with present of 50 fold of annoying contents which highlighting its superior stability/selectivity. More importantly, the developed electrode showed recovery range between 96.0 and

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104.4% within the human blood plasma samples that confirmed the ideal capability of developed platform for accurate detection of AA within biological fluids.

1. Introduction

Ascorbic acid (AA) is among the most frequent small biological molecules that exist within the human blood and perform an important role in the overall physiological performance of body. AA as an essential antioxidant and basic nutrient is vital for human body and its 100 mg daily dosage is required for a healthy life [1]. Shortage of AA within the human blood can lead to scurvy which thereby deteriorate women's fertility and adversely affect the collagen metabolism [2]. Moreover, AA can be used as a biological metric for determination of various kinds of dieses. In this matter, clinical investigations showed that variable concentration of AA within biological fluids is a metric to assess the exact amount of oxidative stress in the metabolism of body [3,4]. Increase in the level of oxidative stress has a well-defined link with diabetes mellitus, cancer, hepatic illnesses and etc. Thereby its bare bone essential to monitor the level of AA within the body through reusable, sensitive, reproducible and highly precise detection platforms [4].

Diverse procedures have been utilized for precise and selective detection of AA within the body fluid, among which liquid chromatography [5], spectrophotometry [6], fluorescence [7] and electrochemical method [8] can mentioned, where the electrochemical one found to be the most promising, sensitive and accurate method by considering its simple process. Additionally, frequently used electrodes such as glassy carbon electrode (GCE) shows miserable electrochemical activity toward distinct detection of AA, thereby, its vital to modify the GCE with highly active electrocatalyst to improve its selectivity and sensitivity toward accurate determination of AA within the blood [9]. So far, diverse kinds of materials were developed and used to improve the sensitivity and capability of GCE among which carbonaceous materials [10] and metal oxides [11] can be mentioned.

Graphene oxide (GO) as a 2D crystal-lattice containing sp^2 hybridize carbon atoms within a heterocyclic structure that decorated with diverse kinds of active function groups (e.g., −OH, −COOH and etc.) found to be an ideal candidate for improving the sensitivity of the GCE electrode. This ideal 2D carbonaceous flake can lead to fast diffusion of the electrolyte and could significantly improve/accelerate the interfacial charge transfer between the electrolyte and electrode [12]. Moreover, GO improves the number of active sites as active/fast electron transfer channels that not only boost the active surface area of the enhanced electrode by modifying the contact with electrolyte, but also promote the reactivity of the improved platform [13].

Likewise, active function groups of GO, e.g., hydroxyl and carboxyl functional groups, providing nucleation sites for homogeneous distribution of different kinds of nanoparticles that can considerably improve the bonding between the GO surface and selected nanoparticles [14]; therefore, surface modification of electrode with GO is considered as a promising strategy for improving the capability/sensitivity of GCE electrode [15]. In addition, metal oxides considered as effective modifying agents for improving the electrocatalytic activity of modified electrodes owing to their ideal chemical properties, economic price and faster electron transfer rate [9], thus, modification of GO with active metal oxides is a promising strategy for development of practical biosensors. In Table 1, a view of recently developed graphene-based platforms for detection of AA can be seen.

Herein, we deposited Ag and hybrid Ag−Fe_3O_4 nanoparticles on the surface of well-exfoliated GO via electrochemical approaches toward precise, sensitive, stable, real-time, repeatable detection of AA within human blood plasma samples using retrievable magnetic biosensor. For this matter, first the developed nanomaterials were well-characterized via diverse analyses to confirm their successful synthesis and thence applied for modification of GCE. The GCE was modified with GO, GO-Ag and GO-Ag-Fe_3O_4 and their performances were checked separately through different electrochemical evaluations. Additionally, the capability of modified GCEs for detection of AA was also checked within the real human blood plasma samples and their limit of detection, sensitivity, stability and selectivity were examined to highlight the superior performance of developed biosensor for accurate detection of AA within the blood.

2. Results and discussions

2.1. Characterization of developed biosensors

In this section, developed materials were well-characterized via diverse analyses to validate their successful production. In this case, Fig. 1 (a) and (b) show the X-ray diffractogram and micro Raman spectroscopy results for the developed graphene oxide (GO). XRD spectrum of GO showed 2Θ peak at 23.727 which is correspond to the (002) plane of GO and confirm the successful exfoliation of GO out of pristine graphite flakes. What is more, outcome of micro Raman spectroscopy clearly showed the finger print of graphene oxide by exhibiting well-resolved peaks at 1353.94, 1613.58 and 2948.63 cm^{-1} that are attributed to the D-band, G-band and D + G band modes of graphene, respectively, with I_D/I_G ratio of 0.95. Additionally, a view of well-exfoliated GO nanoflakes can be seen in Fig. 1 (c) at diverse scales (i.e., 150 and 200 nm) which is in accord with achieved results from XRD and micro Raman spectroscopy analyses and clearly confirm the successful exfoliation/production of GO nanoflakes.

Moreover, outcome of XRD results for Fe_3O_4, Ag and hybrid core-shell Ag−Fe_3O_4 nanoparticles can be seen in Fig. 1 (d). As can be seen in XRD spectrum of Fe_3O_4, well-resolved 2Θ peak/plane at 30.2/(0 2 2), 35.6/(1 1 3), 43.3/(0 0 4), 53.7/(2 2 4), 57.2/(1 1 5), 62.8/(0 4 4) and 73.0/(3 3 5) is correspond to the $Fe_{24}O_{32}$ nanoparticles with cubic crystalline structure which is in accord with reference 96-900-5839; related results can be seen within Table S1. Likewise, EDAX analysis of as developed magnetite nanoparticles confirmed the high quality of developed nanoparticles with 37.36 W%/67.55 A % (with 606.7 intensity) and 62.64 W%/32.45 A% (with 712.3 intensity) oxygen and iron, respectively, which can be seen in Fig. S1. In Fig. 1 (d) part 2, the XRD spectrum of Ag nanoparticles can be seen, where obtained results are tabulated within Table S2. As can be seen in Fig. 1 (d) and Table S2, developed Ag nanoparticles exhibiting fcc symmetry crystalline structure with two identified planes of (1 1 1) and (2 0 0) at 2Θ of 39.4 and 46.5, respectively, which is in accord with reference JCPDS No. 4-783 and a previous study [28]. Besides, in accord to XRD results, EDAX analysis furtherly confirmed the superior purity of developed metallic nanoparticles with 100 W%/A% Ag (Fig. S2).

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Table 1
Recently developed AA detection platforms.

Modified Electrode		Concentration Range (μM)	Limit of Detection (μM)	Ref.
Carbon Part	Modifier			
GO	NiO	40–700	5.50	[9]
G	Chitosan	50–1200	10	[16]
N-doped-G	–	5–1300	2.2	[17]
GO	PANi	150–1050	50	[18]
G	–	9–2314	6.45	[19]
GF	$NiCo_2O_4$	200–750	50	[20]
rGO	La-doped ZO	0.01–700	0.00723	[21]
rGO	Co_3O_4	1–51	0.0487	[22]
rGO	Au NPs	240–1500	51	[23]
G	Tryptophan	200–1290	10.09	[24]
rGO	–	500–2000	250	[25]
GS/GN	–	10–360	0.230	[26]
rGO	Au–Pd	0.03–9.5	0.0157	[27]
GO	Ag or Ag–Fe_3O_4	0.2–60	0.074	Current Study

Abbreviations: graphene oxide (GO), graphene (G), nitrogen doped graphene (N-doped-G), polyaniline (PANi), graphene fiber (GF), reduced graphene oxide (rGO), lanthanum-doped zinc oxide (La-doped ZO) nanoplates (NPs), graphene sheet (GS), graphene nanoribbon (GN).

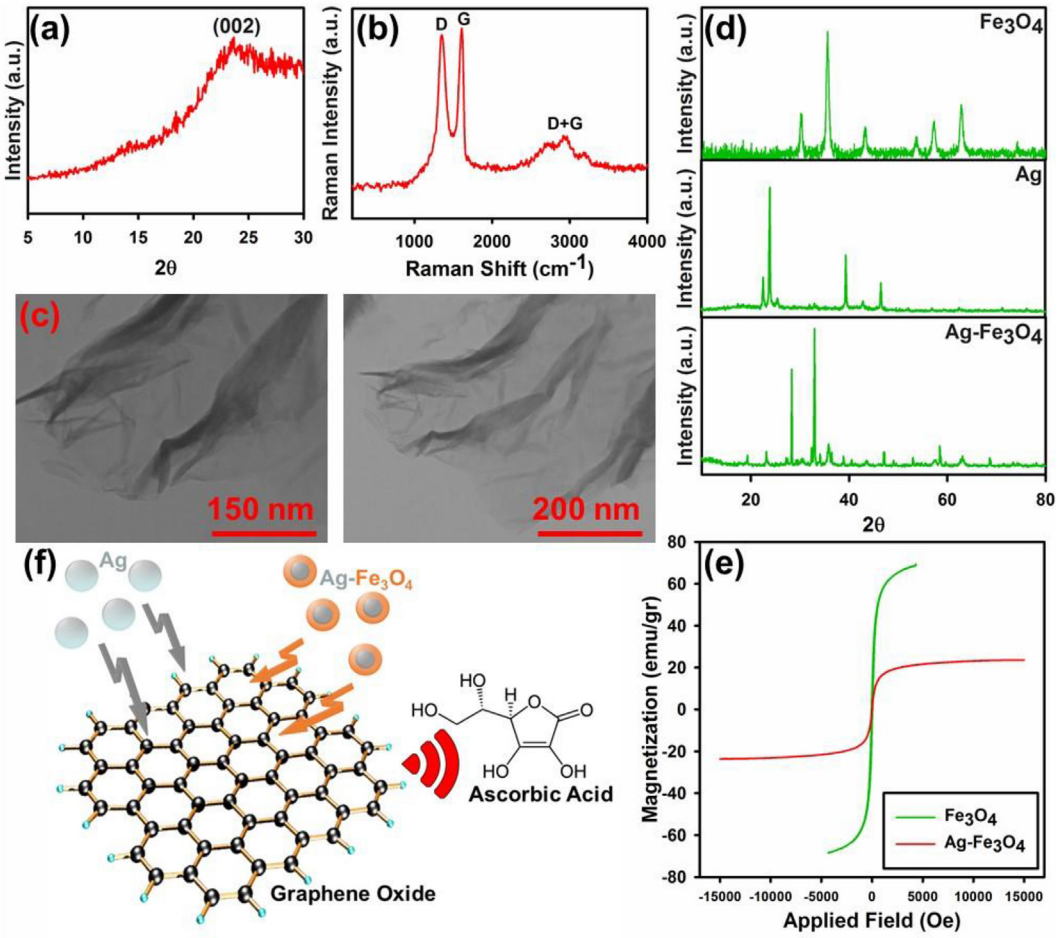

Fig. 1. (a) X-ray diffractogram of GO, (b) micro Raman spectroscopy results of GO, (c) TEM images of well-exfoliated GO nanoflakes at diverse scales (i.e., 150 and 200 nm), (d) XRD spectrums of Fe_3O_4, Ag and Ag–Fe_3O_4 nanoparticles, (e) VSM results of Fe_3O_4 and hybrid Ag–Fe_3O_4 nanoparticles and (f) a view of integration of GO with either Ag or Ag–Fe_3O_4 toward precise detection of AA within diverse media.

In addition, XRD spectrum of hybrid Ag–Fe_3O_4 core-shell nanoparticles (Fig. 1 (d) third part) showed the successful integration of Ag with Fe_3O_4. In this matter, the outcome of XRD results (Table S3) revealed that these hybrid metallic nanoparticles were mainly consisted of $Fe_{24}O_{32}$, $Fe_{72}O_{96}$ and Ag_4O_2 with cubic, tetragonal and cubic crystalline structure, respectively, which exhibited the successful formation of Ag–Fe_3O_4 nanoparticles. In accord with XRD results, EDAX analysis (Fig. S3) of Ag–Fe_3O_4 also confirmed the integration of Fe_3O_4 with Ag. In this case, Ag–Fe_3O_4 is consisted of 3.93 W%/15.27 A% (with 3.8 intensity), 19.18 W

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%/21.33 A% (with 131. intensity) and 57.68 W%/33.21 A% (with 350.2 intensity) oxygen, iron and silver, respectively, which is in accord with obtained data from XRD analysis and justified the formation of Ag–Fe_3O_4 nanoparticles.

Additionally, outcome of VSM analysis showed that developed nanomaterials exhibiting S-like curve with super magnetic nature that make them retrievable biosensors through their high magnetization. In this regard, Fe_3O_4 and Ag–Fe_3O_4 showed specific saturation magnetization (M_s)/remanence (M_r)/squareness ratio (SQR) of 69.48 emu.g^{-1}/31.75 emu.g^{-1}/0.457 and 23.67 emu.g^{-1}/9.47 emu.g^{-1}/0.400, respectively. Additionally, In Fig. 1 (f), a view of integration of GO with either Ag or hybrid Ag–Fe_3O_4 core-shell for detection of AA can be seen.

Furthermore, In Fig. 2, a view of FESEM images of Fe_3O_4, Ag and hybrid Ag–Fe_3O_4 can be seen at diverse scales. As shown in this Fig. 2 (a), Fe_3O_4 exhibiting spherical morphology, while FESEM images of highly pure Ag particles (Fig. 2 (b)) showed that these particles exhibiting semi-spherical morphology with larger size distribution. Moreover, FESEM images of Ag–Fe_3O_4 hybrid nanomaterials showed that integration of Ag with Fe_3O_4 can decrease their size distribution and improve the morphology/size of Ag nanoparticles, which make them ideal candidate for coupling with GO nanoflakes as highly sensitive and practical biosensor (Fig. 2 (c)).

What is more, in Fig. 3, TEM images along with size distribution of Fe_3O_4, Ag and hybrid Ag–Fe_3O_4 nanoparticles can be seen. As shown in Fig. 3(a), in accord with FESEM images of Fe_3O_4, TEM images also exhibiting spherical morphology with size distribution ranging from 11 to 22 nm which confirm the nearly uniform morphology and size distribution of these supermagnetic nanoparticles. On the other hand, pristine Ag nanoparticles did not show uniform size distribution (Fig. 3(b)), where nanoparticles with variable size from less than 14.7 nm up to 31.1 and more than that were detected within these images. Besides, morphology of Ag nanoparticles are not uniform and nanoparticles with diverse morphologies can be seen among them. More importantly, as can be seen in Fig. 3(c), integration of Ag with Fe_3O_4 nanoparticles significantly improved the morphology of Ag nanoparticles from semi-spherical and amorph to fully spherical. These hybrid nanoparticles also showed size distribution ranging from 12.6 nm to 23.4 nm. These data clearly highlighted the potential of proposed method for modification of Ag nanoparticles toward sensing/biosensing applications. Surprisingly, after evaluation of hybrid Ag–Fe_3O_4 nanoparticles, it was also observed that a majority of nanoparticles were integrated together and formed hexagonal Ag–Fe nanoplates with size distribution ranging from 116 nm to 238 nm, which show the potential of the proposed method for

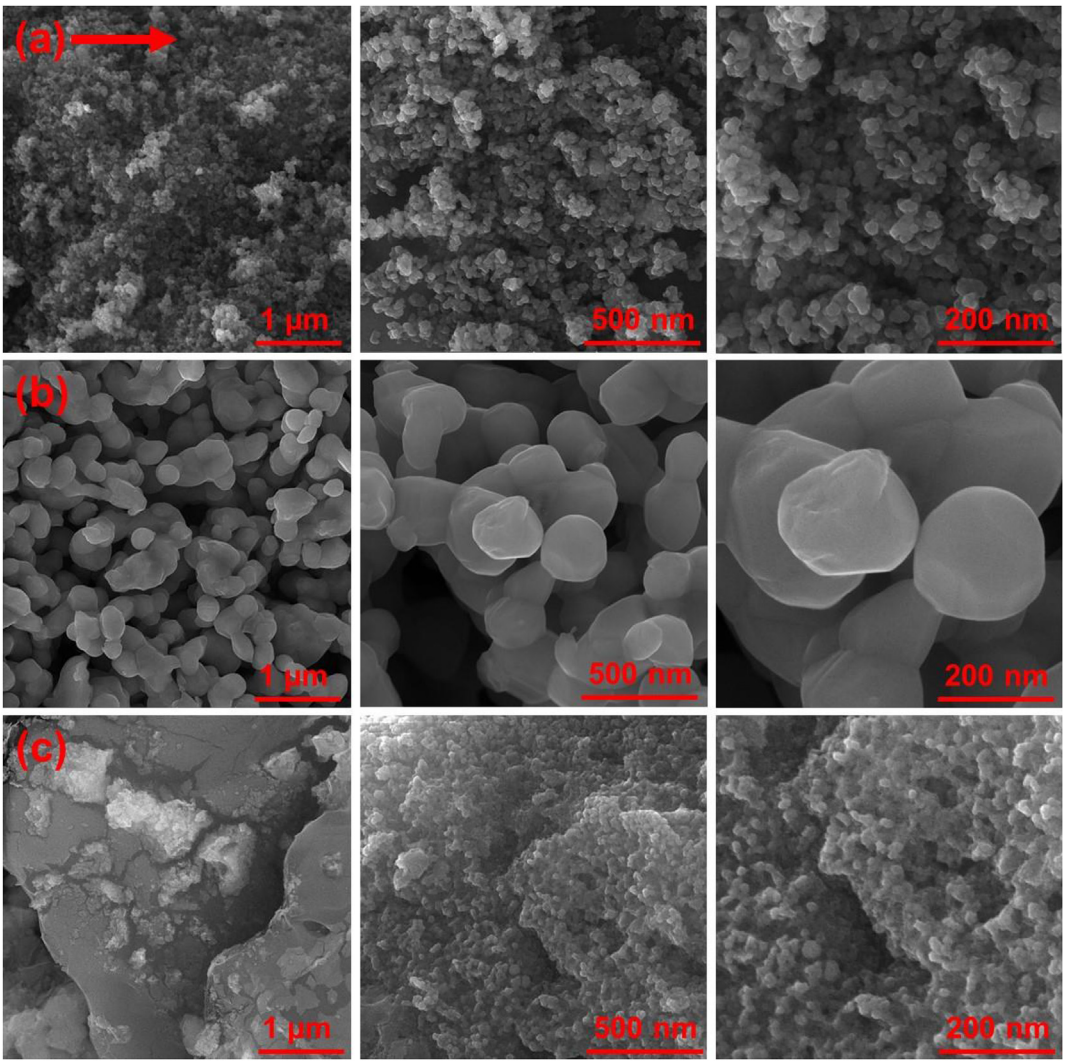

Fig. 2. FESEM images of (a) Fe_3O_4, (b) Ag and (c) hybrid Ag–Fe_3O_4 nanoparticles at diverse scales (i.e., 1 μm, 500 nm and 200 nm).

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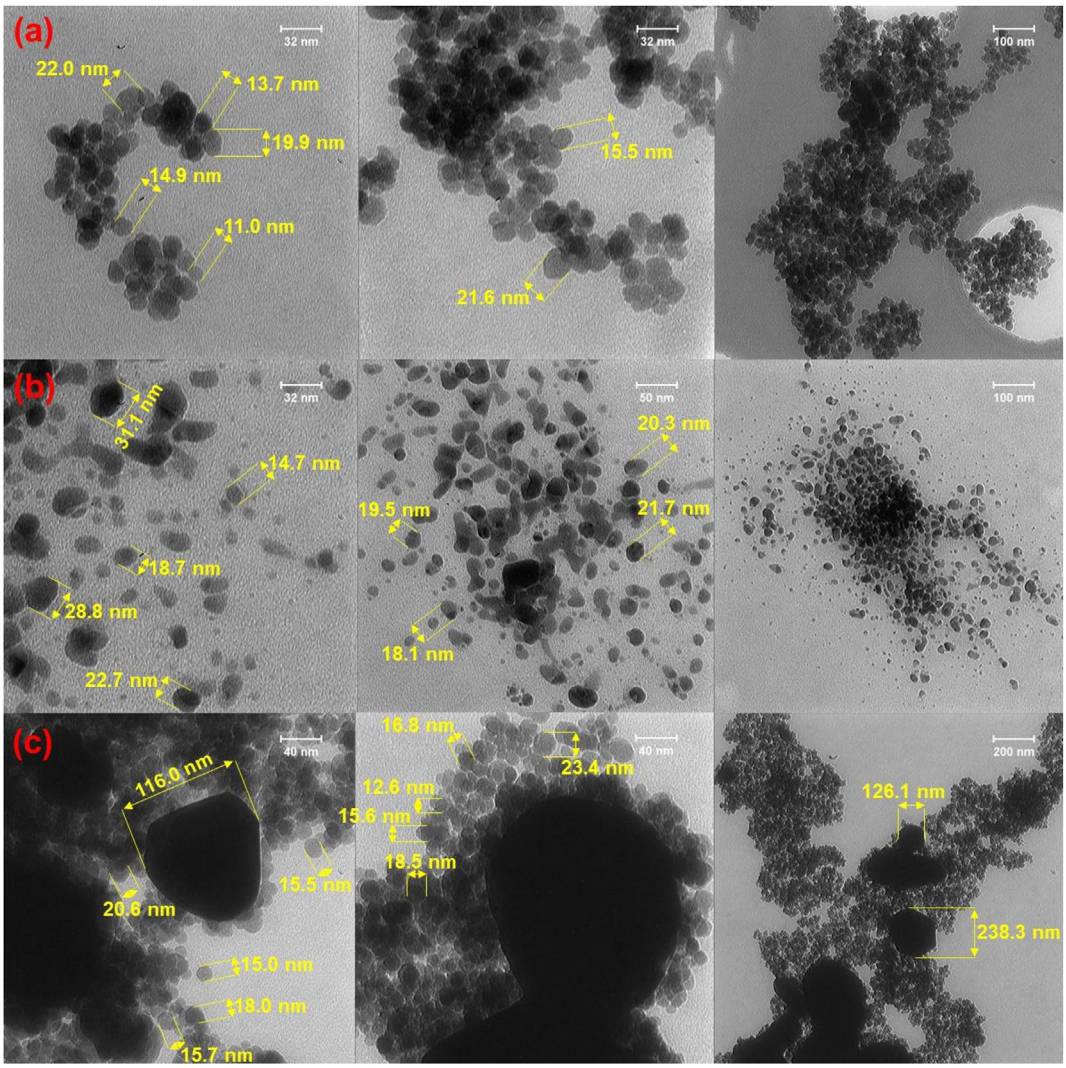

Fig. 3. TEM images of (a) Fe_3O_4, (b) Ag and (c) hybrid Ag−Fe_3O_4 nanoparticles with determined size of fabricated nanoparticles.

production of hybrid supermagnetic hexagonal Ag−Fe nanoplates.

In Addition, in Fig. 4, the elemental map and EDAX analysis of modified GCE with GO-Ag-Fe_3O_4 can be seen. As shown, the final nanocomposite is consisted of 11.7/29.6, 26.0/49.6, 11.73/6.40 and 50.54/14.28 W%/A% carbon, oxygen, iron and silver. In Fig. 4 (a) and (b), a view of elemental distribution map and EDAX analysis of this hybrid nanocomposite can be seen, respectively. Modification of GCE with such conductive and active nanocomposite can boost its catalytic performance for precise and repeatable detection of AA within biological media.

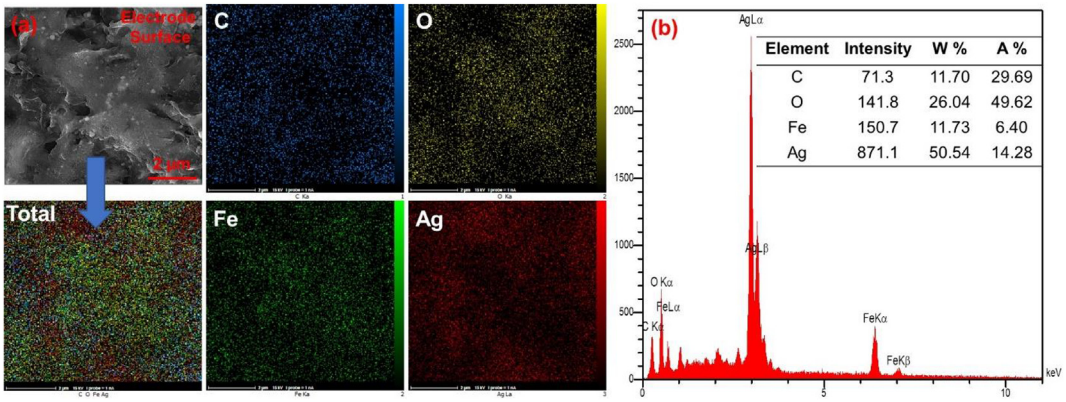

Fig. 4. (a) Elemental mapping and (b) EDAX analysis of modified GCE with developed nanocomposite consisted of GO-Ag-Fe_3O_4.

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2.2. Electrochemical evaluation of developed biosensor

In this section, the electrochemical behaviour of developed biosensors was evaluated via cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. In this matter, the response of electrochemical currents of proposed biosensors to the 5 mM $K_3[Fe(CN)_6]$ as electroactive probe approved the validity of modification stages of developed electrodes; a view of these results illustrated in Fig. 5 (a). The recorded voltammograms on bare GCE exhibited a reversible electrochemical behaviour with about 70 mV peak separation. However, the peak currents of $[Fe(CN)_6]^{3-/4-}$ redox probe were decreased by covering the surface of GCE with GO which is due to the insulative nature of the GO owing to its rich/active chemical functional groups. Besides, the peak separation is more evident and the redox peak potentials of the probe tend to be more positive and negative due to the formation of an insulating layer on the surface of GCE by the GO suspension, which thereby prevents electrons to reach the surface of electrode.

After the modification of GCE-GO with Ag nanoparticles, a notable increase in the current response was remarked that could be due to the high conductivity of nanoparticles and also involvement of the functional groups of GO resulted in much better electron transfer that facilitating the reaction of $[Fe(CN)_6]^{3-/4-}$ redox. Further introduction of Fe_3O_4 magnetite nanoparticles to the system led to a decrease in the current response of probe, which implying the presence of an obstacle for interfacial electron transfer by deposited hybrid Ag—Fe_3O_4 core shell nanoparticles on the surface of GCE. This phenomenon could be due to the negative charge of the hydroxyl groups and subsequent repulsion of the $[Fe(CN)_6]^{3-/4-}$ ions. Additionally, the electrochemically active surface area (ECSA) was determined to evaluate the performance of modified electrode. The effect of the potential scan rate (υ) on the electrochemical properties of electrode in the range of 10–500 mV s^{-1} was pursued by CV analysis in probe solution containing 5 mM $Fe(CN)_6^{3-/4-}$. Based on the slope of I_p vs. $\nu^{1/2}$ plot, (I_p (μA) = $0.0186\upsilon^{1/2}$ + 0.2) and Randles–Sevcik equation ($I_p = 2.69 \times 10^5\ n^{3/2}\ A_{eff} D^{1/2} \nu^{1/2} C$), the effective area of electrode was estimated to be 1.89 mm^2, which was higher than other electrodes. In Randles–Sevcik equation, I_p is the peak current (A), n is the number of electrons transferred, A_{eff} is the effective area (cm^2), D is the diffusion coefficient of 5.0 mM $K_3Fe(CN)_6$ and 0.1 M KCl ($cm^2\ s^{-1}$), v is the scan rate ($V.s^{-1}$) and C corresponds to the bulk concentration of the redox probe (mol cm^{-3}).

The EIS study was also carried out to evaluate the surface of modified electrodes and capability of electron transfer in the solution of $[Fe(CN)6]^{3-/4-}$ from 100 kHz to 0.01 Hz with 5 mV amplitude and potential of 0.25 V at pH 7.0. The Nyquist plots and electrical equivalent circuit obtained for the GCE electrode after each step of modification are presented in Fig. 5 (b). The charge transfer resistance (R_{ct}) value can be directly determined by the diameter of the semicircle of Nyquist plots. It is clearly noticed from Fig. 5 (b) that the electron transfer resistance of GCE, GCE-GO, GCE-GO-Ag and GCE-GO-Ag-Fe_3O_4 modified electrodes are close to 946, 1842.1, 530 and 691 Ω, respectively. According to the obtained results, the GCE-GO electrode showed a higher R_{ct} value compared with the unmodified electrode. This means that insulating nature of GO nanoflakes which coverage the surface of GCE can decline the

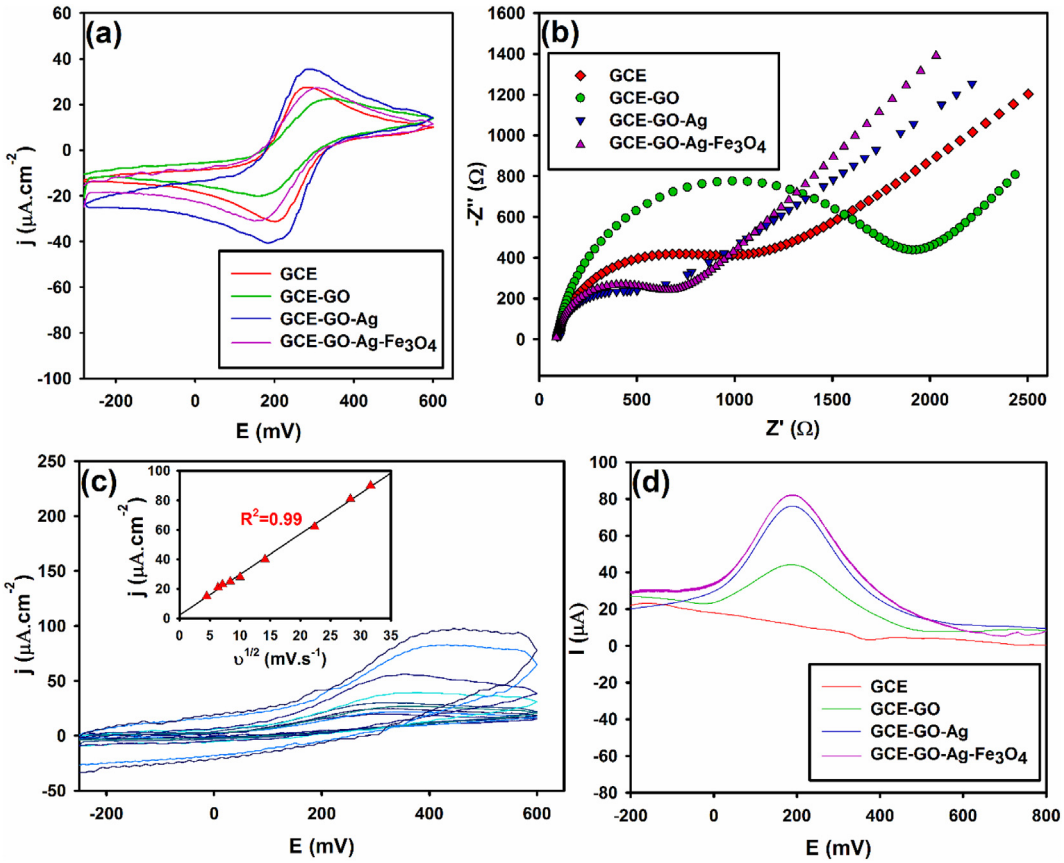

Fig. 5. (a) CV and (b) Nyquist plots of diverse electrodes, including GCE, GCE-GO, GCE-GO-Ag and GCE-GO-Ag-Fe_3O_4 in 0.1 M KCl and 5 mM $Fe(CN)_6^{3-/4-}$; (c) obtained CV at different scan rates in 0.1 M PBS at pH 7.0 on GCE-GO-Ag-Fe_3O_4 electrode in the presence of 50 μM AA, inset show variations of anodic peak current as a function of scan rate; (d) obtained DPV in 0.1 M PBS at pH 7.0 in the absence of any external redox probe on the GCE, GCE-GO, GCE-GO-Ag and GCE-GO-Ag-Fe_3O_4 electrodes.

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electron transfer rate which furtherly confirmed the semi-conductive nature of GO nanoflakes.

However, introduction of silver nanoparticles to the system improved the electron transfer capability, and therefore the final R_{ct} decreased due to the greater surface area of the electrode and the superior conductivity of silver nanoparticles. On the other hand, after modification of GCE-GO with hybrid Ag—Fe_3O_4 core shell nanoparticles, the diameter of the impedance arc increased which highlight the higher interfacial electron transfer resistance of Ag—Fe_3O_4 modified GCE-GO electrode than Ag modified GCE-GO electrode. Additionally, the trend of R_{ct} changes during the preparation of the sensor is consistent from the obtained results of CV test.

The effect of scan rate on the peak potential (Ep) and peak current (ip) of AA at GCE-GO-Ag-Fe_3O_4 have been clearly investigated by drawing/measuring cyclic voltammograms in the solution containing 50 μM AA (pH 7.0). Fig. 5 (c) (inset) reveals that the oxidation peak of AA was increased upon enhancing the scan rate from 20 mV s^{-1} to 1000 mV s^{-1}. The linear variation of the peak currents with respect to the scan rate indicated that a diffusion controlled process was occurred on the surface of GCE-GO-Ag-Fe_3O_4.

Differential pulse voltammetry (DPV) as an accurate technique was utilized for tracing the electrocatalytic response of AA at unmodified electrode and also after deposition of nanocomposites on enhanced electrodes in the absence of any external redox probe. The voltammograms outcomes were obtained for GCE, GCE-GO, GCE-GO-Ag and GCE-GO-Ag-Fe_3O_4 in PBS at an optimized pH of 7.0. Achieved outcomes can be seen in Fig. 5 (d), in which no anodic peak was obtained on the unmodified GCE. Whereas, the GO modified electrode showed weak oxidation peaks because of the oxidation process of hydroxyl group related to the furan ring in AA to carbonyl group, suggesting the poor adsorption of AA on the surface of electrode. However, GCE-GO-Ag and GCE-GO-Ag-Fe_3O_4 modified electrodes showed exceptional current response at 148 mV. The superior electrochemical performance of modified electrodes could be originated from the presence of Ag^+ ion within the system which their ideal catalytic activity can accelerate the charge transfer rate of present molecules within the developed platform through activation of hydroxyl functional groups. It suggested that the GCE-GO-Ag-Fe_3O_4 had an outstanding catalytic performance for the oxidation of AA and thus its precise sensing/biosensing in divers aquatic medias.

The pH of PBS solution is one of the main parameters that affecting the oxidation process/current of AA. The pH dependence of the AA oxidation on the GCE-GO-Ag-Fe_3O_4 was pursued in the pH range of 4.0—8.0 (Fig. 6 (a)). In this case, obtained data from corresponding DPV graphs showed that the enhancement of peak current is accompanied with an increment of the solution pH, while the oxidation peak is changed/shifted to more positive values. The well-defined peak of AA on GCE-GO-Ag-Fe_3O_4 was observed at pH 7.0. Additionally, further increase in the pH of buffer led to a decrease in the oxidation current of AA. Likewise, a linear relationship was also detected between the pH and E_p with regression equation of $E_p/V = -0.0293\ pH + 0.3318$ ($R^2 = 0.9731$). The slope of equation indicated the participation of protons/electrons in the electrochemical process of AA that leads to its sensing via the developed platform. Therefore, pH 7.0 was chosen as an optimum pH for sensing AA within biological media to get favorable sensitivity and better applicability in physiological environment. In Fig. 6 (b) and (c), the effect of pH on the I_p and E_p can be seen.

Additionally, the Effect of accumulation time on the oxidation response of the AA biosensor was also studied. In this matter, the related studies were carried out within the range of 5 min—30 min to accumulate 50 μM AA on the surface of GCE-GO-Ag-Fe_3O_4 at pH 7.0. As illustrated within Fig. S4, anodic peaks and incubation time of the AA was significantly enhanced upon using the developed biosensor. Stable currents were achieved after a certain period of time, confirming the saturation of AA on the surface of GCE-GO-Ag-Fe_3O_4. In this regard, the 20 min time span was selected as the optimum time to accumulate AA and pursue its electrochemical studies.

In order to specify the analytical efficiency of the developed biosensor, electrochemical sensing of AA via GCE-GO-Ag-Fe_3O_4 was verified through DPV analysis. DPV technique can afford intuitive peaks even at low values of analyte by minimizing background current contribution. Sensing AA at various concentrations via GCE-GO-Ag-Fe_3O_4 under optimal conditions is presented within Fig. 6 (d). In this case, it can clearly be seen that the current signal of DPV was linearly increased upon increase in the concentration of AA from 0.2 to 60 μM which demonstrated the ideal sensitivity of the developed platform toward detection of AA within aquatic media. The limit of detection (LOD) and sensitivity of calibration plots were calculated to be 74 nM and 1146.8 μA mM^{-1} cm^{-2}, respectively, at signal/noise ratio of 3. This value along with previously obtained data indicating the superior performance of the developed platform for detection of AA within diverse media.

The measurement of intra-day and inter-day (7 consecutive days) determination of AA at 50 μM were achieved to be 1.17 and 3.07%, respectively, during four repetitive measurements per day through using the proposed GCE-GO-Ag-Fe_3O_4 biosensor. The respective results represent the approved reproducibility of the process as one of the notable benefits of the developed sensor. Moreover, the long-term stability of the fabricated biosensor was evaluated through storing the modified electrode at ambient temperature for 15 days in the solution containing 50 μM AA. According to the obtained results, 91.23% of the initial current of sensor was retained during 15 days of usage, indicating the acceptable stability of the developed sensor. The selectivity of GCE-GO-Ag-Fe_3O_4 biosensor was also tested towards sensing AA at the presence of common interfering/annoying compounds; where in presence of 50-fold concentration of some common interferes such as glucose, fructose, sucrose and lactose, the anodic potentials and currents were retained.

The feasibility of the GO-Ag-Fe_3O_4 in practical analysis was investigated in human blood plasma samples to detect the concentration of AA using standard addition method. All of developed samples were diluted with PBS (pH 7.0) prior to measurements. The obtained results from conducted analysis were tabulated within Table 2. The developed sensor showed recoveries ranging from 96.0 to 104.48% (for $n = 3$), indicating the superior potential of the developed biosensor for real-time detection of AA within the blood plasma samples.

AA commonly exist as ascorbate anion at pH value of 7 with pK_a of 4.2. The oxidation mechanism of AA is pH dependent that shift to more negative values upon the increase in the pH (according to Fig. 6 (a)) [29,30]. The oxidation process of the ascorbate anion occur through two stages; where the first one involves one proton and one electron and the second stage occur through single electron oxidation process [31]. In this matter, outcome of electrochemical process leads to the formation of dehydroascorbic acid at neutral pH values that furtherly cause irreversible hydrolytic process which generates electro inactive 2,3-diketogluonic acid specie [32]. Additionally, the oxidation process of AA occur through a non-reversible inner sphere reaction where its electron transfer kinetic is highly sensitive to the specification of electrode's surface [29,33]. In this regard, lower positive values related to the peak potential is the sign of faster electron transfer rate/kinetic. A view of AA's oxidation mechanism that leads to its electrochemical sensing can be seen in Fig. 7.

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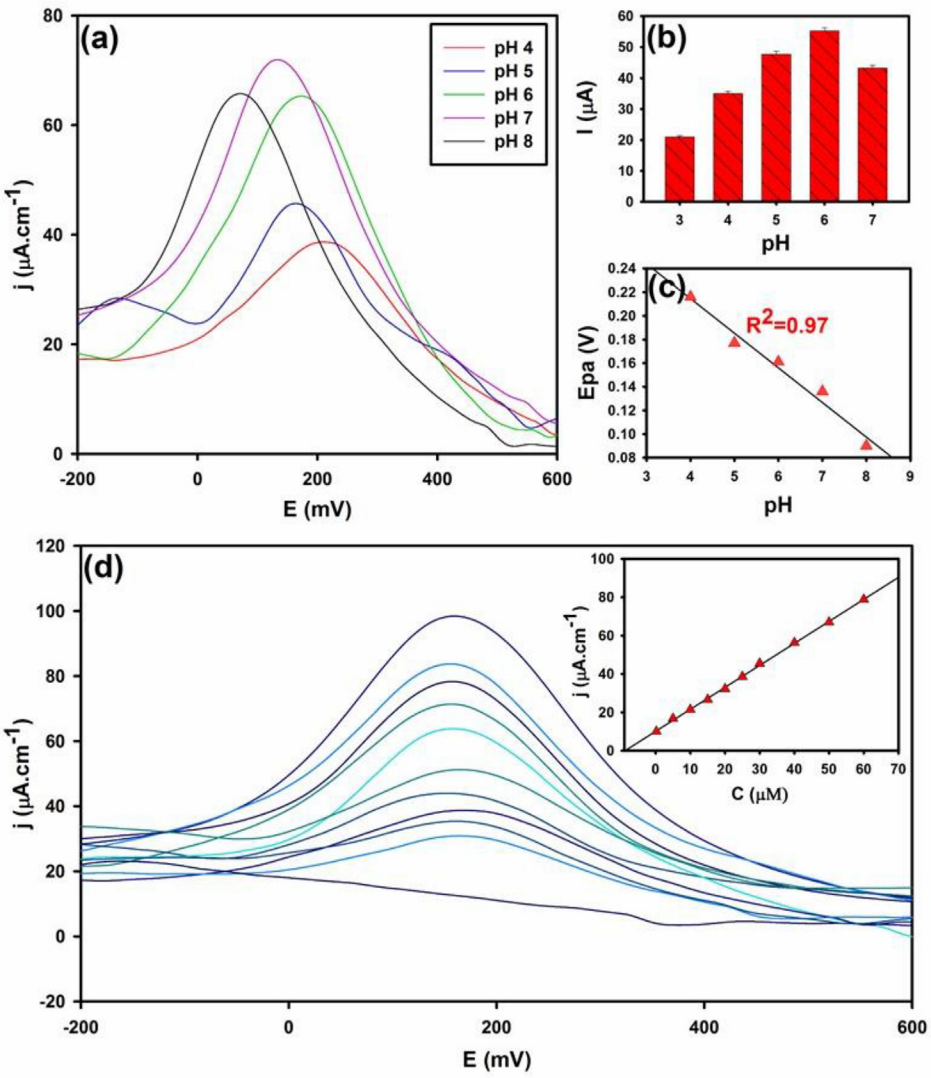

Fig. 6. (a) DPV plots of AA as a function of solution pH; influence of pH on (b) peak current, (c) peak potential and (d) DPV responses of GO-Ag-Fe_3O_4 modified GCE to AA at variable concentrations from 0.2 μM to 60 μM; inset shows the calibration curves of AA (n = 3) in 0.1 M PBS (pH 7.0).

Table 2
Analytical results for AA determination in human plasma sample.

Sample No.	Added (μM)	Found (μM)	Recovery (%)	RSD % (n = 3)
1	0.0	50.10	–	–
2	5.0	54.9	96.00	3.40
3	10.0	60.06	99.60	2.44
4	50.0	102.34	104.48	3.55

Besides, oxygen-based functional groups of GO can considerably improve the electrocatalytic and electro-chemical performance of biosensors [33,34]. These oxygen-based functional groups lead to preferential electrocatalytic process for the oxidation of the endiol group. The required heterogeneous charge for the oxidation of endiol groups mainly aries from the proton-coupled electron transfer process, in which the oxygen-based functional groups lead to withdraw of two protons from endiol groups, initiate the oxidation reaction of AA and decline the overpotential [34]. Moreover, H-bond formation between the oxygen-based functional groups of carbonaceous materials and endiol functional group of analyte has a significant role for improvement of electron transfer rate of endiol containing chemical compounds. On the other hand, along with superior role of oxygen-based functional groups in the electrochemical process, some other factors such as reduction in the accessibility of the analyte, electrostatic interactions and morphological alterations can also affect the electrochemical process [33].

3. Conclusions

AA is an essential biological molecule for the body that can also be used as a metric for determination of oxidative stress and thus various kinds of disease within the body. In current study, we deposited Ag and Ag—Fe_3O_4 hybrid nanoparticles on the surface of well-exfoliated GO toward modification of GCE for accurate detection of AA within the human blood plasma. In primary stages, the developed nanomaterials were well-characterized via different analyses to justify their successful preparation followed by examination of divers kinds of modified glassy carbon-based electrodes (i.e., GCE-GO, GCE-GO-Ag and GCE-GO-Ag-Fe_3O_4) as potential substrates for detection of AA within the blood plasma samples. Achieved results showed the superior sensitivity of about

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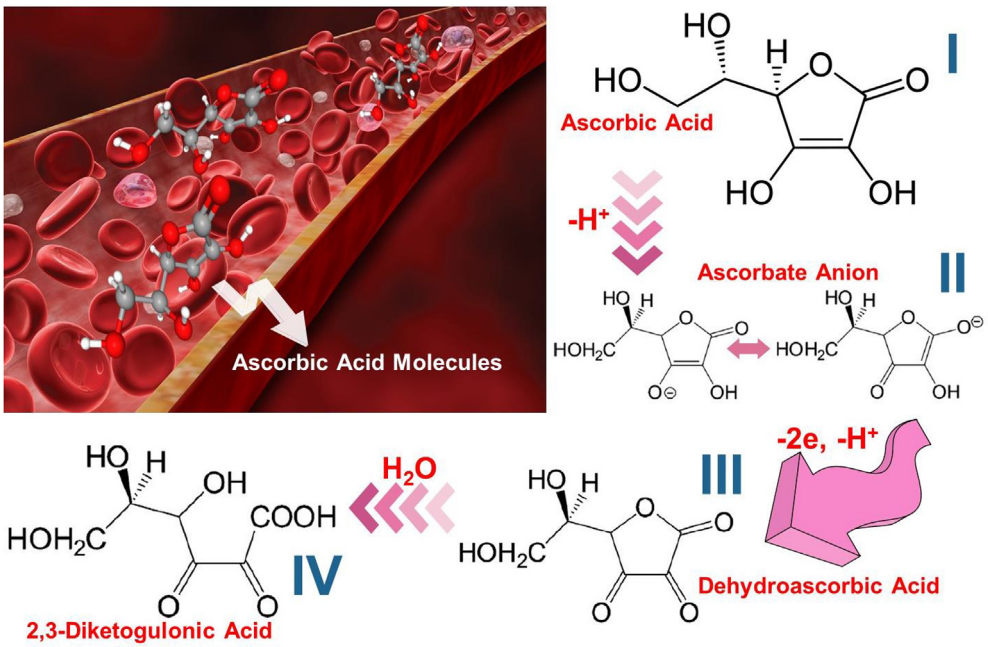

Fig. 7. Oxidation mechanism of AA that leads to its electrochemical sensing.

1146.8 μA mM^{-1} cm^{-2} with LOD of 74 nM for the developed platform that highlighting the fantastic sensitivity of the modified electrode at nano molar level.

Likewise, the modified electrode showed ideal stability where it kept more than 91.23% of its performance after 15 days and detected the oxidation peak of the AA even upon the existence of higher dosage of annoying contents up to 50 folds. The developed electrode also showed favorable function during detection of AA within the blood plasma samples where it recovered the AA between range of 96.00–104.48%. Obtained results clearly showed that the modified electrode can sense the AA through sensitive, selectable, stable, magnetic retrievable, recoverable and dividable approach which make it ideal for real-time monitoring of AA within biological fluids/media.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Seyyed Alireza Hashemi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Seyyed Mojtaba Mousavi:** Conceptualization, Resources, Funding acquisition. **Sonia Bahrani:** Conceptualization, Methodology, Software, Validation, Investigation, Writing - original draft. **Seeram Ramakrishna:** Supervision, Validation. **Aziz Babapoor:** Resources, Funding acquisition. **Wei-Hung Chiang:** Supervision.

Acknowledgement

Details of experimental section including characterization techniques and evaluations methods along with further details of developed nanomaterials/biosensor can be seen within the supporting information.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.02.018.

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