Graphene quantum dots decorated with magnetic nanoparticles: Synthesis, electrodeposition, characterization and application as an electrochemical sensor towards determination of some amino acids at physiological pH

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Abstract

This study reports on the synthesis and characterization of a novel nano-composite, Fe₃O₄ magnetic nanoparticles/graphene quantum dots (Fe₃O₄ MNP-GQDs), for sensing of some amino acids. For the first time, as-synthesized GQDs and Fe₃O₄ MNPs-GQDs was electrodeposited on the glassy carbon electrode (GCE) by cyclic voltammetry (CV) regime in the potential range from -1.0 to 1.0 V. Fe₃O₄ MNP-GQDs is engineered to specifically and effectively capture and enhancement the electrochemical signals of some amino acids at physiological pH due to the synergy among GQDs and magnetic nanoparticles. We have illustrated that the obtained Fe₃O₄ MNPs-GQDs exhibited a much higher electroactivity individual GQDs and Fe₃O₄ MNPs for the electrooxidation and detection of amino acid which was about 10 fold higher than for GQDs. Magnetic and specific properties of the Fe₃O₄ MNP-GQDs can be exploited to capture and pre-concentration the amino acids onto its surface, which are important for detection of multi-amino acids.

KEYWORDS

magnetic nanoparticle, graphene quantum dot, electrochemical sensor, amino acid.

1. Introduction

Graphene quantum dots (GQDs) are nanometer-sized fragments of graphene. GQDs are a kind of 0D material with characteristics deriving from both graphene and carbon dots (CDs) [1]. Taking advantage of the electrochemical properties similar to graphene, GQDs are widely used as a kind of suitable electrode material, not only in fuel cells [2], supercapacitors [3, 4], and photovoltaic cells [5], but also in the field of electrochemical sensors [6, 7]. Furthermore, GQDs with tunable sizes, *ie.*, 2.2 ± 0.3 , 2.6 ± 0.2 , and 3 ± 0.3 nm, can act as multivalent redox species using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements, and present exciting opportunities for building electrochemical sensors [8]. More recently, GQDs have been used in the area of bioanalysis [6-9]. However, their applications in the analytical field have not been explored until now. Due to their unique properties, sensors based on GQDs can achieve a high level of performance.

GQDs increase the contact area with the analyte, so they could increase the electrochemical active surface to interact with some electroactive analytes [10]. Since the increase in geometric surface area is very important parameter in electrochemistry, therefore modification of different substrates (such as glass, carbon, graphite etc.) by GQDs can increase the rate of electrochemical reaction.

Besides the applications of GQDs, integration of nanoparticles (MNPs) into GQDs has recently become a hot topic of research due to their new and/or enhanced functionalities that cannot be achieved by either component alone, and therefore holds great promise for a wide variety of applications in catalysis, optoelectronic materials, and so on [11-14]. Magnetic nanoparticles (MNPs) especially iron oxide (either Fe_3O_4 or Fe_2O_3), and its nanocomposite with GQDs, were applied in enhanced optical limiting, MRI (magnetic resonance imaging), drug delivery, energy storage and removal of contaminants from wastewater [15-17]. Although a number of researchers have proposed methods for the preparation of magnetic GQDs finding applications in various fields, none of them have managed to apply these hybrids for electrochemical sensing or biosensing purposes. As far as we know, little or no attention has been paid to the use of magnetic GQDs for electrochemical sensing. Herein, for the first time, we report an electrochemical preparation of Fe_3O_4 MNPs-GQDs film and its interface for the recognition of some amino acids.

As a laboratory technique, the analysis of amino acid plays an important role in biochemical, pharmaceutical and biomedical fields. Also, amino acids exist in a free form or bound in peptides, proteins, or non-peptide bonded polymers [17]. Naturally occurring L-amino acids are required for protein synthesis and are precursors for essential molecules, such as co-enzymes and nucleic acids. Non-protein amino acids may also occur in animal tissues as metabolic intermediates or have other important functions [18-39]. Mostly, the methods were based on the technology developed by Moore and Stein, which includes post-column derivatization and detection in the visible region on an amino acid analyzer. These analyses are reliable, but costly and time-consuming. Also, although these methods have the advantages of sensitivity and accuracy, their complicated operation, limit their extensive application. There is, therefore, a need for the rapid and accurate determination of amino acids. The electrochemical methods, with respect to their sensitivity, accuracy, simplicity and ease of on-site determination, have received considerable attention for the analysis of amino acids [40-49]. The oxidation and adsorption behaviors of amino acids on electrode surfaces are relevant to the interfacial behaviors of

biological molecules and also to the medical and industrial problems associated with the adsorption of the amino acids on the surfaces [50-52]. But, the use of bare, unmodified electrodes as electrochemical sensors for amino acids had a number of limitations. Low sensitivity and reproducibility, slow electron transfer reactions, low stability over a wide range of solution compositions and high over potential at which the electron transfer process occurs were the limitations identified.

Considering that the electrochemical method possesses unique advantages for precise control over the redox reactions, we therefore hypothesize that, electrochemical deposition of Fe_3O_4 MNP-GQDs under controllable conditions may provide an alternative approach for tailoring the surface properties of thin films. On the other hand, these improved performances encouraged us to explore the possible leading role played by the presence of Fe_3O_4 MNP on the structure of GQDs. Therefore, in the present study, the magnetic graphene quantum dot (Fe_3O_4 MNPs-GQDs) was synthesized successfully and characterized by using Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and atomic force microscopy (AFM). For the first time, as-synthesized GQDs and Fe_3O_4 MNPs-GQDs was electrodeposited on GCE by cyclic voltammetry (CV) in the potential range from -1.0 to 1.0 V and the prepared films were used for detection of amino acid at physiological pH. Herein, we explore the electrocatalytical activity of Fe_3O_4 MNPs-GQDs. We have illustrated that the as-obtained Fe_3O_4 MNPs-GQDs exhibited a much higher electroactivity individual GQDs and Fe_3O_4 MNPs for the electrooxidation and detection of amino acid which was about 10 fold higher than for GQDs. More importantly, a substantial (+0.21 V) decrease in the overvoltage of the amino acid oxidation reaction (compared to ordinary electrodes) was observed using Fe_3O_4 MNPs-GQDs - GCE.

2. Experimental details

2.1. Chemicals and reagents

All chemicals were purchased from Merck (Darmstadt, Germany) and used without further purification. Alumina slurry was purchased from Beuhler (Illinois, USA) and raw material of amino acids was purchased from Merck (Germany). All solutions were prepared with deionized water. The stock solution of Amino acid (0.003 g/ mL) was prepared by dissolving an accurate amount of selected amino acids in an appropriate volume of 0.1 M phosphate buffer solution (PBS), pH=7.4 (which was also used as supporting electrolyte), and then stored in the dark place at 4°C. Additional dilute solutions were prepared daily by accurate dilution just before use. Also the other stock solutions were prepared by dissolving an accurate amount equal to molecular weight of each one in an appropriate volume of 1000 mL deionized water and then all stored in the dark place at 4 °C.

2.2. Apparatuses and methods

Electrochemical measurements were carried out in a three-electrode cell setup. The system was run on a Personal Computer using NOVA1.7 software. Saturated Ag/AgCl as a reference electrode and the counter electrode (also known as auxiliary electrode), which usually made of an inert material was platinum. All potentials were measured with respect to the Ag/AgCl which

was positioned as close to the working electrode as possible by means of a luggin capillary. Glassy carbon electrode (GCE) (from Azar electrode Co., Urmia, Iran) was used as the working electrode. The transmission electron microscope (TEM) images were obtained on Leo 906, Zeiss, (Germany). Atomic force microscopy (AFM) experiments were performed at contact mode by Nanowizard AFM (JPK Instruments AG, Berlin, Germany) mounted on Olympus Invert Microscope IX81 (Olympus Co., Tokyo, Japan).

2.3. Synthesis GQDs and Fe₃O₄ MNPs-GQDs

An easy bottom-up method was used for the preparation of GQDs. At first, GQDs were synthesized by pyrolyzing citric acid and dispersing the carbonized products into alkaline solutions. Briefly, 2 g of citric acid was put into a beaker and heated to 200 °C by a heating mantle until the citric acid changed to an orange liquid. Then, for preparing GQDs, 100 mL of 10 mg/mL NaOH solution was added into the orange homogenous liquid dropwise with continuous stirring. The obtained GQD solution was stable for at least one month at 4 °C. Then, The Fe₃O₄ MNPs-GQDs composites were synthesized through a one-step co-precipitation procedure. First, GQDs (0.1 g) was dispersed in 150 mL deionized water by sonication for 10 min. Then, 1.214 g FeCl₃.6H₂O was added to GQDs solution at room temperature under a nitrogen flow with vigorous stirring. Then, temperature was increased to 80 °C, and 0.485 g of the FeCl₂.4H₂O was added slowly to the solution containing Fe³⁺/GQDs, which was vigorously stirred for an additional 30 min. Finally, the ammonia solution was added dropwise to adjust the pH of the solution to 10 for the synthesis of magnetite Fe₃O₄ MNPs-GQDs. (See Scheme 1)

2.4. Characterization of GQDs and Fe₃O₄ MNPs-GQDs

Fig. 1(A and B) presents the AFM and TEM images of synthesized GQDs. The corresponding AFM image shows a single GQD monolayer thin film. Ninety percent of the particles represent dark brown color which assigned to a size range below 10 nm. Also, the morphologies of the Fe₃O₄ MNPs-GQDs used in this work were characterized using AFM and TEM and the images are depicted in Fig. 1D and E. From these images it's found that, differing from the two dimensional layered structures of GQDs, the Fe₃O₄ MMNPs and GQDs aggregated into three dimensional structures. Most probably, as schematically depicted in Fig. 1D-F, the Fe₃O₄ MNPs are surrounded by GQDs to form composite particles. The sizes of the Fe₃O₄ MNPs-GQDs particles reached 30 nm.

In addition, the surface chemistry of Fe₃O₄ MNPs-GQDs was investigated using FTIR. The typical FT-IR spectra of magnetic nanoparticles are shown in Fig. 2. As can be seen the Fe–O band at Fe₃O₄ MNPs-GQDs (611 cm⁻¹) shifted to a higher wavelength in comparison with Fe₃O₄ (580 cm⁻¹), indicating the bonding of Fe₃O₄ to C–O–H groups on the GQDs surface. An absorption bond appeared at 3411 cm⁻¹ corresponding to hydroxyl groups on Fe₃O₄ and the Fe₃O₄ MNPs-GQDs surface and a peak at 1618 cm⁻¹ corresponded to the vibration of water molecules adsorbed on Fe₃O₄ and Fe₃O₄ MNPs-GQDs surfaces. A strong bond at 1605 cm⁻¹ corresponded to the stretching frequencies of C–C on the Fe₃O₄ MNPs-GQDs surface. Peaks at 908 cm⁻¹ and 1065 cm⁻¹ can be attributed to the stretching frequencies of C–C on Fe₃O₄ MNPs-GQDs and the peaks at 1258 cm⁻¹ and 1384 cm⁻¹ corresponded to the C–O stretching and O–H bending vibrations.

2.5. Preparation of GQDs and Fe₃O₄ MNPs-GQDs modified GCE (GQDs-GCE and Fe₃O₄ MNPs-GQDs-GCE)

GCE (2 mm in diameter) was polished to a mirror-like finish with 0.3 and 0.05 μm alumina slurry and then thoroughly rinsed with double distilled water. Then it was successively sonicated in acetone and double distilled water and was allowed to dry at room temperature. Finally, 5 mL homogenous GQD and Mag-GQD films were electrodeposited onto GCE by cyclic voltammetry (CV) in the potential range from -1.0 to 1.0 V at a scan rate of 200 mV s^{-1} for 30 cycles (Fig. 3). When the cyclic potential scan reached 30 cycles, the peak currents hardly changed. The first positive scan, an oxidation peak (I) at + 0.51 V was observed, which is attributed to the oxidation of the Fe₃O₄ MNPs-GQDs. In the subsequent reversal scan, one reduction peaks (II) were observed at - 0.7 V, which may be attributed to the reduction of species. In addition, it's found that, peak I was decreased cycle by cycle, and reflecting the continuous growth of the Fe₃O₄ MNPs-GQDs on the surface of GCE. These facts indicate that Fe₃O₄ MNPs-GQDs was successfully deposited on the surface of GCE by the electrochemical method.

After electrochemical deposition of Fe₃O₄ MNPs-GQDs on the GCE, the Fe₃O₄ MNPs-GQDs - GCE was employed for taking SEM images for monitoring the distribution of Fe₃O₄ MNPs and morphology of the engineered electrode surface. The SEM images of the engineered electrode surface have been shown in Fig. 4 at different magnitudes. This Figure shows that magnetic nanoparticles were distributed into GQDs and confirmed attachment of Fe₃O₄ MNPs to the GQDs. Meanwhile the figure shows that some of Fe₃O₄ MNPs have been aggregated. The overall size of Fe₃O₄ MNPs was found to be less than 100 nm. The Fe₃O₄ MNPs were well dispersed on the electrode surface. The effective surface area was 0.098, 0.12 and 0.289 cm^2 for GCE, GQDs-GCE, and Fe₃O₄ MNPs-GQDs-GCE, respectively.

3. Results and discussion

3.1. Electrochemical behavior

Following a qualitative assessment prepared Fe₃O₄ MNPs-GQDs-GCE with GQDS-GCE; the electrochemical behavior of these sensors was investigated in further detail. The cyclic voltammograms of GCE, GQDs-GCE and Fe₃O₄ MNPs-GQDs-GCE were recorded between -1.0 and 1.0 V using the scan rate of 100 mVs^{-1} in the 0.1 M PBS (pH=7.4) and selected amino acids (L-Cysteine, L-Tyrosine, L-Glycine, and L-Phenylalanine). Fig. 5 and 6 compares the cyclic voltammetric response of the Fe₃O₄ MNPs-GQDs-GCE with GQDs-GCE. The Fe₃O₄ MNPs-GQDs-GCE display well fined oxidation peaks for all four analytes.

As can be seen in Fig. 5A-D, on the bare GCE electrode no redox behavior was observed. On the other hand, as well as on the GQDs-GCE (Fig. 5, curve a) and Fe₃O₄ MNPs-GQDs-GCE (Fig. 6, curve b) one pair redox peaks was appeared at 0.13V vs. Ag/AgCl. The comparison of recorded CVs using GQDs-GCE and Fe₃O₄ MNPs-GQDs-GCE in the presence of selected L-Cysteine, L-Tyrosine, L-Aspartic Acid, and L-Phenylalanine (Fig. 6, curve b) shows a new anodic peak at 0.628, 0.638, 0.672, 0.445 V vs. Ag/AgCl which attribute to the anodic oxidation of L-Cysteine,

L-Tyrosine, L-Aspartic Acid, and L-Phenylalanine, respectively. This observation is also attributed to the high conductivity and inherent ability of Fe₃O₄ MNPs-GQDs. From the above two figures, it can be concluded that without catalytically agent (Fe₃O₄ MNPs), bare GCE or GQDs-GCE cannot result in the successful electrooxidation of selected amino acid. When Fe₃O₄ MNPs containing catalyst agent is used to modify GQD, the differences in peak currents are obviously enlarged, suggesting that Fe₃O₄ MNPs-GQDs can provide an acceleration center to electron shuttling [40, 42, and 47]. These results show the anodic peak currents at the surface of Fe₃O₄ MNPs-GQDs-GCE are significantly enhanced. These results indicate that Fe₃O₄ MNPs-GQDs film could accelerate the rate of electron transfer of selected amino acids and have good electrocatalytic activity for redox reaction of L-Cysteine, L-Tyrosine, L-Aspartic Acid, and L-Phenylalanine [40-49]. Therefore, Fe₃O₄ MNPs-GQDs is a suitable mediator to shuttle electron between amino acid and working electrode, and facilitate electrochemical regeneration following electron exchange with amino acid. That might be related to the excellent properties of Fe₃O₄ MNPs-GQDs such as high specific surface area and electrical conductivity [40, 42, and 47]. Also, the recognition efficiency is further improved at the Fe₃O₄ MNPs-GQDs-GCE with a peak current ratio of 4.1 (Fig. 3D), it is no doubt that Fe₃O₄ MNPs play important roles in the improvement of catalytically activity of GQDs towards electrooxidation of amino acid. As a decisive catalytical component in the composite film, Fe₃O₄ MNPs provides a catalytically platform for selective detection of amino acid meanwhile, GQDs can amplify the electrochemical signals produced during the electrochemical sensing process. In one word, the synergetic effect of GQDs and Fe₃O₄ MNPs leads to a successful and effective detection of amino acid.

So, Fe₃O₄ MNPs-GQDs could be substituted for other electrode modifiers. In addition, in the presence of Amino acid, it was observed that the anodic current and the associated anodic charge increased dramatically, whereas the cathodic current and the corresponding charge decreased. In the presence of Amino acid, the I_{pa}/I_{pc} ratio was 78.0; in the absence of Amino acid, this ratio was decreased to 28.0. This result indicates that amino acid is oxidized by active Fe(III) moiety through a cyclic mediation redox process. Iron species are immobilized on the electrode surface, and the one with a higher valence oxidizes to Fe(III) *via* a chemical reaction followed by the generation of low valence iron (Fe(II) species). Along this line, the high-valence oxide is regenerated through the external electrical circuit. Accordingly, Amino acid is oxidized *via* an EC' mechanism. Finally, we noticed that the peak current values exhibited a linear dependence on the concentration of amino acid, suggesting the utility of the modified electrodes for analytical applications. Thus, it is likely that the electrogenerated Fe(III) species is the active moiety that efficiently speeds up the oxidation of Amino acid. Any increase in the concentration of Amino acid causes a proportional and nearly linear enhancement of the anodic wave.

The observed electrochemical behavior for Fe₃O₄ MNPs-GQDs -GCE in PBS (pH=7.4) could be attributed to the iron phosphate redox system. At first, Fe₃O₄ MNPs directly reduced at the electrode surface according to the following reaction:

Then, Fe²⁺ ions produced could combine with phosphate ions in the buffer solution:

Here after, the solid FePO_4 at the surface of electrode could be responsible for observed redox behavior. Therefore, the electrocatalytic activity of Fe_3O_4 MNPs-GQDs can be ascribed to the combination of the electron-acceptor ability of Fe_3O_4 MNPs and confinement effects due to the attachment to the GQDs matrix. On the basis of the previous considerations, the electrocatalytic activity of Fe_3O_4 MNPs-GQDs must result from the superposition of the effect associated with more external MNPs units and that due to boundary-associated ones. On the other hand, since the catalytic effect associated with Fe_3O_4 MNPs consists essentially of an enhancement of the anodic peak for amino acid oxidation, the catalytic effect can tentatively be associated with a redox reaction in solution phase between Fe_3O_4 and any intermediate species resulting from the initial electron transfer of amino acid.

It is important to point out that, we have a negative approach in CVs of other amino acids (L-Serine, L- Arginine, L-valine, and L-Glycine) which were recorded and no response was observed which indicated no response of proposed sensor (Fe_3O_4 MNPs-GQDs-GCE) towards detection of these amino acids (See Fig. 7).

Scan rate is one of parameters significantly affecting electrooxidation of various compounds. Also, useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behaviors of selected amino acids at different scan rates were investigated on the surface of Fe_3O_4 MNPs-GQDs-GCE by CV. Fig. 8A presents typical CV of a Fe_3O_4 MNPs-GQDs-GCE in 0.1 M PBS (pH=7.4) at various potential sweep rates from 2-1000 mV s^{-1} . The peak's currents are proportional to sweep rates in the range of 20-500 mV s^{-1} , as shown in Fig. 7B, pointing to the electrochemical activity of the surface redox couple. From the slope of this lines and using:

$$I_p = \left(\frac{n^2 F^2}{4RT} \right) \nu A \Gamma^* \quad (1)$$

where Γ^* is the surface coverage of the redox species and ν being the potential sweep rate [53,54] and taking average of both cathodic and anodic results, Γ^* values of around $8.5 \times 10^{-10} \text{ mol cm}^{-2}$ have been derived.

Fig. 9 illustrates cyclic voltammograms of L-Cys, L-Tyr, L-Asp, and L-Phe using the Fe_3O_4 MNPs-GQDs-GCE recorded at different potential sweep rates. According to Fig. 9, Fig. 10 A and B indicates anodic peak currents increased linearly with the square root of the potential sweep rate (50-500 mVs^{-1}) for L-Cys, which indicates a mass transfer-controlling process of oxidation via diffusion. In addition the value of the electron-transfer coefficient for the reaction can be obtained from the following equation: [55]

$$E_p = \left(\frac{RT}{2\alpha F} \right) \ln \nu + \text{constant} \quad (2)$$

This is valid for a totally irreversible diffusion-controlled process. Using the dependency of anodic peak potential on the Neperian logarithm of the potential sweep rate (Fig. 10C), the value of the electron-transfer coefficient was obtained as 0.6. On the basis of the slopes of the linear dependency of the anodic peak currents on the square root of the potential sweep rates, and the Randles-Sevcik equation: [56]

$$I = (2.99 \times 10^5) \alpha^{1/2} n^{3/2} A C^* D^{1/2} \nu^{1/2} \quad (3)$$

where I_p is the peak current, A is the electrode surface area, D is the diffusion coefficient, and C^* is the bulk concentration of L-Cys, the diffusion coefficient (D) for L-Cys was calculated to be $0.34 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. Also, plotting the current function (peak current divided by the square root of the potential sweep rate) against the square root of the potential sweep rate revealed negative slope confirming the electrocatalytic nature of the process. These results indicated that prepared film could accelerate the rate of electron transfer of L-Cys and have good electrocatalytic activity for redox reaction of L-Cys. Similar results were obtained for L-Tyr, L-Asp, and L-Phe which the results were summarized on Table 1. Also oxidation mechanisms for the studied amino acids are presented in scheme 2.

For further confirmation, chronoamperometry was used for investigation the electrochemical behavior of selected amino acids using Fe_3O_4 MNPs-GQDs (Fig. 11). Single steps chronoamperograms were recorded by setting the working electrode potentials to desired values and were used to measure the catalytic rate constant on the modified surface over a concentration range of 0.05-0.1 M. The plot of net current versus $t^{-1/2}$ which has been obtained by removing the background current by the point-by-point subtraction method gives a straight line. This indicates that the transient current must be controlled by a diffusion process. By using the slopes of these lines; we can obtain the diffusion coefficients of the selected amino acids according to the Cottrell equation: [57]

$$I = nFAD^{1/2} C^* \pi^{-1/2} t^{-1/2} \quad (4)$$

where D is the diffusion coefficient, and C^* is the bulk concentration. The mean value of the diffusion coefficients for L-Tyr, L-Asp, and L-Phe were cited on the Table 1

3.2. Analytical application

The calibration curve for amino acids in PBS (pH=7.4) was obtained by DPV. Fig. 12 shows typical DPV curves for different concentrations of L-Cys, L-Tyr, L-Asp, and L-Phe in PBS using Fe_3O_4 MNPs-GQDs-GCE. The dependency between peak current and concentration was rectilinear for selected amino acids. The lower limits of quantitation (LLOQ) were found to be in sub-micromolar for oxidation peaks of selected amino acids. The results were summarized in Table 1.

3.3. Reproducibility and stability

Another advantage of the proposed modified electrode was that the resulting modified electrode showed good long term stability. Stability of the proposed electrode was tested by measuring the decrease in voltammetric current during repetitive DPV measurements of amino acids with Fe_3O_4 MNPs-GQDs-GCE stored in solution or air. For example, this electrode, within 24 h, is used for the determination of 1 mM amino acids in buffer solutions. Obtained results show that this electrode has remarkable stability without significant change in the voltammetric currents. When the electrode was stored in the atmosphere, the current response remained almost unchanged for about 20 days. RSD of repeated peak currents was (6.1 %). The high stability of

the Fe₃O₄ MNPs-GQDs-GCE could be related to the strong affinity of Fe₃O₄ MNPs for GQDs and the low solubility of the Fe₃O₄ MNPs-GQDs-GCE in water. To check the inter-electrode reproducibility of the modified electrode, four modified electrodes were tested simultaneously by recording DPVs in buffer solution, containing 1 mM of amino acids. The relative standard deviation was 4.82%. Therefore, 1 mM imparts a suitable stability onto DPVs measurements of amino acids.

4. Conclusions

In summary, Fe₃O₄ MNPs-GQDs was electrodeposited on GCE by cyclic voltammetry (CV) in the potential range from -1.0 to 1.0 V and used for detection of some amino acid at physiological pH. The Fe₃O₄ MNPs-GQDs-GCE showed better performance for the electrochemical oxidation of amino acid, when compared with bare GCE and GQD-GCE. More importantly, the proposed nano-sensor was used for determination of selected amino acids at physiological pH, without the necessity for sample pre-treatment or any time-consuming extraction or evaporation steps prior to the analysis, with satisfactory analytical results. The analytical performance of modified electrode indicates that it can be used as sensitive amperometric detector for detection of amino acids when coupled with flow system. Also due to remarkable electrocatalytic properties of Fe₃O₄ MNPs-GQDs-GCE, prepared sensor can be used for determination of some other amino acids which are necessary for interference study.

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Table 1: Kinetic and analytical parameters of selected amino acids oxidation using Fe₃O₄ MNPs-GQDs-GCE

Kinetic and analytical parameters	L-Cys	L-Tyr	L-Asp	L-Phe
electron-transfer coefficient (α)	0.60	0.55	0.32	0.61
diffusion coefficient (D)/ cm ² s ⁻¹ using CV	0.34×10 ⁻⁵	0.67×10 ⁻⁵	0.12×10 ⁻⁵	0.49×10 ⁻⁵
diffusion coefficient (D)/ cm ² s ⁻¹ using CHA	0.28×10 ⁻⁵	0.44×10 ⁻⁵	0.90×10 ⁻⁵	0.11×10 ⁻⁵
Linear rang/ μ M	0.01-100	0.09-230	1-50	0.5-650
LLOQ/ μ M	0.01	0.09	1	0.5

Figure Captions:

Fig. 1: TEM, AFM images, and schematic representations structure of the GQDs (A, B, C) and Fe₃O₄ MNPs-GQDs (D, E, F), respectively.

Fig. 2: FT-IR spectra of Fe₃O₄ MNPs and Fe₃O₄ MNPs-GQDs

Fig. 3: CVs for 5 mL of Fe₃O₄ MNP-GQDs using a GC electrode scanned continuously at 200 mVs⁻¹ between -1 to +1 V. Number of scan is 30.

Fig. 4: SEM images of Fe₃O₄ MNP-GQDs modified GC electrode.

Fig. 5: CVs of GCE in the presence of 5 ml PBS (0.1M, pH=7.4) + (0.004 g/ml) of amino acids. Scan rate is 100 mVs⁻¹.

Fig. 6: CVs of GQD-GCE (curve a) and Fe₃O₄ MNPs-GQDs-GCE (curve b) in the presence of 5 ml PBS (0.1M, pH=7.4) + (0.004 g/ml) of amino acids. Scan rate is 100 mVs⁻¹.

Fig. 7: CVs of Fe₃O₄ MNPs-GQDs-GCE in the presence of 5 ml PBS (0.1M, pH=7.4) + (0.004 g/ml) of other amino acids (L-Val, L-Ser, L-Arg, and L.Gly). Scan rate is 100 mVs⁻¹.

Fig. 8: **A)** CVs of a Fe₃O₄ MNPs-GQDs-GCE in 0.1M PBS in various potential sweep rates (from inner to outer): 2, 5, 10, 20, 30, 40, 50, 60, 70, 90, 100, 150, 200, 250, 300, 350, 400, 450, 50, 600, 700, 800, 900, and 1000 mVs⁻¹, respectively. **B)** Dependence of anodic/cathodic peak currents during the forward and reverses sweep vs. potential sweep rate.

Fig. 9: **A)** CVs of a Fe₃O₄ MNPs-GQDs-GCE in the presence of 0.004 g/ml of **[A)** L-Cys, **B)** L-Tyr **C)** L-Asp, and **D)** L-phe]+0.1M PBS (pH=7.4) in various potential sweep rates (from inner to outer): 2, 5, 10, 20, 30, 40, 50, 60, 70, 90, 100, 150, 200, 250, 300, 350, 400, 450, 50, 600, 700, 800, 900, and 1000 mV s⁻¹, respectively.

Fig. 10: **A)** Dependence of anodic/cathodic peak currents of 0.005 g of L-Cys during the forward and reverses sweep vs. potential sweep rate. **B)** Dependence of anodic/cathodic peak currents of 0.005 g of L-Cys during the square roots of forward and reverses sweep vs. potential sweep rate. **C)** Dependence of the peak potential on $\ln v$ for the oxidation of L-Cys at Fe₃O₄ MNPs-GQDs-GCE. **D)** Current function vs. $v^{0.5}$ for 0.1M PBS containing 0.005 g of L-Cys.

Fig. 11: Single step chronoamperograms of the Fe₃O₄ MNPs-GQDs-GCE in different concentrations of **[A)** L-Cys, **B)** L-Tyr **C)** L-Asp, and **D)** L-phe]: (0.1, 0.2, 0.25, 0.3, 0.33, 0.5, 0.66, 1, 1.33, 2, and 4 mM), respectively. Supporting electrolyte: 0.1 M; PBS (pH = 7.4); scan rate: 20 mV s⁻¹. Potential step for L-Cys, L-Tyr, L-Asp, and L-Phe were 0.61, 0.68, 0.14, and 0.19V, respectively. **B)** Dependency of transient current on $t^{-0.5}$.

Fig. 12: DPVs obtained for determination of **[A)** L-Cys, **B)** L-Tyr **C)** L-Asp, and **D)** L-phe] in 0.1M PBS, , respectively. **A)** L-Cys concentrations are as: 0.06, 0.07, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18, and 0.2. **B)** L-Tyr concentrations are as: 0.01, 0.02, 0.03, 0.04, 0.05, 0.07, 0.08, 0.09, and 0.1. **C)** L-Asp concentrations are as: 0.2, 0.4, 0.7, 1, 5, 10, 20, 50, and 70. **D)** L-Phe concentrations are as: 0.2, 0.4, 0.5, 0.8, 1, 2, 5, and 10.

Scheme 1: Schematic setup for the synthesis of GQD and Fe₃O₄ MNPs-GQDs

Scheme 2: A provisional mechanism for the electrooxidation of **[A)** L-Cys, **B)** L-Tyr, **C)** L-Asp, and **D)** L-phe] on Fe₃O₄ MNPs-GQDs-GCE.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

I/A
 $\uparrow$
 t/s

Fig. 11

Fig. 12

I/A
 $\uparrow$
 E/V

Scheme 1

Scheme 2