# Application of TiO₂/Fe₃O₄/MWCNTs Nanocomposite Conductive Mediator for Fabrication of Kojic Acid Sensor

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This research work focused on kojic acid determination using carbon-paste electrode (CPE) improved with TiO₂/Fe₃O₄/MWCNTs nanocomposite and ionic liquid (TiO₂/Fe₃O₄/MWCNTs/IL-CPE). Redox behavior of kojic acid as an antioxidant food additive was examined by different electrochemical techniques. The recording results confirmed TiO₂/Fe₃O₄/MWCNTs/IL-CPE could improve redox behavior of kojic acid in current and potential cases. The TiO₂/Fe₃O₄/MWCNTs/IL-CPE showed linear relation with kojic acid concentration in the range 0.5 µM - 300.0 µM with detection limit 0.2 µM using the DPV. The TiO₂/Fe₃O₄/MWCNTs/IL-CPE was used to determine kojic acid in real food samples.

## 1. INTRODUCTION

Electrochemical methods are promising analytical techniques for which has the potential to replace expensive and complicated instruments in terms of electroanalytical performance [1-10]. Electroanalytical methods are now a days widely used in drug and environmental analysis due to numerous advantages like cheaper instrumentation, fast response, robustness, reproducibility, high sensitivity, wide linear dynamic range [11-20]. The I-V methods works based on direct redox reaction signal of the electroactive compounds on the sensor surface [21-30].

For the first time in 1985 year, Prof. Adams suggested carbon paste electrode as a new types of heterogeneous carbon-based electrode that make by mixture of carbon powder and an appropriate water-immiscible oil binder. The use of carbon paste as an electrode was reported in 1958 by Adams. The carbon paste electrodes can be easily and quickly fabricated to obtain electrode surface. CPE usually shows low residual current, and can be used in conjunction with various types of modifiers to yield

porous surface. Because of the above mentioned benefits, carbon paste electrode are preferred over other solid electrodes [31-35]. The high electrical resistance of bare CPE created a low conductivity for it and reduced ability of CPE for trace assay of electroactive species. Researchers usually mixed CPE with other substances as new way for overcoming to this problem.

Changing in conductivity of electrodes with appropriate biocompatible resources promotes the electrochemical activity of electroactive compounds to proceed without hindrance, as they reduces the background currents, and work in a large potential domain. This leads to increase in ability of modified sensor for trace determinations of electroactive compounds [36-40].

Over the years, nanomaterials like conductive metal oxides with interest properties [41] have been used to modify the CPEs to enhance better electroanalytical properties. The properties of nanoscale materials have made them attractive for electrochemical applications that had not been observed before. This is due to the emergence of new features such as improved selectivity, high active surface area, optimum signal to noise ratio and catalytic activity. The mentioned features of this type of materials have been introduced them as a serious option in designing the new generation of electrochemical sensors [42-50].

Carbon nanotubes have been introduced as a new generation of nanomaterials with unique and interesting properties such as high electrical conductivity and high specific surface area as a serious option for manufacturing the new generation of electrochemical sensors. The use of its composites has made a significant difference in the field of analytical sensors [51-56].

Ionic liquids with properties such as good electrical conductivity and very high viscosity have been proposed as a conductive substrate for various applications, especially the preparation of electron exchange sensors. On the other hand, the proper viscosity of ionic liquids provides a suitable condition for the construction of modified carbon paste electrodes [57, 58].

Kojic acid (KA) with phenolic structure showed many properties such as antioxidant activity, inhibiting the catecholase activity of tyrosinase and skin-lightening effect and suggested for different application such as cosmetic and food industries [59]. This compound is used as an additive in many foods such as soy sauce, soy bean paste and sake. Also, this phenolic compound has also been used as a plant growth regulator, analytical chemical, antibiotic and pesticide. There are reports of adverse effects of this compound and the possibility of cancer in humans with overuse of food samples. Therefore, it is interest to develop a convenient and sensitive method for the measurement of kojic acid [60-65]. In the presence study, we report fabrication of a sensitive and selective electroanalytical sensor for kojic acid and have explored the sensor application in analyzing this phenolic compound in real samples. The sensor was fabricated by modification of carbon paste electrode with $TiO_2/Fe_3O_4/MWCNTs$ nanocomposite and ionic liquid. The $TiO_2/Fe_3O_4/MWCNTs/IL-CPE$ sensor gave an excellent voltammetry response to this phenolic antioxidant in a neutral conditions solution.

## 2. EXPERIMENTAL

### 2.1. Instruments and materials

Electrochemical measurements and tests were performed on a PGSTAT 302N Autolab, connected to an electrochemical system software. pH readings were performed using a Metrohm 710

pH-meter. An Ag/AgCl/KCl (3.0 M) electrode, $TiO_2/Fe_3O_4$/MWCNTs/IL-CPE and a platinum wire were used as the reference, working and auxiliary electrodes, respectively.

All chemicals, including kojic acid were obtained from Merck Co., and n-hexyl-3-methylimidazolium hexafluoro phosphate from Sigma were of analytical grade purity. The phosphate buffer solutions (PBS) were ready from orthophosphoric acid and its salts in the different pH range.

The $TiO_2/Fe_3O_4$/MWCNTs nanocomposite were synthesis by reported strategy by Luo et al. in two separated steps [66]. Typical SEM of the $TiO_2/Fe_3O_4$/MWCNTs is shown in Fig.1.



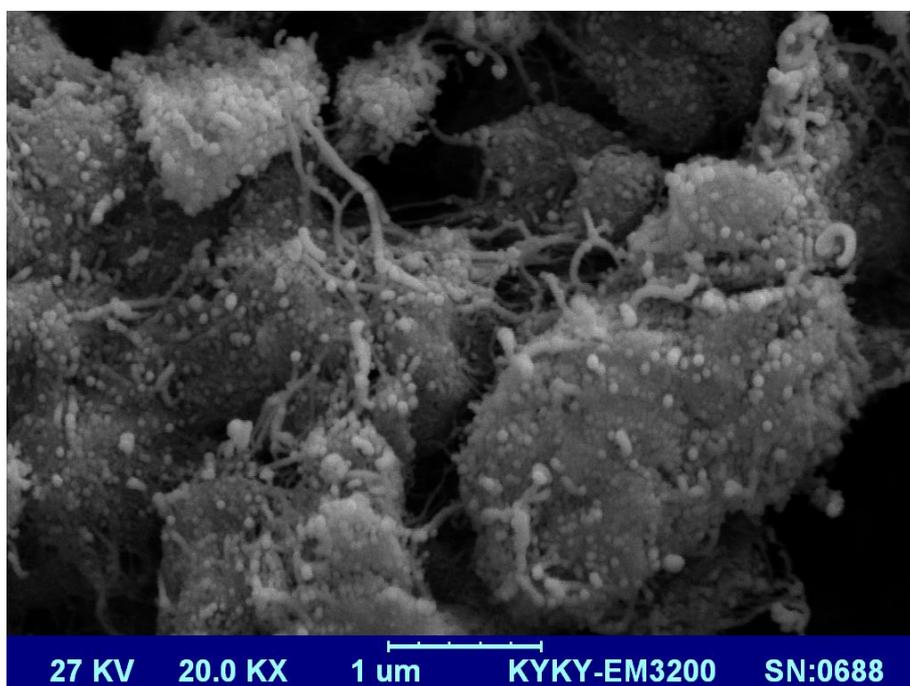**Figure 1.** SEM image of TiO2/$Fe_3O_4$/MWCNTs NANOCOMPOSITE

## 2.2. Preparation of the Electrode

$TiO_2/Fe_3O_4$/MWCNTs/IL-CPE were prepared by mixing 0.04 g of $TiO_2/Fe_3O_4$/MWCNT nanocomposite with 0.96 g graphite powder and approximately, ~0.8 mL of ionic liquid with a mortar and pestle. The paste was then packed into the end of a glass tube that modified with coper wire as electrical contact system.

## 2.3. Preparation of Real Samples

2 mL edible oil + 2 mL of edible oil with 20 mL hexane solution was mixed into beaker and then then mixture solution centrifuge for 5 min (2500 rpm). In continuous we mixed 10 mL of supernatant + 10 mL of ethanol in straining condition for 10 min and then centrifuge for 5 min (2500 rpm). In continuous hexane solution (10 mL) was added and mixture was shaken for 30 min. For final rela sample analysis, 2 mL extracted solution + 2 mL ethanol was prepared and used for real sample analysis.

5.0 g chilli sauce was added in 100.0 mL ethanol flask and the mixture was ultrasonicated for 45 min and filtrated with 0.25 µm filter according to Elyasi et al. report procedure [67].

## 3. RESULTS AND DISCUSSION

### 3.1. Electrochemical behavior of kojic acid at the surface of various electrodes

The electrochemical behaviour of kojic acid depends on the pH of the aqueous solution [37]. Thus, it is essential to optimize the solution pH in order to gain better insights of the electro-oxidation process of kojic acid [68]. The electrochemical voltammetric behaviour of kojic acid was examined by using $TiO_2/Fe_3O_4/MWCNTs/IL$-CPE in 0.1 M PBS at different pH ranging from 2 to 9. The results showed that neutral pH is optimum for electro-oxidation of kojic acid in comparison to the basic or acidic medium. Thus, pH 7.0 was selected for carrying out further analysis of kojic acid.
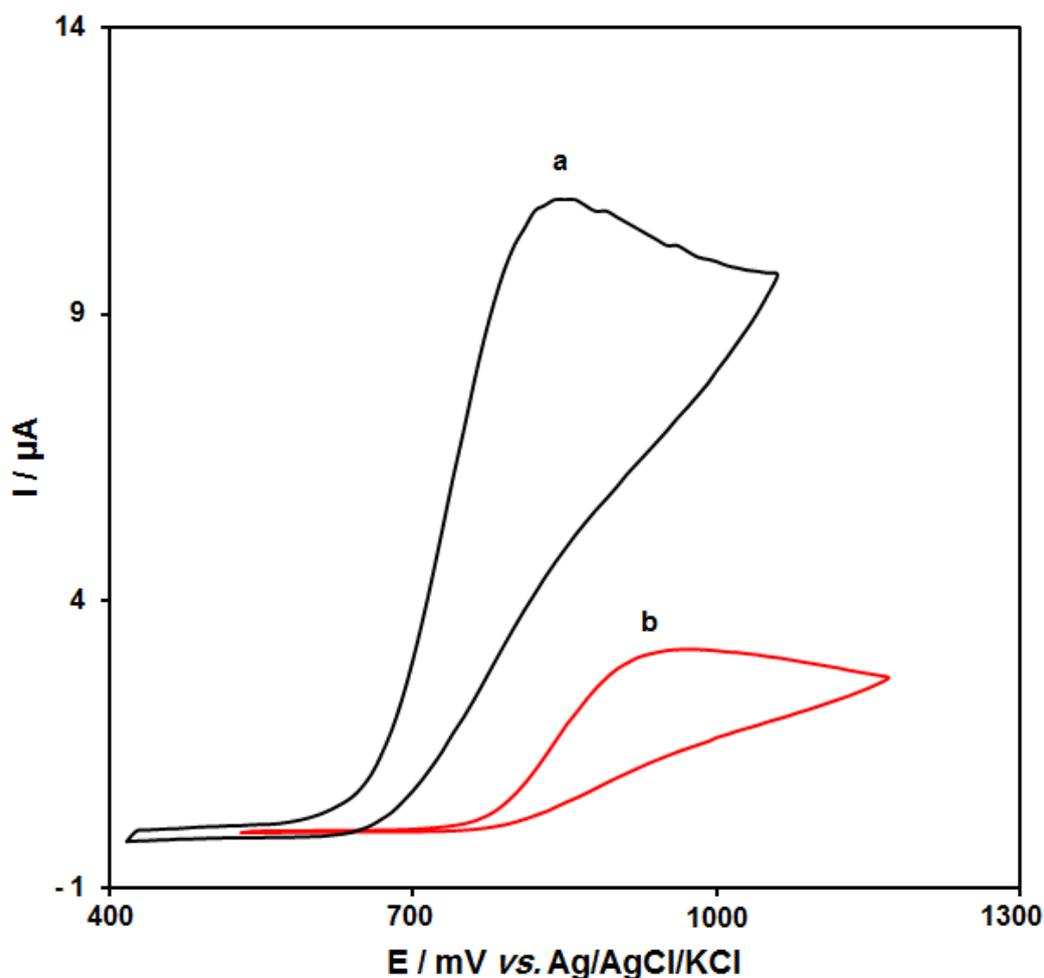


**Figure 2.** CVs of a) $TiO_2/Fe_3O_4/MWCNTs/IL$-CPE and b) CPE in the presence of 100.0 µM of kojic acid at pH 7.0. Rate of scan in all cases was found to be 50 mV s$^{-1}$.

Figure 2 shows responses of CV towards electro-oxidation of 100.0 μM kojic acid at the unmodified CPE (curve b) and $TiO_2/Fe_3O_4/MWCNTs/IL-CPE$ (curve a). The oxidation peak potential of kojic acid occurs at 850 mV, which is around 120 mV more negative than the unchanged CPE, indicating better electroactivity of the modified electrode ($TiO_2/Fe_3O_4/MWCNTs/IL-CPE$). Also, the peak current showed significant enhancement with the modified electrode [69-71], which also reaffirms their superiority over unmodified CPE.

### 3.2. Effect of scan rate

The impact of potential scan rates on the electro-oxidation of kojic acid was monitored by linear sweep voltammetry (LSV) (Figure 3). Results indicated enhancement of the peak current with increase in potential scan rate from 5-700 mV/sec. The linear dependency of the peak current ($I_p$) with the square root of potential scan rate ($v^{1/2}$) indicates diffusion controlled [72-78] electro-oxidation of kojic acid.
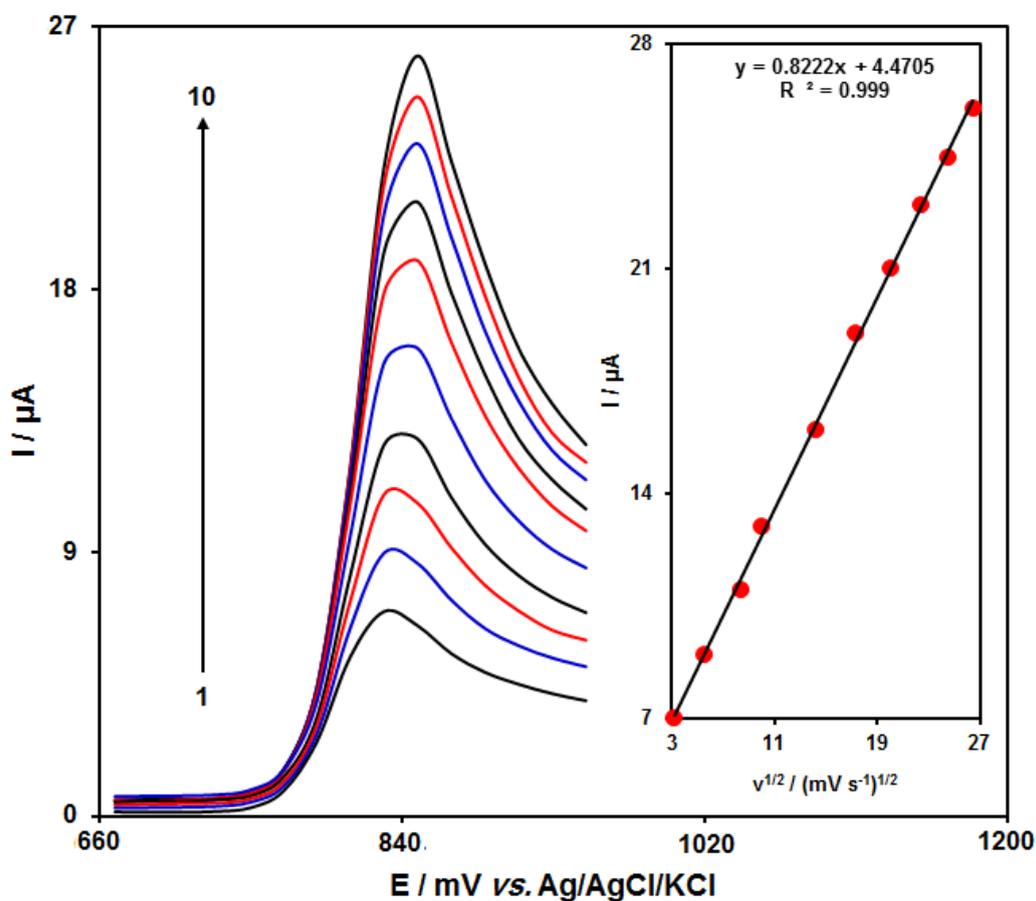


**Figure 3.** LSV of $TiO_2/Fe_3O_4/MWCNTs/IL-CPE$ in solution containing 100.0 μM kojic acid at various scan rates; 1) 5, 2) 10, 3) 30, 4) 70, 5) 100, 6) 200, 7) 300, 8) 400, 9) 500, 10) 600, and 11) 700 mV s$^{-1}$. Inset: oxidation kojic acid current vs. $v^{1/2}$.

*3.3. Chronoamperometric measurements*

The chronoamperograms of TiO$_2$/Fe$_3$O$_4$/MWCNTs/IL-CPE in the presence different concentration of kojic acid displayed in Figure 4 by adjusting the working electrode potential at 0.9 V.
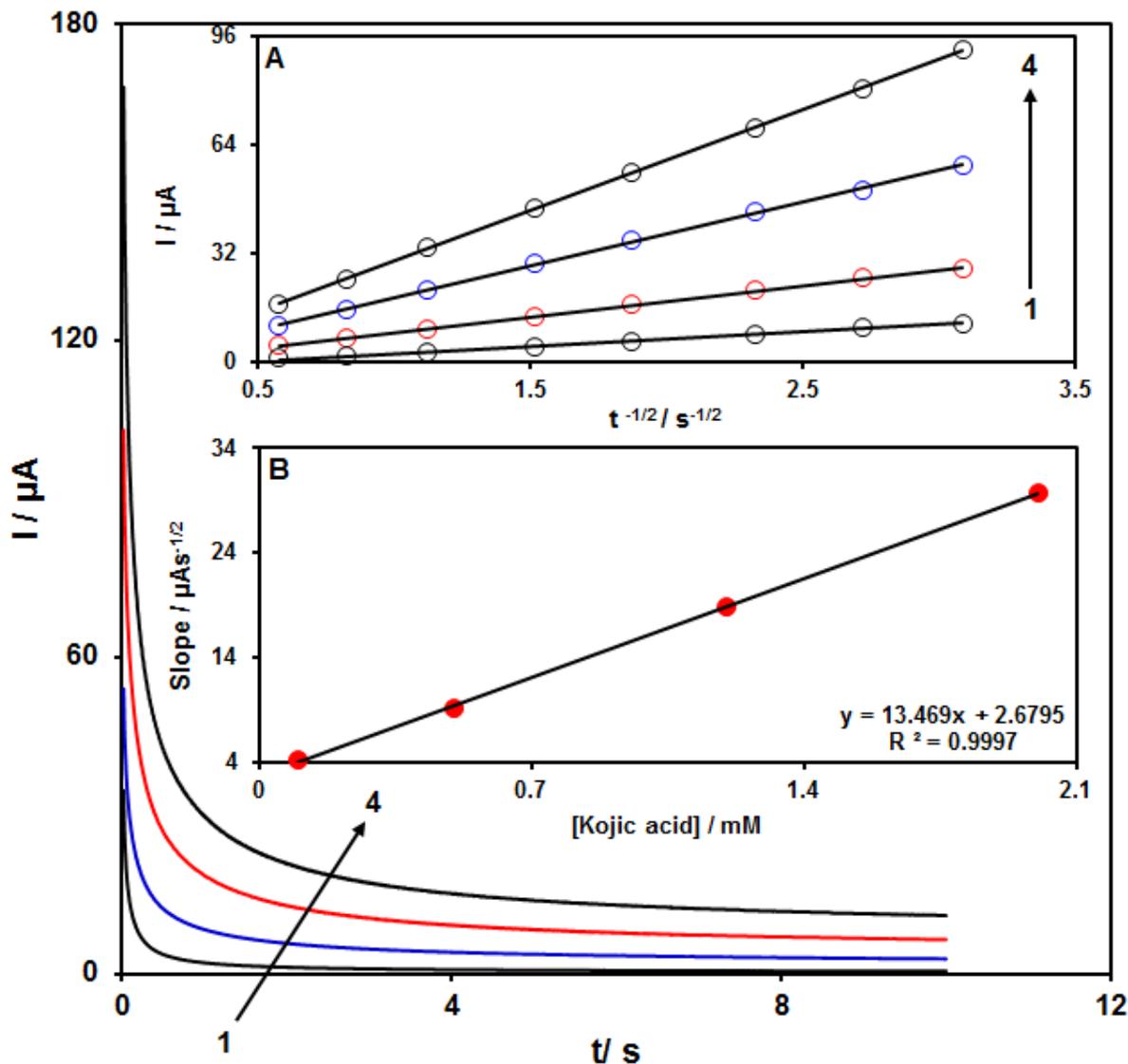


**Figure 4.** Chronoamperograms obtained at TiO$_2$/Fe$_3$O$_4$/MWCNTs/IL-CPE for (1) 0.1; (2) 0.5; (3) 1.2 and (4) 2.0 mM of kojic acid. Insets: (A) Plots of I vs. t$^{-1/2}$ obtained from chronoamperograms 1–4. (B) Derived result relative to slopes from figure 4B.

The value of kojic acid speed in solution constant (diffusion coefficient; D) was calculated by the Cottrell equation (I $=nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$) in a the mass transport limited condition. Baes on Cottrell equation, the plots of I versus t$^{-1/2}$ for various concentrations of kojic acid were useful determination of kojic acid diffusion coefficient (Figure 4A and B). Based on concluded slopes in Figure 4 B that derived from Figure 4A data, mean values of D were $1.9\times10^{-6}$ cm$^2$/s for kojic acid that is an acceptable data for aqueous solution [37].

## 3.4. Calibration curves

Using the peak currents recorded with the $TiO_2/Fe_3O_4/MWCNTs/IL-CPE$, the analyte concentration in two aqueous samples were determined by differential pulse voltammetry (DPV). DPV offers several advantages of enhanced sensitivity and performance (Fig. 6). Also, DPV method suggested better LOD in an electrochemical analysis systems [79-82].
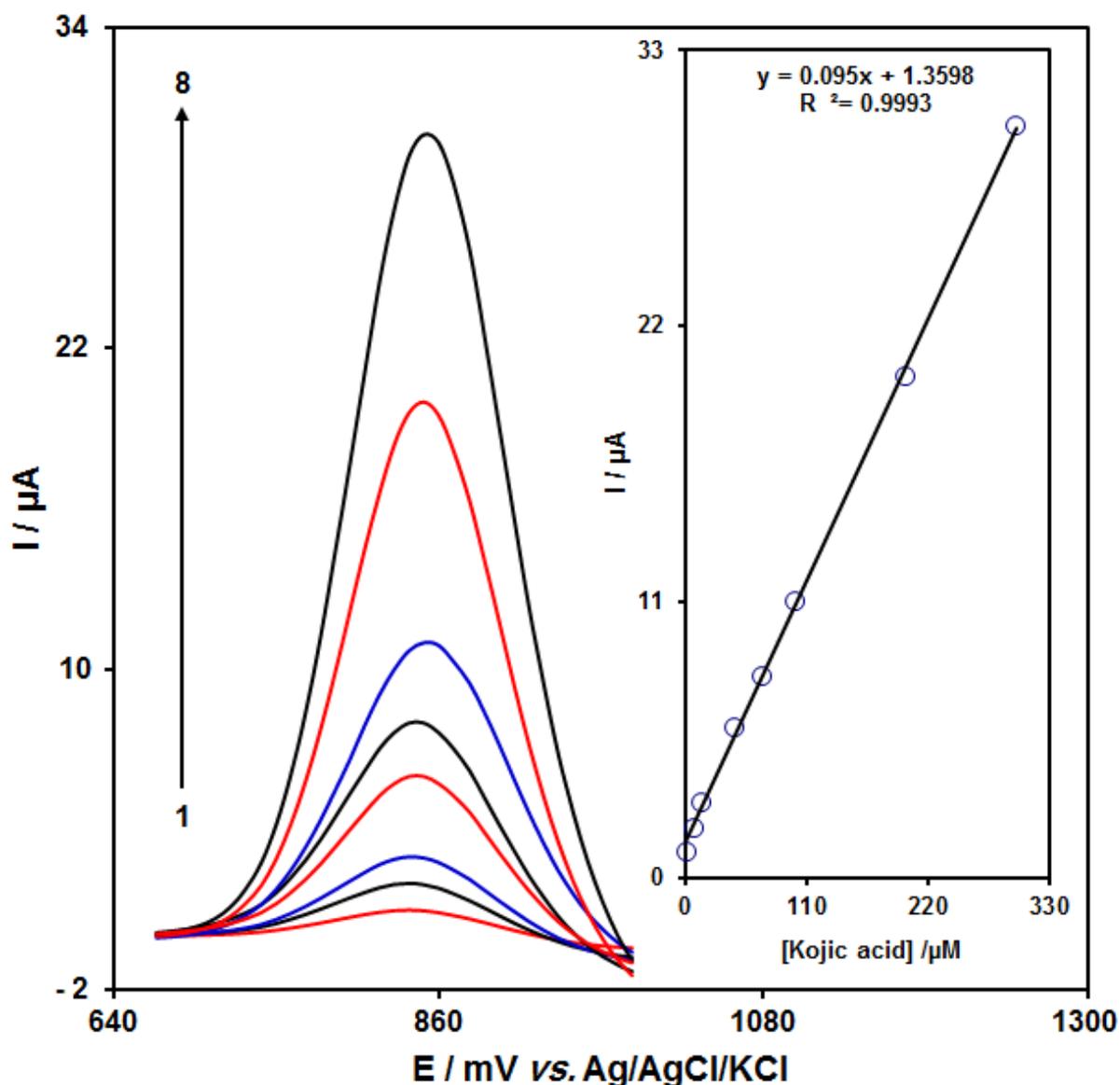


**Figure 5.** DPVs of $TiO_2/Fe_3O_4/MWCNTs/IL-CPE$ containing 1) 0.5, 2) 7.5, 3) 15.0, 4) 45.0, 5) 70.0, 6) 100.0, 7) 200.0 and 8) 300.0 µM of kojic acid. Inset: Plot of the electrocatalytic peak current as a function of kojic acid concentration.

The results indicated a linear relationship between the DPV peak currents and kojic acid concentration in the range of 0.5-300.0 µM with a correlation coefficient of 0.9993. The limit of detection for the analyses was determined to be 0.2 µM .

*3.5. Analyzing real sample*

The method was used to evaluate the application of $TiO_2/Fe_3O_4/MWCNTs/IL$-CPE in determining kojic acid in real samples such as in Bean edible oil and Chilli sauce. The standard addition technique was applied.

**Table 1.** Determining kojic acid in real samples through $TiO_2/Fe_3O_4/MWCNTs/IL$-CPE All the concentrations are in μM (n=3).

| Sample | Spiked | Found | Recovery (%) | R.S.D. (%) |
|---|---|---|---|---|
| Edible oil | 0 | 3.0 | - | 2.9 |
| | 2.0 | 4.9 | 98.0 | 3.1 |
| | 4.0 | 7.2 | 102.9 | 2.1 |
| | 6.0 | 8.8 | 97.8 | 1.9 |
| | 8.0 | 11.2 | 101.8 | 2.8 |
| Chilli sauce | 0 | 4.0 | - | 3.3 |
| | 2.0 | 6.2 | 103.3 | 1.7 |
| | 4.0 | 7.8 | 97.5 | 2.9 |
| | 6.0 | 10.2 | 102.0 | 2.6 |
| | 8.0 | 11.8 | 99.2 | 2.4 |

Table 1 reports the results. Acceptable recoveries of kojic acid were observed, and reproducible results was shown with regard to the mean relative standard deviation (R.S.D.).

**4. CONCLUSION**

In this project we introduce a new sensor amplified with $TiO_2/Fe_3O_4/MWCNTs$ nanocomposite and ionic liquid introduce as analytical approach for kojic acid determination. The $TiO_2/Fe_3O_4/MWCNTs/IL$-CPE showed better electrochemical performances due to the presence of $TiO_2/Fe_3O_4/MWCNTs$ nanocomposite and its interaction with ionic liquid on the electrode. The $TiO_2/Fe_3O_4/MWCNTs/IL$-CPE displayed great electrocatalytic activity to the oxidation of kojic acid by major improvement in the oxidation signal. The $TiO_2/Fe_3O_4/MWCNTs/IL$-CPE displayed a great stability, and good analytical parameters for the detection of kojic acid.

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