Research Article

# Application of carboxylic acid-functionalized of graphene oxide for electrochemical simultaneous determination of tryptophan and tyrosine in milk

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## Abstract

In this work, a new method for the simultaneous determination of tryptophan (Trp) and tyrosine (Tyr) in milk has been reported. Trp and Tyr are essential amino acids in human nutrition, but their electrochemical signal overlapped. For solve this problem, we developed modified Screen Printed Carbon Electrode (SPCE) with Graphene Oxide–COOH/Chitosan (GO–COOH/Chitosan) electro-deposition. The morphology and electrochemical performance of modified electrode were characterized by scanning electron microscopy, energy dispersive spectrometry, FT-IR, electrochemical imped-ance spectroscopy and cyclic voltammetry. The electrochemical simultaneous determination of Trp and Tyr has been investigated by using differential pulse voltammetry. The carboxylic acid functionalized GO modified SPCE was utilized to catalyze the oxidation of Trp and Tyr. Compared with SPCE/GO/Chitosan and SPCE/Chitosan sensor, the new sensor has enhanced sensitivity, low detection limit and high selectivity. In addition, the SPCE/GO–COOH–Chitosan sensor enhanced separation of the oxidation peak of Tyr and Trp and showed a remarkable increase in peak current for electro-active compounds, thus, it can be used for simultaneous voltammetric determination. Under the optimized experimental conditions, a linear correlation between oxidation peak current and concentration of Tyr and Trp in the ranges 0.1–60 μM and 0.4–40 μM with a detection limit of 0.05 μM (S/N = 3) and 0.1 μM (S/N = 3) were achieved, respectively. Finally, the proposed electrochemical sensor was applied for quantification of Tyr and Trp in milk samples, where standard solutions were spiked to the milk samples and recoveries were obtained.
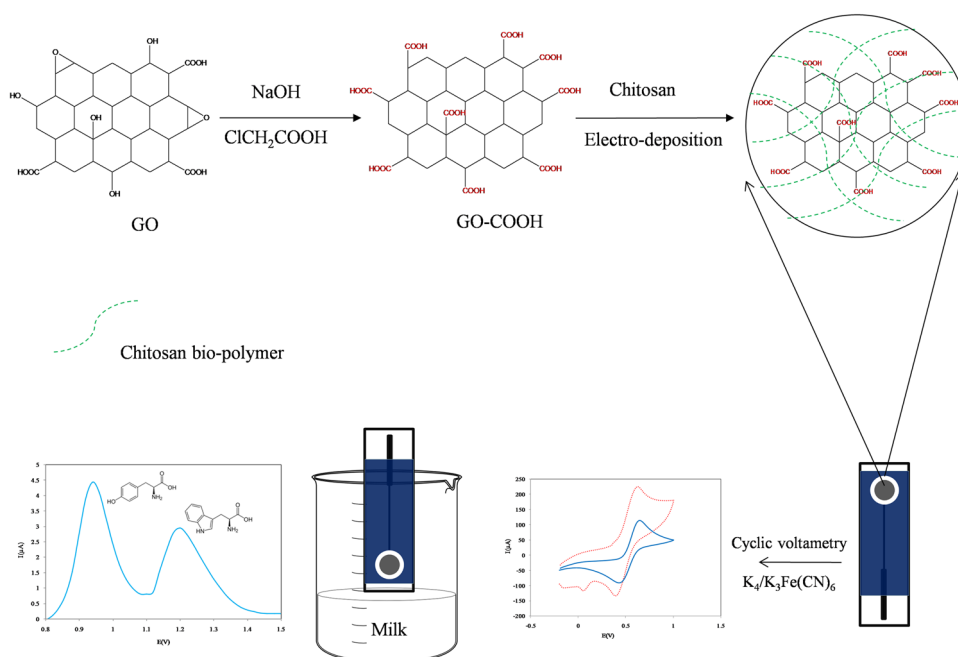
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**Graphic abstract**

# 1 Introduction

Trp has to exist in human nutrition owing to be an essential amino acid and keeping the nitrogen level balanced in human beings [1]. However, the high intake or improper metabolism of Trp can result in waste products in the brain causing hallucinations, delusions, and schizophrenia [2]. Therefore, developing a convenient, rapid, and inexpensive method with high selectivity and sensitivity for the determination of Trp would be desirable in a variety of fields such as food processing, biochemistry, pharmaceutical industry, and clinical analysis [3, 4]. In contrast, Tyr is synthesizable in the body from another amino acid, phenylalanine. Tyr is a constituent of many proteins in the body and is a precursor to many neurotransmitters, including, serotonin, dopamine, norepinephrine and epinephrine [5]. Because of the coexisting of these two amino acids in the pharmaceutical industry, food processing, and biological samples, strategies have been developed for selective determination of them in the presence of one another. More importantly, with the aim of maintaining a healthy lifestyle, analytical methods have been applied in determining the amino acids in foodstuffs. In particular, there have been some reports on the determination of Trp and Tyr in milk [6–8].

There are several reported methods for the determination of amino acids including, chromatographic methods [9], gas chromatography-mass spectrometry [10], chemiluminescence [11], capillary electrophoresis [12], LC–MS/MS [13] and electrochemical methods [14–17]. Among these, electrochemical techniques have attracted considerable attention for the determination of amino acids due to its high sensitivity, precision, ease of operation, low costs and short analysis times. However, the electrochemical response is not pleasing because there is a heterogeneous and slow transferring of electron at the electrode surface and consequently, high over potentials of the electrochemical oxidation [18, 19]. Chemical modification of electrodes with suitable materials could solve these problems. Commonly, many nano structure materials such as polymer a nanocomposite [20–25], metal nanoparticles [26–28], and graphene nanocomposite [15, 29–35] have been introduced to enhance the electrode performance.

Graphene has aroused an explosive interest in recent decades due to its high stability [36], flexible structure, low manufacturing cost and large surface area [37]. Particularly, graphene has turned to an ideal electrochemical platform [38] because it fits electrically supreme conductivity [39] (550 S cm$^{-1}$), and a substantial amount of electrochemically desired edge carbons per mass of

graphene to facilitate a low over potential transfer of electron between molecules to an electrode [41, 42]. Chemical functionalization of GO is of a major concern in order to improve the GO properties while preserving the chemical structure of carbon framework [32, 43]. The GO analytical performance can be enhanced by controlled immobilization of various electro-active materials on existing active sites for developing electrochemical sensors and biosensors [44–47]. One of the most common functionalization is utilizing nanoparticles as a promising way to make nanoscale composite electrodes [21, 48]. By enhancing properties of these host material, they are applicable in catalysis, opto- electronic materials, surface enhanced Raman Scattering, and biomedical fields [27, 31, 49, 50]. It is worth noting that the modification of GO with carboxyl groups enhances both selectivity and sensitivity for electrochemical determination of Trp and Tyr.

Recently, the electrodeposition technique has received much attention due to their wide use in the manufacture of sensors and biosensors. Chitosan is one of the most widely used materials for electrodeposition. Owing to its pH-responsive deposition properties, chitosan can be electrodeposited as a nanocomposite film on the cathode with other substance through consummation of $H^+$ at the surface of cathode [51].

Herein, we have designed a novel electrochemical approach for simultaneous determination of Trp and Tyr in milk via applying electrodeposition of GO–COOH/Chitosan nanocomposite on SPCE. For this purpose, GO was functionalized with carboxylic groups to increase selectivity and sensitivity of the resultant electrode. This modification allows the electrochemical reaction to occur at a lower potential range.

## 2 Experimental

### 2.1 Material and reagent

Graphite powder, potassium permanganate ($KMnO_4$), hydrochloric acid (HCl), Chitosan,$H_2SO_4$, Tyrosine and Tryptophan were purchased from Sigma-Aldrich.

### 2.2 Apparatus

The electrochemical measurements were carried out on potentiostat/galvanostat (Autolab PGSTAT-204NGPES and FRA software, Eco-Chemie, Utrecht, Netherlands) in a cell equipped with a three-electrode system contained a working Screen printed electrode (SCEs), a platinum wire as a counter electrode and a Ag/AgCl (3 M) as a reference electrode.

### 2.3 Nanocomposite synthesis

GO was obtained by using modified Hummers method from pure graphite powder [52, 53]. To be precise, at first, graphite powder (1.0 g) was poured into 23 mL concentrated $H_2SO_4$ and stirred for several minutes. The temperature was kept below 5 °C by ice bath. Then, 3.0 g of potassium permanganate ($KMnO_4$) was slowly added into the mixture under continuous stirring until the solution became dark green. Then, 184 mL ultra-pure water was gradually added and stirred at 35 °C for 2 h. In the next step, the mixture is kept in a reflux system at 95 °C for 15 min. Finally, the reaction was completed by addition of 30 mL $H_2O_2$ and 140 mL distilled water by which color changes to bright yellow. The solid product was washed with 10% HCl and deionized (DI) water several times. The washed GO was dried in a vacuum oven at 60 °C for 48 h to obtain the powder of GO. Following that, for carboxylation of GO, 200 mL of GO (1 mg/mL) was dissolved in purified water under bath sonicated for 1 h. In the following step 50 g NaOH was added to the mixture solution and stirred. Then, to change the OH group with COOH at surface of GO nanocomposite, chloroacetic acid was added to the GO suspension and then sonicated for 3 h. The last product (GO–COOH) was collected and washed three times with ultrapure water and then dried in an oven at 60 °C. For sensor preparation, the Surface of SPCEs was modified by nanocomposite. Firstly, nanocomposite was prepared by mixing of GO–COOH (2 mg/mL) and Chitosan (0.2% w/w) and sonicated for 30 min and stirring for 2 h. Then, the modified electrode was prepared by choronoamprometry electrode position of the nanocomposite on the SPCE surface at constant potentials − 1.5 V and different electrode position times (10–200 s) [42].

### 2.4 Electrochemical measurement

The electrocatalytic properties of modified electrode were investigated by using cyclic voltammetry (CV) and Electrochemical Impedance spectroscopy (EIS). Simultaneous determination of L-tryptophan and L-tyrosine were performed by differential pulse voltammetry (DPV).

## 3 Results and discussion

### 3.1 Surface characterization of the modified screen printed electrode

Prior to the electrochemistry determination of amino acids, characterization of nanocomposite and modified electrode can be carried out by FT-IR, FE-SEM, CV and EIS.
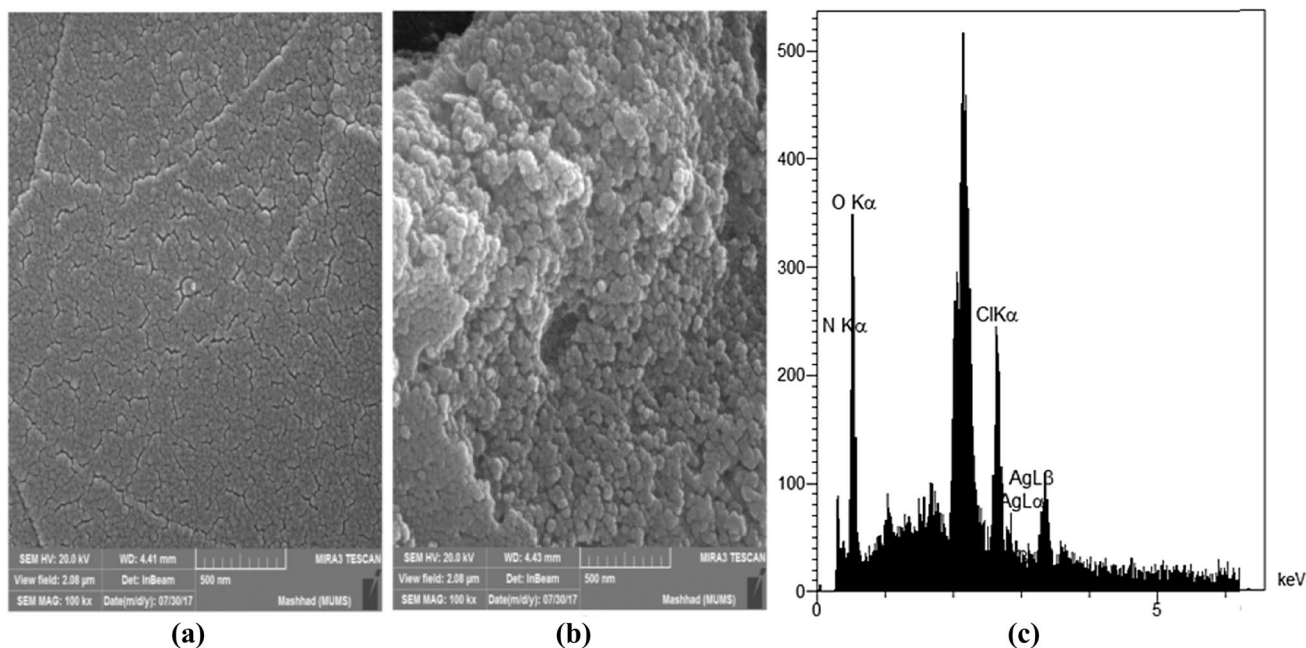
**Fig. 1  a** FE-SEM images of Bare SPCEs, **b** FE-SEM images of SPCEs/GOX–COOH/Chitosan and **c** EDS spectrum of SPCEs/GOX–COOH/Chitosan
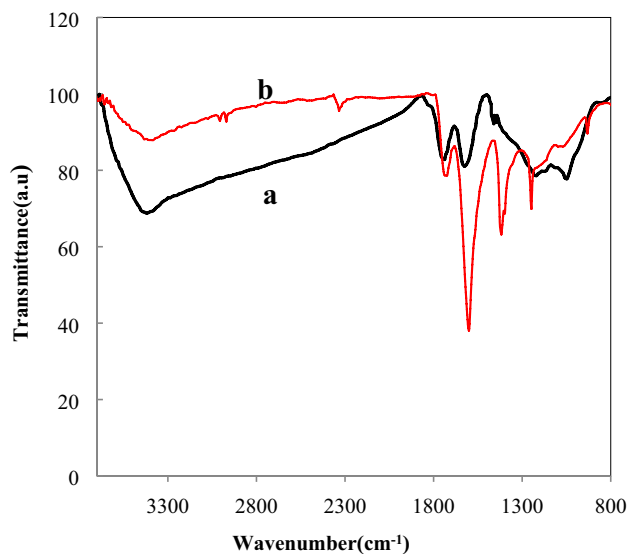


**Fig. 2**  FT-IR spectra of **a** GO and **b** GO–COOH

**Fig. 3**  Nyquist diagrams for the electrochemical impedance measurements of **a** Bare SPCEs and **b** SPCEs/GOX–COOH/chitosan electrode, electrochemical species: 5 mM $K_4/Fe(CN)_5/K_3Fe(CN)_6$. 0.1 M KCl biasing potential: 0.25 V amplitude: 5 mV. Frequency range: 0.1 Hz–10 kHz

Surface morphology of the modified screen printed electrode was examined with the help of FE-SEM. FE-SEM has been used to study the surface morphology of bare SPCE (1a) and SPCE/GOX–COOH/Chitosan (1b). Figure 1 shows the FE-SEM images of electrodeposition process of chitosan/GO–COOH nanocomposite on SPCEs bare electrode, which is confirming the successful electrodeposion of nanocomposite on the surface of SPCEs.

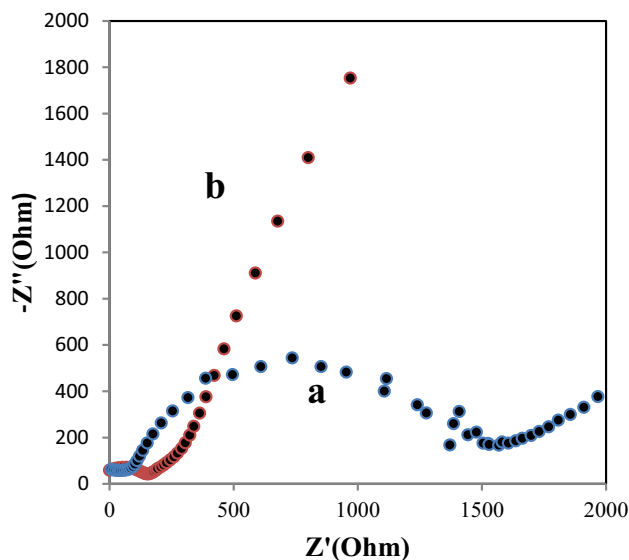The chemical composition of the SPCE/GOX–COOH/ Chitosan was analyzed using an energy dispersive spectrometry (EDS). The EDS spectrum show elements that appear are N, C, O and Ag (Fig. 1c).

FT-IR is a common instrument for determination of different oxygen-containing functional groups in the GO and GO–COOH. The functionalization of GO by carboxylic acid groups was showed by FT-IR spectra. Figure 2 displays the

FT-IR spectra of the GO(a) and GO–COOH(b). As expected, in the spectrum of GO, the broadband centered about 3425 cm$^{-1}$ is recognized to the stretching vibrations of O–H.

The band at 1730 cm$^{-1}$ and 1627 cm$^{-1}$ can be assigned to the vibrations of C=O and aromatic C=C.

After carboxylation of GO, the FT-IR spectra of GO–COOH underwent two changes: one was the peak of C–O–C groups at 1249 cm$^{-1}$ which converted into COOH under carboxylation procedure, and the other was the thin vibration of C=C at 1627 cm$^{-1}$ shifted to 1600 cm$^{-1}$.

EIS is an efficient tool used for characterization of electrical properties of materials and their interfacial regions with electrodes. In addition, it gives information about surface coverage, existing holes/defects on the surfaces, and the mechanism and kinetics of film formation procedures. Figure 3 shows EIS measurements of two electrodes including bare SPCEs (a) and SPCEs/GOX–COOH/Chitosan electrode. Nyquist plots have been utilized to study the change in charge transfer resistance ($R_{ct}$) at sensor solution interfaces with electrode surface modification.

The semicircle of SPCEs/GOX–COOH/Chitosan (b) was obviously smaller than that of the bare SPCEs (a). It meant that the use of GOX–COOH/Chitosan in the modified electrode provided excellent binder between the electrode and electrolyte, leading to an improvement of the electron transfer rate. EIS measurements were carried out in the solution containing a mixture of. 0.1 M KCl 5 mM $K_4$/$Fe(CN)_5$/$K_3Fe(CN)_6$ as a redox probe.

Cyclic voltammetry (CV) is an effective and convenient method for the probing feature of the modified electrode surface. In this study, electrochemical redox behavior of theSPCEs/GOX–COOH/Chitosan electrode was investigated with cyclic voltammetryby using a solution of 0.1 M KCl and 5 mM $Fe[(CN)6]^{3-/4-}$ redox probe at scan rate of 0.05 V s$^{-1}$. After the bare electrode modified with nano-composite, the anodic and cathodic peak current was increased. Thus, this result proved that the electron transfer rate was improved with GO–COOH electro-deposition (Fig. 4).
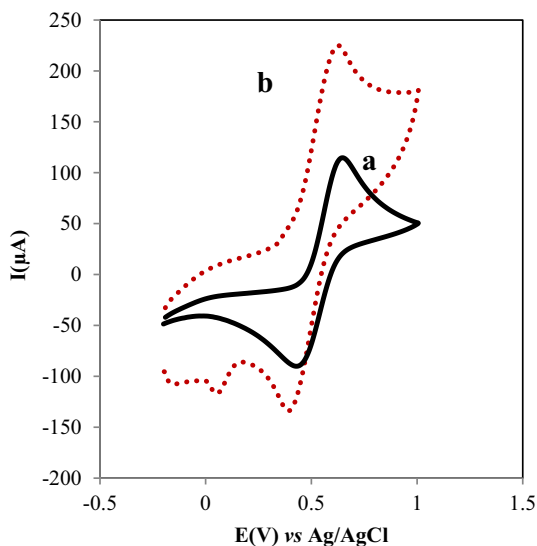
## 3.2 Electrochemical determination of Trp and Tyr

Electrochemical determination of Trp and Tyr was performed with the modified GO–CCOH/Chitosan electrode at different concentration by using DPV.
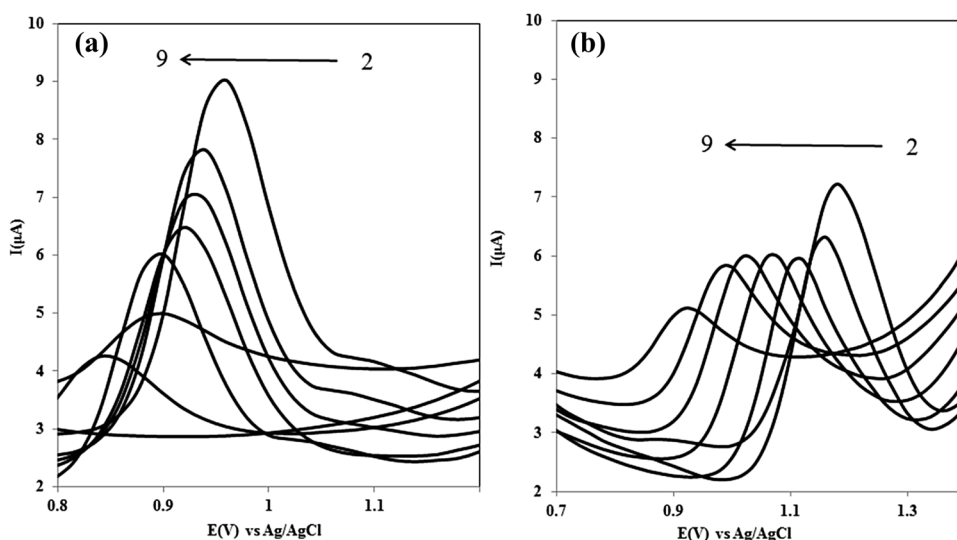


**Fig. 4** The cyclic voltammetry curves of **a** Bare SPCEs and **b** SPCEs/ GOX–COOH/chitosan electrode into 5 mM[Fe(CN)$_6$]$^{3-/4-}$. 0.1 M KCl at 50 mV/s

**Fig. 5** **a** Differential pulse voltammetry of Tyr (20 μM) in different pH values (2 → 9), **b** differential pulse voltammetry of Trp (20 μM) in different pH values (2 → 9)

### 3.3 Optimization of the experimental conditions

#### 3.3.1 GO/COOH/chitosan electrodeposition condition

The thickness film of nanocomposite on SPCE has effect on DPV current. Thus, the relationship between of DPV current peak (Ip) of Trp and Tyr with potential and electro-deposition time was investigated. The Ip of the components increased when the time was increased within the range from 10 to 100 s and response had decreased thereafter. Higher responses and more acceptable calibration curves were achieved with electro-deposition potential at − 1.5 V.

#### 3.3.2 Effect of pH

Amino acids depending on the pH solution can have different forms. In the acidic environment, the amino group gets protonated and changed to $NH_3^+$. In contrast, in the basic environment, the carboxylic acid gets deprotonated and changed to $COO^-$. Since pH plays a vital role, the effect of pH solution variation on the electrochemical oxidation of Trp and Tyr at the electrode was investigated. The maximum $I_p$ of Trp and Tyr were obtained at pH 2.0. Moreover, $E_p$ had shifted to a negative potential as the pH increased linearly with a slope of 37 mv/pH and 22 mv/pH for Trp and Tyr, respectively (Fig. 5a, b).

#### 3.3.3 Effects of accumulation potential and accumulation time

The effects of accumulation potential on the DPV current of Trp and Tyr at the SPCEs/GO–COOH/Chitosan were investigated over the range of − 0.6 to 0.4 V. The results indicated Ip of Trp and Tyr are increased as the potentials shifted negatively. However, to achieve the best signal-to-noise, the accumulation potential 0.00 V was selected for future experiments. Moreover, the effect of accumulation

**Fig. 6** **a** DPVs for increasing concentration of Tyr at SPCE/GO–COOH/Chitosan sensor in buffer solution (pH = 2.0). (Inset) corresponding calibration graph. **b** DPVs for increasing concentration of Trpat SPCE/GO–COOH/Chitosan sensor in buffer solution (pH = 2.0). (Inset) corresponding calibration graph
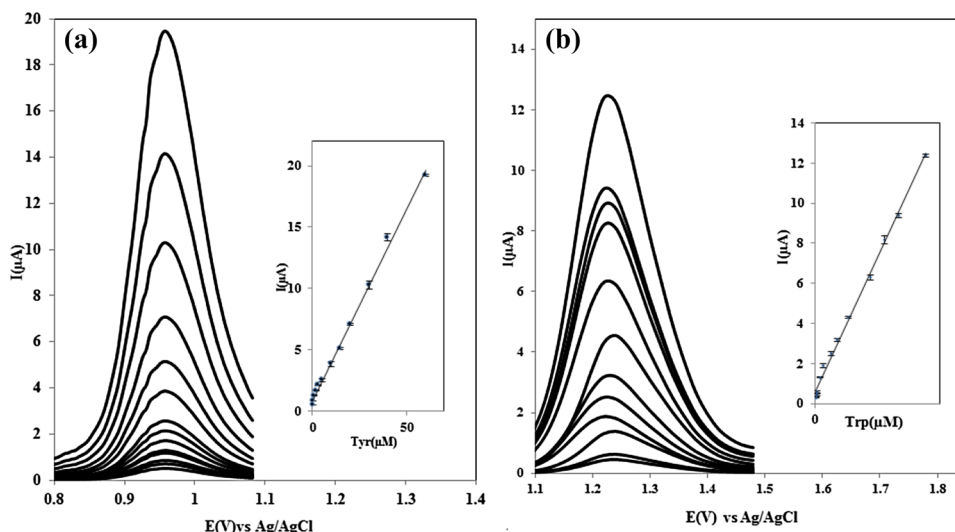


**Table 1** Comparison of the performances of some Trp and Tyr electrochemical sensors

| Electrode modification | LOD for Trp (µM) | Linear range for Trp (µM) | LOD for Tyr (µM) | Linear range for Tyr (µM) | Refs. |
|---|---|---|---|---|---|
| ERGO/GCE | 0.1 | 0.2–40.0 | 0.2 | 0.5–80.0 | [1] |
| SWCNHs/GCE | 0.05 | 0.5–50 | 0.4 | 2.0–30 | [8] |
| POM-rGO/GCE | 2.0 | 10–1000 | 2 | 10–1000 | [15] |
| MSNs/CPE | 0.034 | 0.05–400 | 0.150 | 0.5–600 | [25] |
| BDD | 10 | 400–3000 | 1 | 100–700 | [28] |
| Nafion/TiO$_2$-graphene/GCE | 0.7 | 5–140 | 2.3 | 10–160 | [35] |
| GO–COOH/Chitosan/SPCE | 0.1 | 0.4–40 | 0.05 | 0.1–60 | This study |

*SWCNT* single-walled carbon nanotubes, *GCE* glassy carbon electrode, *MSNs* mesoporous silica nanoparticles, *ERG* electrochemically reduced graphene oxide, *POM* polyoxometalate, *rGO* reduce grapheme oxide, *BDD* boron-doped diamond

time was investigated. Initially, the Ip increased at the 60 s and then the peak currents reach a plateau.

### 3.4 Linear range, detection limit and reproducibility of the method

The linear range and detection limit of Trp and Tyr at the optimum conditions were determined by using DPV technique. DPV waves and the corresponding calibration curves obtained for various concentrations of Tyr and Trp
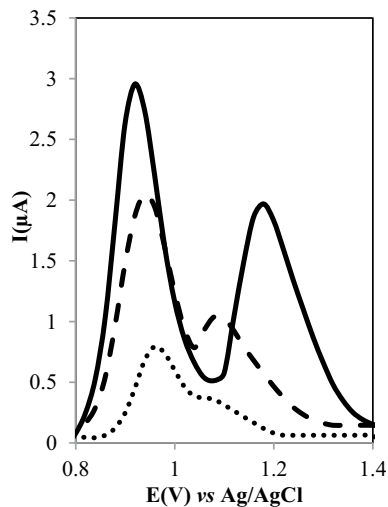


**Fig. 7** DPVs of; SPCE/Chitosan (dotted), SPCEs/GO/Chitosan (dash-line) and SPCEs/GO–COOH/Chitosan(solid line) in a mixture solution of Tyr and Trp (each 5 μM) in 0.01 M HCl solution (pH 2.0)

at SPCE/GO–COOH/Chitosan in 0.01 M HCl (pH 2.0) as supporting electrolyte (Fig. 6). By drawing the anodic current signal versus the concentration, a linear dynamic range from 0.1–60 μM with a limit of detection (LOD) of 0.05 μM (S/N = 3) was obtained for Tyr (Fig. 6a) and for Trpa linear relationship was found over the range of 0.4–40 μM with a limit of detection (LOD) of 0.1 μM (S/N = 3) (Fig. 6b).

To evaluate the electrode-to-electrode reproducibility, five fabricated electrode used for determinations of Tyr (5 μM) and Trp (5 μM) by DPV technique. The relative standard deviations (RSD) of 3.2% and 2.8% for Tyr and Trp were obtained, respectively. The repeatability of the analytical signals was studied by ten successive determinations of Tyr (5 μM) and Trp (5 μM) and RSD of 1.2 and 1.1% were obtained, respectively.

The comparison of SPCE/GO–COOH/Chitosan sensor with other modified electrodes for determination of Tyr and Trp were shown in Table 1. It can be seen that the GO–COOH/Chitosan indicated a wider linear range and a lower detection limit for Trp and Tyr than some previous works. In addition, an important advantage of this method is the best resolution for the voltammetric peaks of Tyr and Trp. Thus, it is suitable and effective for simultaneous determination of two amino acids.

In order to describe about the utilization of the prepared modified electrodes for simultaneous voltammetric detection of Tyr and Trp, differential pulse voltammogram was recorded for; SPCE/Chitosan (dash-dotted), SPCE/GO/Chitosan (dotted line) and SPCEs/GO–COOH/Chitosan (solid line) in a mixture solution of Tyr and Trp (each 5 μM) in 0.01 M HCl solution (pH 2.0) as shown in

**Table 2** Maximum tolerable concentration of interfering species

| Interfering species | Interference concentration (μM) | Tyr recovery (%) | Trp recovery (%) | Interfering species | Interference concentration (μM) | Tyr recovery (%) | Trp recovery (%) |
|---|---|---|---|---|---|---|---|
| Ascorbic acid | 200 | 92 | 95 | Citric acid | 1000 | 95 | 96 |
| Uric acid | 200 | 95 | 98 | Lactic acid | 1000 | 93 | 92 |
| Dopamine | 100 | 95 | 105 | $PO_4^{3-}$ | 500 | 94 | 98 |
| Cysteine | 100 | 96 | 93 | $CO_3^{2-}$ | 500 | 94 | 93 |
| Aspartic acid | 200 | 95 | 95 | $Cl^-$ | 500 | 102 | 99 |
| Glutamine | 1000 | 91 | 96 | $SO_4^{2-}$ | 500 | 97 | 96 |
| Threonine | 200 | 92 | 93 | $Mn^{2+}$ | 200 | 95 | 91 |
| Proline | 1000 | 95 | 95 | $Fe^{2+}$ | 200 | 93 | 99 |
| Histidine | 1000 | 96 | 97 | $Zn^{2+}$ | 1000 | 98 | 94 |
| Valine | 1000 | 95 | 96 | $Ca^{2+}$ | 2000 | 99 | 102 |
| Serine | 1000 | 99 | 98 | $Mg^{2+}$ | 500 | 98 | 95 |
| lucine | 1000 | 100 | 97 | $Na^+$ | 500 | 100 | 99 |
| Arginine | 200 | 100 | 96 | $K^+$ | 2000 | 95 | 97 |
| Methionine | 200 | 100 | 98 | $Cu^{2+}$ | 100 | 99 | 100 |
| Phenylalanine | 200 | 93 | 95 | $Cr^{3+}$ | 100 | 96 | 95 |

**Table 3** Simultaneous determination of Tyr and Trp in Cows and Goat milk

| Milk samples | Added Tyr (µM) | Added Trp (µM) | Found Tyr (µM) | Found Trp (µM) | Tyr recovery % | Trp recovery % |
|---|---|---|---|---|---|---|
| Cow | 0 | 0 | 3.43 ± 0.05 | 0.92 ± 0.07 | – | – |
| | 1 | 1 | 4.37 ± 0.06 | 1.9 ± 0.04 | 94 | 98 |
| | 2 | 2 | 5.33 ± 0.09 | 2.78 ± 0.07 | 95 | 93 |
| | 4 | 4 | 7.41 ± 0.07 | 4.87 ± 0.03 | 99.5 | 94.5 |
| Goat | 0 | 0 | 2.75 ± 0.05 | 1.42 ± 0.10 | – | – |
| | 1 | 1 | 3.74 ± 0.09 | 2.35 ± 0.06 | 99 | 93 |
| | 2 | 2 | 4.7 ± 0.07 | 3.38 ± 0.08 | 97.5 | 98 |
| | 4 | 4 | 6.8 ± 0.05 | 5.36 ± 0.10 | 101.25 | 98.5 |

Fig. 7. As seen, the DPVs wave of SPCE/Chitosan, application of GO–COOH, results in the increase of the peak current and obtained good anodic peak resolution of Tyr and Trp, therefore this electrode is suitable for simultaneous voltammetric determination of Tyr and Trp.

### 3.5 Interference studies

The influences of matrixes on sensor response were also investigated in the solutions containing a mixture of 5 µM of Tyr and 5 µM of Trp in the present of various interferences such as aspartic acid, ascorbic acid, uric acid, dopamine, threonine, cysteine, glycine, lysine,glutamine, glycine, histidine, proline, valine, serine, lucine, arginine, methionine, phenylalanine, citric acid, lactic acid, $PO_4^{3-}$, $CO_3^{2-}$, $Cl^-$, $SO_4^{2-}$, $Mn^{2+}$, $Fe^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$, $Cu^{2+}$ and $Cr^{3+}$ (Table 2).

The results show that the concentration of the interfering species did not cause any interference.

### 3.6 Simultaneous determination of Tyr and Trp in milk samples

Applicability of the SPCE/GO–COOH/Chitosan sensor was examined for determination of Tyr and Trp content in milk. At first, the milk samples were diluted 500 times with HCl (0.01 M) solution and transferred to the voltammetric cell, and then sample analysis was investigated by standard addition of both amino acids in milk by using DPV method. The results are summarized in Table 3. The good recovery values show good efficiency of the sensor for selective determination of two amino acids in milk samples.

## 4 Conclusion

The present study demonstrates a new modified SPCE based on GO–COOH/Chitosan and its application for the simultaneous determination of Tyr and Trp. According to the results, the sensor obtained by GO–COOH/Chitosan electrode-position shows high sensitivity, good reproducibility and enhanced separation of the oxidation peak of Tyr and Trp with respect to the sensors prepared based on GO/Chitosan and Chitosan.

Moreover, GO–COOH/Chitosan nanocomposite exhibited excellent electro-activity and linear voltammetric response towards of Tyr and Trp. The method was successfully applied to the determination of Tyr and Trp in Cow and Goat milk. In particular, the proposed method offers advantages such as simplicity, sensitivity, and stability in Tyr and Trp detections from spiked milk samples.

## Compliance with ethical standards

**Conflict of interest** Ebrahim Fooladi declares that he has no conflict of interest. BiBi Marzieh Razavizadeh declares that she has no conflict of interest. Monireh Noori declares that she has no conflict of interest. Saeid Kakooei declares that he has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent** All of the authors are fully aware and agree for submitting the manuscript to *SN applied Science* journal.

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