

## Determining Caffeic Acid in Food Samples Using a Voltammetric Sensor Amplified by Fe<sub>3</sub>O<sub>4</sub> Nanoparticles and Room Temperature Ionic Liquid

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In this study, the fabrication of a carbon paste electrode (CPE) amplified with Fe<sub>3</sub>O<sub>4</sub> nanoparticles (Fe<sub>3</sub>O<sub>4</sub>-NPs) and 1-methyl-3-butylimidazolium bromide (MBIBr) is reported as a food analytical sensor for determining caffeic acid (CA). The electrochemical behavior of CA on the Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE is investigated via voltammetric techniques. Also, the capability of the Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE for CA determination in different food samples is checked. The Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE shows a powerful ability for improving the oxidation signal of CA with a low limit of detection  $8.0 \times 10^{-9}$  mol/L. The recorded signals show linear a dynamic range  $2.0 \times 10^{-8}$  -  $1.0 \times 10^{-4}$  mol/L with linear coefficients of 0.9941 for CA determination at surface of Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE. Additionally, the Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE shows remarkably good stability, rapid response, repeatability, and selectivity.

**Keywords:** Caffeic acid, Fe<sub>3</sub>O<sub>4</sub> nanoparticle, 1-methyl-3-butylimidazolium bromide, Food electrochemical sensor

### 1. INTRODUCTION

Caffeic acid (CA) is a catechol derivative organic compound found in all plants which plays an important role in the biosynthesis of lignin [1]. As a natural organic compound, CA is found abundantly in coffee, argan oil, thyme, sage, spearmint, and sunflower seeds [2]. This acid shows antioxidant, immunomodulatory, and anti-inflammatory activities [3]. Due to its potent antioxidant activity, it is used in different types of food samples especially sinapic acid and wine [4]. CA also shows anticancer effect

on cancer cells and viruses [5]. Therefore, extensive scientific research has focused on investigation of presence of CA in different samples as well as the properties of this antioxidant/anticancer natural compound. The analytical methods are used for examining the presence and determining CA concentration in different samples, whose results can be useful for therapeutic applications [6-10]. Due to the catechol structure of CA, it shows a good redox response which can be useful for developing electrochemical methods in CA determination [11-13]. On the other hand, electrochemical strategy can be of more interest compared to other analytical methods due to its low cost and fast response [14-20]. Meanwhile, modification of electrochemical sensors can improve the selectivity of electrochemical methods for their simultaneous determination in real samples [21-29]. Also, nanomaterials with wide range applications as well as interesting properties [30-36] such unique electrical conductivity can be helpful for fabrication of powerful electroanalytical sensors [37-40]. There are many scientific reports for creating influential and selective electroanalytical sensors in which nanomaterials and other conductive or mediators are used [41-46].

In this regard and continuing our research group works [47-50], an electroanalytical food sensor was fabricated for caffeic acid determination. For improving the sensor sensitivity, we used Fe<sub>3</sub>O<sub>4</sub>-NPs and MBIBr as two conductive mediators. The results showed high performance ability of the sensor in CA determination. In the final step, we checked the ability of the sensor for analysis of real samples. We used standard addition method for CA determination in white and red wines. The results confirmed the high-performance ability of Fe<sub>3</sub>O<sub>4</sub>-NPs/ MBIBr/CPE for CA determination in food samples.

## 2. MATERIALS AND METHODS

### 2.1. Reagents

1-Methyl-3-butylimidazolium bromide, iron trichloride, caffeic acid, iron (II) sulfate, paraffin oil and sodium hydroxide were purchased from Sigma-Aldrich. The phosphoric acid (for preparation of phosphate buffer solution) and graphite powder were purchased from Merck company. The Fe<sub>3</sub>O<sub>4</sub> nanoparticle was synthesized by co-precipitation method recorded in our previous published paper [51]. The trichloride and iron (II) sulfate solution a with molar ratio 2:1 molar was prepared in Erlenmeyer (50 mL) and then stirred for 45 min at 25<sup>0</sup> C. Next, NaOH (2.0 mol/L) was added drop-wise into the Erlenmeyer under nitrogen gas with the solution being stirred for 20 min. The black samples were filtered and then dried at 100°C for 20 h.

### 2.2. Apparatus

The electrochemical signals were recorded by potentiostat/galvanostat Vertex-Ivium. In all of the voltammetric investigations, we used Ag/AgCl/KCl<sub>sat</sub> as the reference electrode. Also, Pt wire and Fe<sub>3</sub>O<sub>4</sub>-NPs/ MBIBr/CPE were used as counter and working electrodes, respectively. The FESEM figure for the morphological investigation was recorded by Mira 3-XMU.

### 2.3. Fabrication of $Fe_3O_4$ -NPs/MBIBr/CPE

The  $Fe_3O_4$ -NPs/MBIBr/CPE was fabricated by mixing 0.95 g graphite powder + 0.50 g  $Fe_3O_4$ -NPs in the presence of ethanol as solvent. At 35 °C and after 30 min, the ethanol solvent evaporated after which suitable amounts of MBIBr and paraffin oil were added as binders into pestle and mortar. The sample was hand-mixed for 50 min and the obtained paste was used as the working electrode in the presence a copper wire.

### 2.4. Real sample preparation

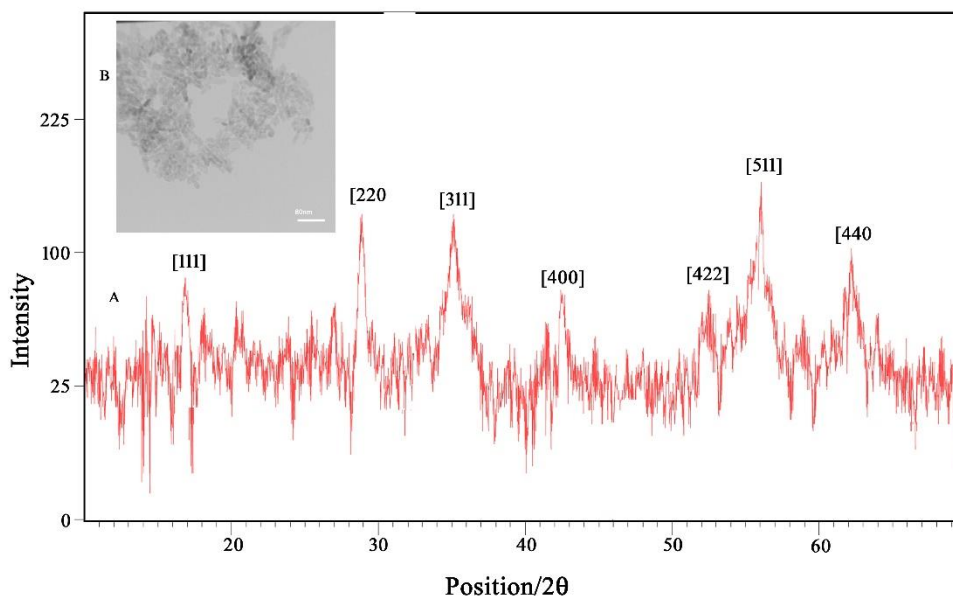
Red and white wines were selected for real sample analysis. The samples were centrifuged at 2000 rpm for 10 min and then filtered. The solution was used directly for real sample analysis.

## 3. RESULTS AND DISCUSSION

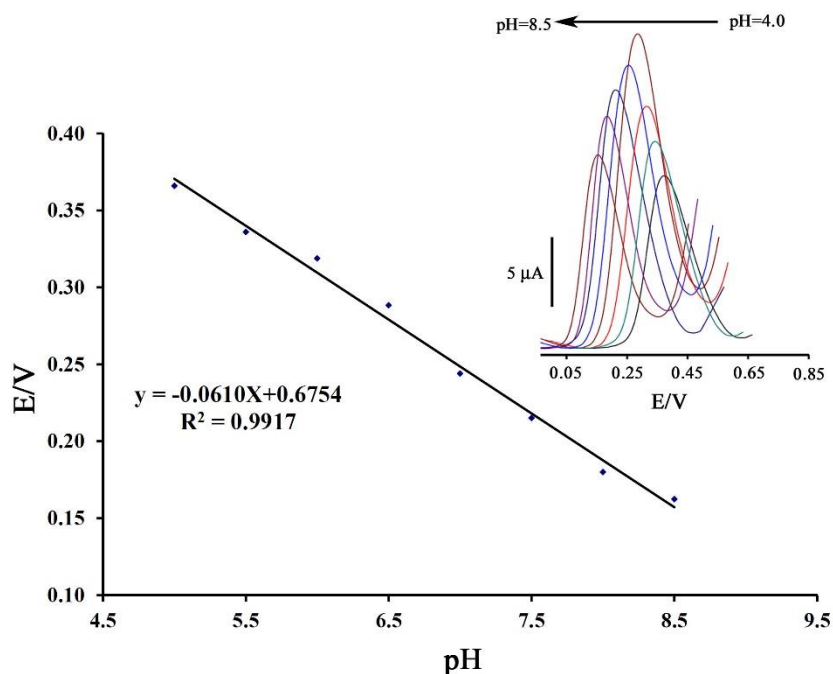
### 3.1. Characterization of nanoparticles

The purity and structure of  $Fe_3O_4$ -NPs was characterized by FESEM and XRD methods. Figure 1A displays the FESEM image of the synthesized nanoparticles. Good distribution and spherical shape of  $Fe_3O_4$ -NPs can be observed in this figure. Further, the presence of seven planes with miller indices 111; 220; 311; 400; 422; 511; and 440 in XRD pattern matching the JCPDS card No. 74-0748 for  $Fe_3O_4$ -NPs can be observed in Figure 1B [52]. The particle size of  $Fe_3O_4$ -NPs was determined as ~ 13.2 nm by Scherrer formula.

### 3.2. Electrochemical behavior of caffeic acid

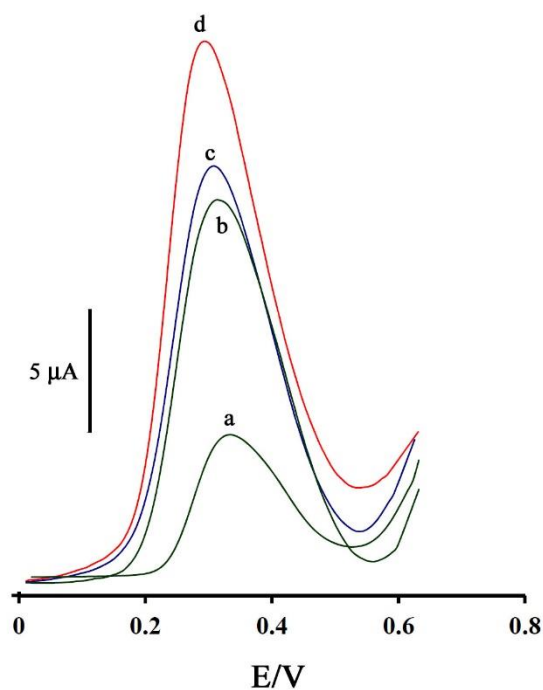


**Figure 1.** A) XRD pattern and B) TEM image of  $Fe_3O_4$  nanoparticle



**Figure 2.** E-pH curve for electro-oxidation 100  $\mu\text{mol/L}$  CA. Inset) Differential pulse voltammograms of 100  $\mu\text{mol/L}$  at surface of  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$  in the different pH range.

Due to presence of a phenolic part in CA structure, the electrochemical oxidation signal of CA may change with pH alteration [53]. Thus, the differential pulse voltammograms of CA were recorded within pH range 5.0-8.5 (Figure 2 inset).



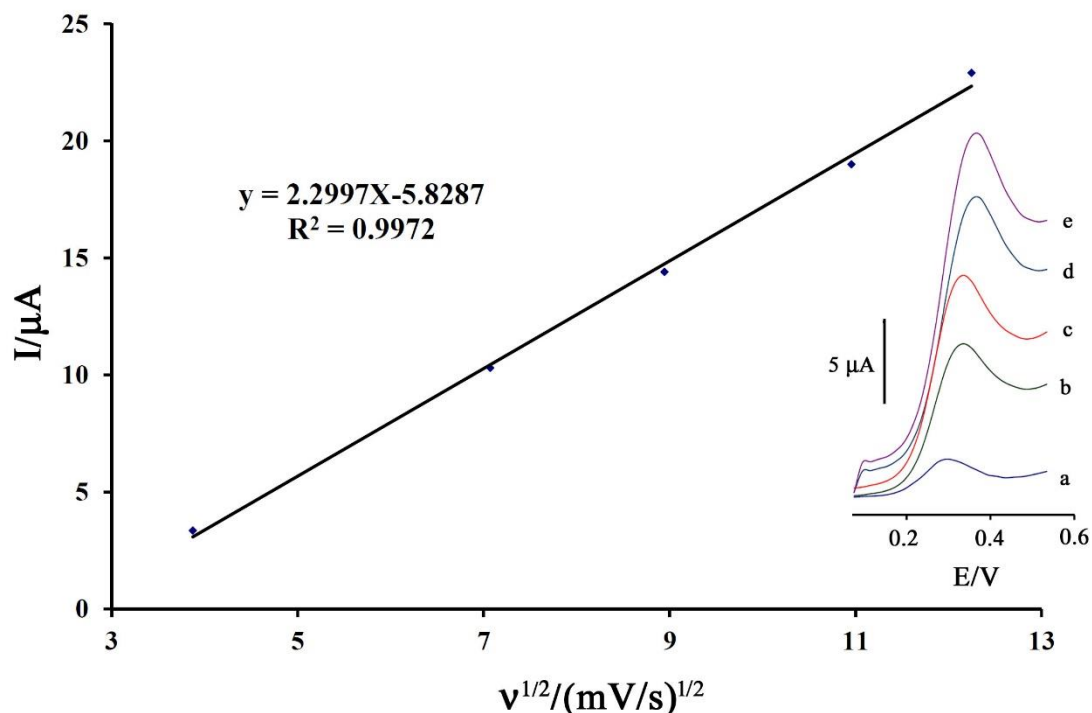
**Figure 3.** DP voltammograms of 100  $\mu\text{mol/L}$  CA at surface of CPE (a),  $\text{Fe}_3\text{O}_4\text{-NPs/CPE}$  (b),  $\text{MBIBr/CPE}$  (c) and  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$  (d).

The results showed with moving from pH=4.0 to pH=8.5, the oxidation reaction of CA facilitated. The results show a negative slope between the oxidation potential signal of CA vs. pH with equation  $E_{pa}=0.0610 \text{ pH} + 0.6754$  confirming the presence of equal proton and electron content in the electro-oxidation mechanism of CA due to the obtained slope is near to the anticipated Nernstian value. In addition, and according to inset data of Figure 2, the maximum sensitivity was observed at pH=6.5 which was selected for further investigations.

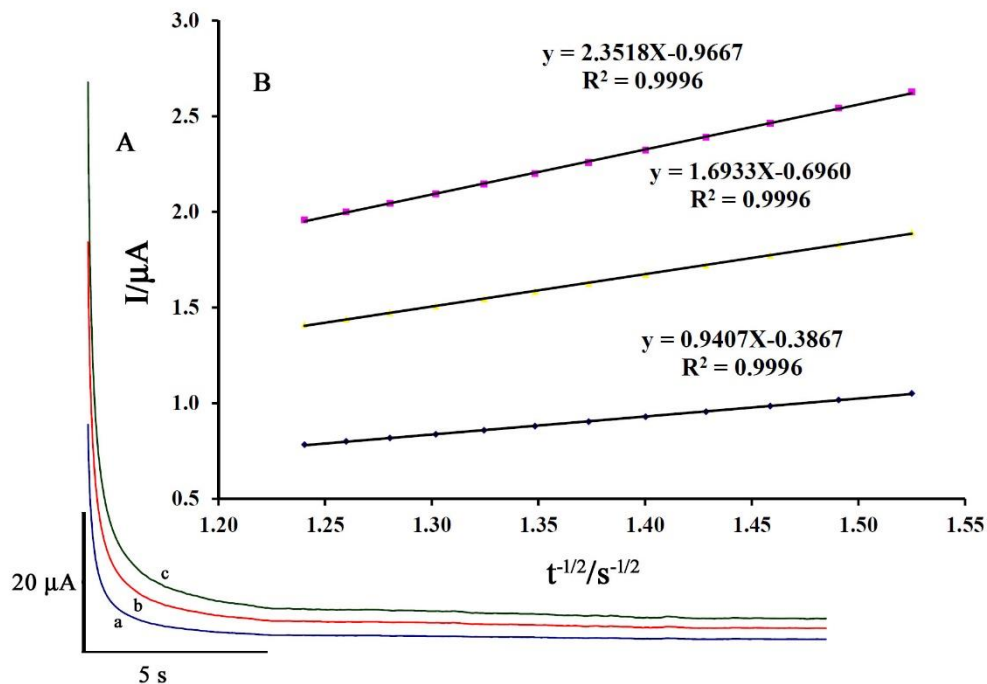
The modification effect of CPE by  $\text{Fe}_3\text{O}_4$  nanoparticles and 1-methyl-3-butylimidazolium bromide on the oxidation signal of CA was investigated by recording DP voltammograms of 100.0  $\mu\text{mol/L}$  CA at the surface of CPE (Figure 3, curve a),  $\text{Fe}_3\text{O}_4\text{-NPs/CPE}$  (Figure 3, curve b), MBIBr/CPE (Figure 3, curve c) and  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$  (Figure 3, curve d). The oxidation potentials 338, 312, 310 and 295 mV as well as oxidation currents 5.88  $\mu\text{A}$ , 15.2  $\mu\text{A}$ , 16.3  $\mu\text{A}$  and 21.3  $\mu\text{A}$  were detected for CA upon moving from curve a to curve d. These improvements can be related to the high conductivity of  $\text{Fe}_3\text{O}_4\text{-NPs}$  as a mediator and MBIBr as a binder.

The linear sweep voltammograms of CA were recorded at the surface of  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$  within the scan rate range 15-150 mV/s (Figure 4 inset). The linear relationship between the current as well as  $v^{1/2}$  and equation  $I_{pa} = 2.2997 v^{1/2} - 5.8287$  ( $R^2=0.9972$ ) can be observed in Figure 4 confirming the diffusion control process [54-59] for electro-oxidation of CA at the surface of  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$ .

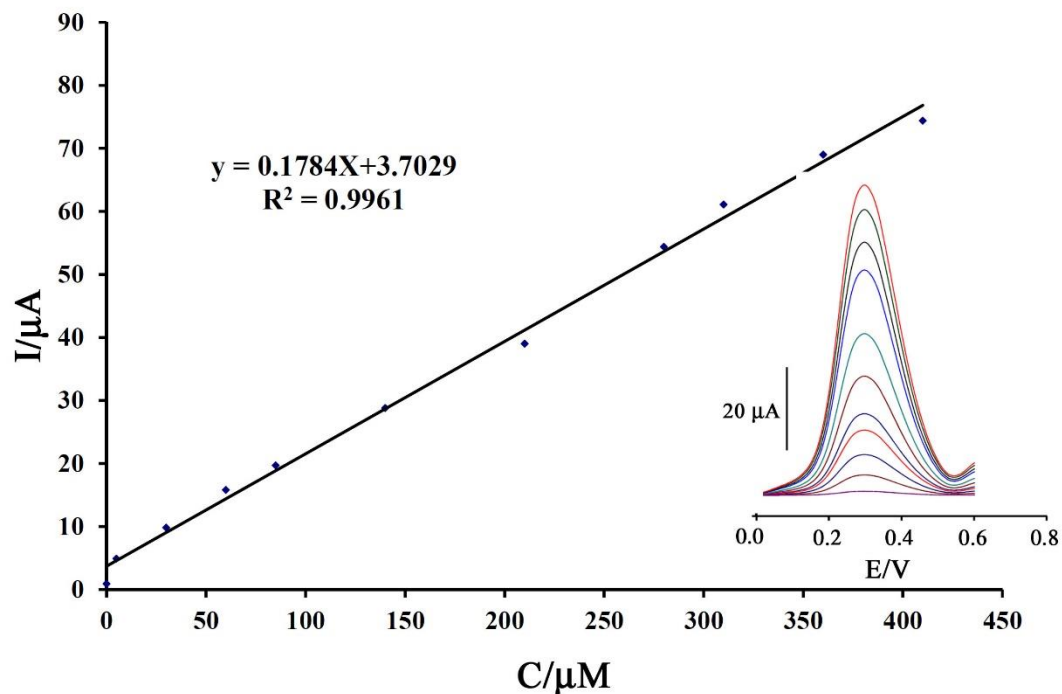
The diffusion coefficient of CA in this research was determined by recording chronoamperograms 50.0, 100.0 and 150.0  $\mu\text{mol/L}$  of CA at the surface of  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$  with an applied potential 450 mV (Figure 5 A). The plots of  $I-t^{-1/2}$  (Cottrell plots) relative to CA at the surface of  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$  are present in Figure 5 B. According to these plots and Cottrell equation, the value of D was determined  $2.02 \times 10^{-6} \text{ cm}^2/\text{s}$ .



**Figure 4.** Plot of  $I-v^{1/2}$  for electrooxidation of 200  $\mu\text{mol/L}$  CA at surface of  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$ . Inset) LS voltammograms 200  $\mu\text{mol/L}$  CA at scan rates a) 15, b) 50, c) 80, d) 120 and e) 150 mV/s.



**Figure 5.** A) Chronoamperograms of a) 50.0, b) 100.0 and c) 150.0  $\mu\text{mol/L}$  of CA at surface of  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$ . B) Cottrell's plot obtained at the  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$



**Figure 6.** A) The  $I_{pa}$  vs. CA concentration obtained from DP voltammograms. B) The DP voltammograms of solution containing CA at the  $\text{Fe}_3\text{O}_4\text{-NPs/MBIBr/CPE}$ ; a) 0.03; b) 5.0; c) 30d)60.0; e) 85.0; f) 140.0; g) 210.0; h) 280.0; i) 310.0; j) 310.0; k) 360.0 and l) 410.0  $\mu\text{mol/L}$ .

Hence, the Differential pulse voltammetry was used as powerful electroanalytical strategy to determine the CA containing various concentrations of antioxidant compound from  $3.0 \times 10^{-8}$  -  $4.1 \times 10^{-4}$

mol/L (Figure 6). The recorded oxidation signals of the CA was increased with increasing the antioxidant concentration ranging from  $3.0 \times 10^{-8}$  -  $4.1 \times 10^{-4}$  mol/L with the  $R^2 = 0.9961$ . In addition, the detection limit  $6.0 \times 10^{-9}$  mol/L was calculated for determination of CA at surface of Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE. These analytical values are comparable to the data reported by other research groups (see Table 1).

**Table 1.** Comparison of the efficiency of previous reported electrochemical sensors in the determination of CA

Electrode	Mediator	LDR ( $\mu$ M)	LOD ( $\mu$ M)	[Ref]
Glassy carbon	fluorine-doped graphene oxide	0.5-100.0	0.018	[53]
Glassy carbon	Graphene Oxide/Polydopamine	0.005-450.5	1.2	[60]
Screen Printed Carbon Electrode	Co <sub>3</sub> O <sub>4</sub> microballs	0.2-272	0.04	[61]
Carbon paste	Fe <sub>3</sub> O <sub>4</sub> -NPs/MBIBr	0.03-410.0	0.008	This work

To investigate the response stability of the Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE as an analytical sensor, DPV of 100  $\mu$ mol/L CA was explored after 35 days. The results related to the oxidation signals of CA revealed that the changes in the currents were less than 4% which is an acceptable value for an electroanalytical sensor. In addition, the RSD%  $\sim$ 2.4 was detected for determination of 50  $\mu$ mol/L CA when we used six different electrodes with a similar process preparation, confirming good reproducibility for the fabricated sensor.

The selectivity of Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE as an analytical tool was investigated for determining 20  $\mu$ mol/L CA. The results confirmed that 500 folds of Na<sup>+</sup>, Br<sup>-</sup>, vitamin B<sub>9</sub>, methionine, valine and tryptophan have not any interference for CA determination in the suggested sensor.

For further evaluating the Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE as an electro-analytical tool, it was used for CA determination in real food samples. The recovery data between 99.28-102.13% for CA determination via the standard addition method are observed in Table 2. It suggests the powerful ability of Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE for analysis of CA in the real food samples.

**Table 2.** The determination of CA in wine samples using Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE

Samples	CA added $\mu$ mol/L	CA expected $\mu$ mol/L	CA founded $\mu$ mol/L	Recovery%
Red wine	---	---	67.33 $\pm$ 1.05	---
	10.00	77.33	78.98 $\pm$ 1.67	102.13
White wine	---	---	56.14 $\pm$ 0.97	---
	10.00	66.14	65.67 $\pm$ 1.01	99.29

#### 4. CONCLUSION

In this work, we benefitted from high conductivity of Fe<sub>3</sub>O<sub>4</sub>-NPs and MBIBr as a modifier and binder for amplification of a carbon paste electrode. The fabricated Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE was tested as an electroanalytical tool for CA determination. The results showed a high-performance ability of Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE for nanomolar determination of CA. Further, the Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE was used for CA determination within the concentration range  $3.0 \times 10^{-8}$  -  $4.1 \times 10^{-4}$  mol/L. In the final step, the ability of Fe<sub>3</sub>O<sub>4</sub>-NPs/MBIBr/CPE as an analytical tool was tested for CA determination in food samples, with recovery data between 99.28-102.13% confirming the powerful ability of the sensor as a food analytical sensor.

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