Anal. Bioanal. Electrochem., Vol. 12, No. 3, 2020, 289-300

Analytical &
Bioanalytical
Electrochemistry

2020 by CEE
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Full Paper

New Two-fold Amplified Electroanalytical Sensor for Determination of Xanthine in the Presences of Caffeine in Food Samples

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Received: 21 December 2019 / Received in revised form: 5 March 2020 /
Accepted: 7 March 2020 / Published online: 31 March 2020

Abstract- Fast and sensitive response electrochemical sensor based on $CoFe_2O_4$ nanoparticle and 1-buthyl-3-methyl imidazolium bromide room temperature ionic liquids carbon paste electrode ($CoFe_2O_4$/NP/ILs/CPE) is fabricated for the determination of Xanthine (Xt) in the presences of Caffeine in fish and fruit samples. In the first step, $CoFe_2O_4$/NP was characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD) and results showed synthesized nanoparticle with diameter between 26.92-29.22 nm. The electro-oxidation of Xt and Caffeine occurred at a potential about 0.7 V and 1.1 V, which was much better in comparison with bare carbon paste electrode in phosphate buffer (pH=6.0) at the surface of the ($CoFe_2O_4$/NP/ILs/CPE). Other electrochemical techniques were also investigated including cyclic voltammetry, differential pulse voltammetry (DPV) and chronoamperometry at $CoFe_2O_4$/NP/ILs/CPE. At optimized conditions for Xt and Caffeine analysis, DPV peak currents showed a wide linear dynamic ranging from 8.0 nM to 500 µM and detection limit for Xt was 3.0 nM and of course we obtained good dynamic range between 0.1 to 300 µM and good limit of detection 0.05 µM for caffeine. As a result, the proposed electrode could determine Xt in the presences of Caffeine in real samples with good selectivity and high sensitivity.

Keywords- Xanthine; Caffeine; Carbon paste electrode; Fish samples; Fruit samples

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1. INTRODUCTION

In recent years, attention to electrochemical sensors has increased due to high sensitivity, good selectivity and potential portability of the kits for this type of sensor [1-5]. On the other hand, electrochemical methods are the best option due to their easy operation and a low cost for analyzing drug and biological compounds and food additives compared to other analytical methods [6-10]. In recent year, new types of nanomaterials amazing features and many applications [11-20], it helps greatly to the performance of electrochemical sensors [21-30]. Nanomaterials with unexpected and attractive properties have revolutionized the sciences [31-40]. Improving the performance of electrochemical sensors can be one of the amazing effects of nanotechnology [41-50].

Product of purine degradation, Xanthine is formed from hypoxanthine by Xt oxidoreductase [51]. Purine nucleoside phosphorylase is another way of creating Xt. Xt arouses the respiratory center and widely is utilized to cure Childhood Sleep Apnea. Because of its widespread impacts, the medical range of Xt is limited, so that they just are used as a second way to treat asthma and its medical level is between 10 and 20 μg/mL blood. Nausea, nervousness, tremor and tachycardia/arrhythmia are symptoms of toxicity. When Xt is used in high concentrations, it can harm the consumers' healthiness, for instance, contraction force, airways and heart rate and cardiac arrhythmias can be influenced hugely by overuse of Xt [52]. However various studies indicate different toxicity in several subgroups, as well as a wide variety of synthesized Xt (some non-methylated) have also been established to search for substances with higher selectivity for adenosine receptor subgroups or phosphodiesterase enzyme [53-55]. According to what is said, precision in Xt is of great importance.

Another most widely used additive is caffeine. Caffeine is a drug stimulant and lightly bitter methylxanthine derivative which can be found in some soft drinks naturally [56]. Research shows that more than 80 percent of the people on this planet use caffeine on a daily basis [57]. The Europeans with approximately 4.6 kg/person/year intake of caffeine are the largest consumers of this substance in the world. Humans' body can absorb caffeine quickly. The amount of human salivary, demonstrating the degree of absorption, increases approximately 40 minutes after intake of caffeine. Overdose of caffeine can pose a risk such as miscarriage and cancer and hyperactivity in children so Carefulness in its use is important. Recently, many food additives have been used in food industries for a better sale and good income, but the consumer interest is to use healthy food to reduce the risk of illness [58-61]. Many researchers are seeking new methods to measure the food additives [62-70].

In this essay, high sensitive electrochemical nanostructure sensor fabricated for determination Xt in presence caffeine in fruit juice and fish meat. The sensor amplified with $CoFe_2O_4$ nanoparticle and 1-buthyl3-methyl imidazolium bromide as two conductive binders and results confirm the ability of $CoFe_2O_4$/NP/ILs/CPE for determination of Xt and caffeine simultaneously.

2. EXPERIMENTAL

2.1. Chemicals

All of the chemicals employed in this analysis were bought from Sigma and Merck. During the analysis, 1.0×10^{-2} M of Xt and caffeine solution was being prepared daily. Also, various pH values of Phosphate buffer solutions (PBS, 0.1 mol L^{-1}) were used in the initial step. Paraffin (Merck) and 1-buthyl-3-methyl imidazolium bromide (Sigma) were utilized to prepare the suggested sensor as pasting liquids.

2.2. Apparatus

A PGSTAT apparatus (μ-3AUT 71226) (Eco Chemie, the Netherlands) was used to perform different electrochemical techniques including differential pulse voltammetry (DPV), cyclic voltammetry (CV) and chronoamprometry. This analysis was carried out based on a three-electrode system connected to the mentioned apparatus, so that CoFe2O4/NP/ILs/CPE, platinum and Ag/AgCl/KCl_{sat} (Azar Electrode CO.) were utilized as working electrode (WE), counter electrode and reference electrode, respectively. A private computer was employed to run this system using Nova software.

2.3. Preparation of the sensor

A novel sensor ($CoFe_2O_4$/NP/ILs/CPE) was prepared by mixing 0.94 g graphite and 0.06 g $CoFe_2O_4$ nanoparticles with 16% v/v ionic liquid and 84% V/V paraffin at room temperature to obtain wetted carbon paste. The mixture was then filled into a glass tube to prepare a modified sensor for determination of xanthine. To obtain a new surface of novel sensor, it should be polished on a flat surface.

2.4. Preparation of the real samples

Firstly, we divided the tuna fish sample (canned) into small pieces. Secondly, the proteins were precipitated when we added 10 mL of 0.5 M per chloric acid to the mentioned sample. In the next stage, we stirred the obtained sample for 15 min. Finally, the sample was ready when it had been centrifuged at 5500 rpm for 10 min.

Fruit juice bought from local markets in Iran was used as the second real sample. This sample was prepared using filtration. In other words, the filtrate directly was employed in real sample analysis. In both cases, standard addition considered as the second method to evaluate the selectivity and sensitivity of the novel sensor application.

All the usual materials present in real samples with potential interference with the electrooxidation signal of xanthine in presence of caffeine should be investigated for the tables.

3. RESULTS AND DISCUSSION

3.1. $CoFe_2O_4$ nanoparticles characterization

Fig. 1A shows SEM images of $CoFe_2O_4$ nanoparticles that confirming the accurate formation of the nanoparticles in spherical shaper and diameter between 26.92-29.22 nm and X-ray pattern show the presences of planes with miler indexes [111]; [220]; [311]; [222]; [400]; [422]; [511] and [440] relative to $CoFe_2O_4$ nanoparticle (Fig. 1B) [71].

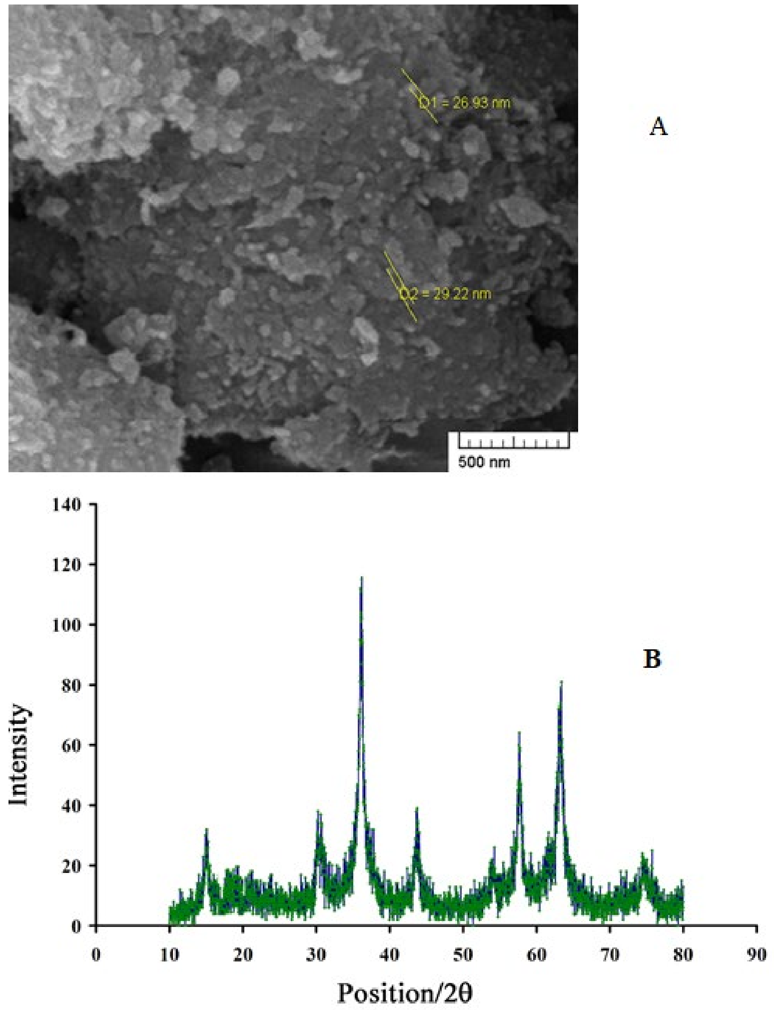

Fig. 1. A) SEM image of $CoFe_2O_4$ nanoparticles and B) X-ray pattern of $CoFe_2O_4$ nanoparticles

3.2. Voltammetric assay

The oxidation behavior of Xt was examined in buffer phosphate solution with different pH values of 4.0-8.0 at the surface of $CoFe_2O_4$/NP/IL/CPE (Fig. 2). The results confirm that the peak potential of the redox couple was pH-dependent with a slope of −0.0516. V/pH at 25 °C which was equal to the Nernstian value for a one-electron, one-proton electrochemical reaction.

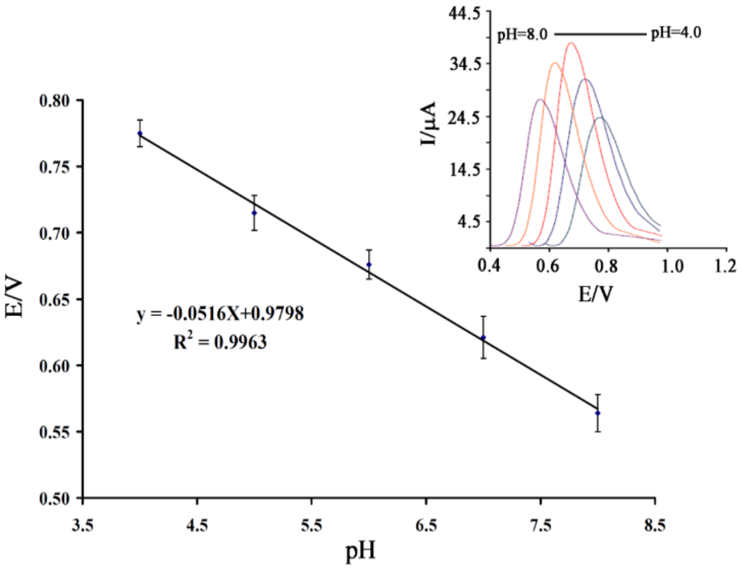

Fig. 2. The plot of potential, E_p, *vs.* pH for the electro-oxidation of 240 µM Xt at the surface of $CoFe_2O_4$/NP/IL/CPE (n=4). Inset: influence of pH on differential pulse voltammetry at the surface of the proposed sensor

Electrochemical voltammetric results show that there is an optimization condition at pH 6.0, meaning that the most value of the current and the least value of the potential were obtained at this pH level. Fig. 2 shows the pH optimization selected at pH=6 in this work to investigate other electrochemical parameters.

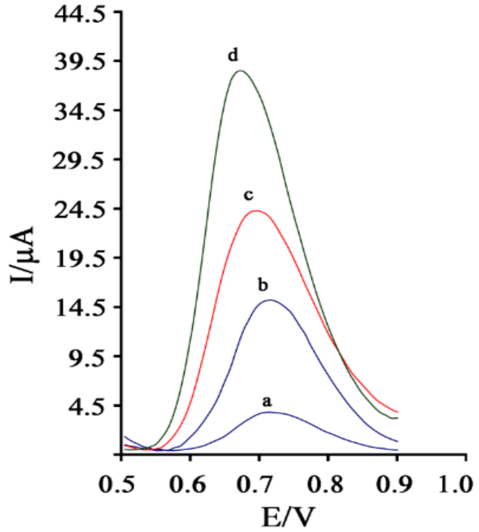

Fig. 3. The Differential pulse voltammetric signals of (d) $CoFe_2$O4/NP/IL/CPE, (c) IL/CPE, (b) $CoFe_2O_4$/NP/CPE, and (a) CPE in 240 µM Xt (pH 6.0)

Electrochemical voltammogram of Xt was investigated at different surfaces of carbon paste electrode in 240 µM of Xt (Fig. 3). The results show that by using unmodified electrode (CPE, curve a), there is a lesser amount of the current and greater amount of the potential.

Use of ionic liquid for this sensor (IL/CPE, curve c) improved current value, with a more improved current value obtained using $CoFe_2O_4$ nanoparticle carbon paste electrode ($CoFe_2O_4$/NP/CPE, curve c).

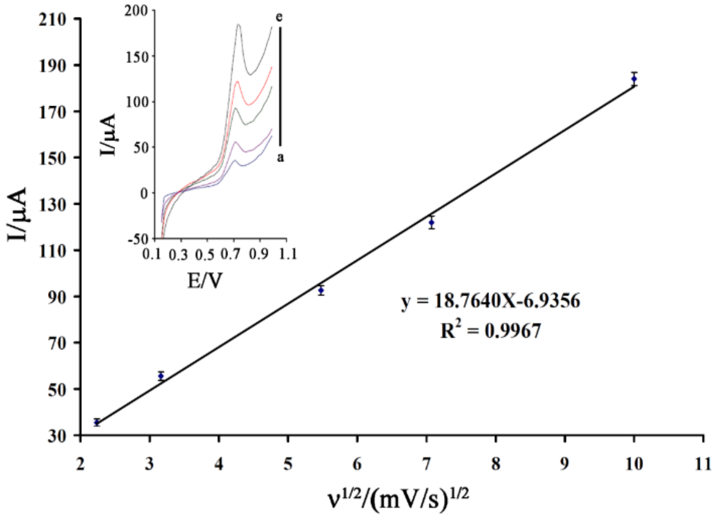

Fig. 4. The plot of I_{pa} *vs*. $\nu^{1/2}$ for the oxidation of Xt at $CoFe_2O_4$/NP/IL/CPE (n=4). The inset shows the linear sweep voltammograms of Xt (150 µM) in 0.1 M phosphate buffer (pH 6.0) at different scan rates of a) 5, b) 10, c) 30, d) 50, and e) 100 mV/s

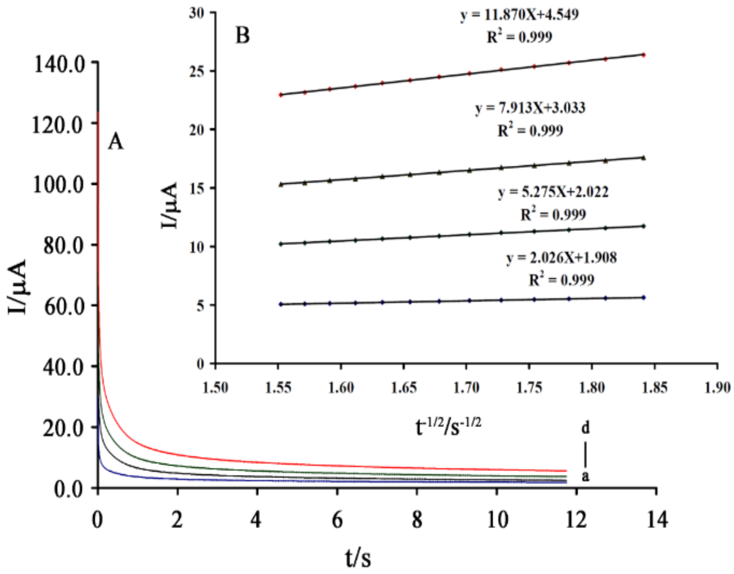

Fig. 5. The chronoamperograms obtained at $CoFe_2O_4$/NP/IL/CPE in the presence of (a) 50, (b) 100, (c) 150, (d) 200 µM Xt in the buffer solution (pH 6.0)

Finally, the highest value of current and the least value of potential were obtained through a combination of ionic liquid and nanoparticles for modification of electrode ($CoFe_2O_4$/NP/IL/CPE). Fig. 4 shows the linear sweep voltammogram of 240 µm Xt (Fig. 4 inset). In continuous the effect of the scan rate (ν) on the oxidation current of xanthine was also studied (Fig. 4). The results showed that the peaks current increased linearly with increasing the square root of the scan rate ranging from 5 to 100 mVs^{-1}. There was a relationship between the peak current (Ip) and $v^{1/2}$ showing the diffusion-controlled process for the electro-oxidation of Xt on the surface of the modified electrode ($CoFe_2O_4$/NP/IL/CPE) in this work. It is necessary to know the rate-determination step. So the Tafel plot was drawn, the slope of which was equal to 2.3 RT/n(1−α)F to obtain a value of α equal to 0.8.

Choronoamprogram of Xt is another electrochemical parameter that should be studied at ($CoFe_2O_4$/NP/IL/CPE) at potential 700 mV and in buffer phosphate solution (pH=6) to obtain diffusion coefficient of D, we use four different concentrations of Xt (50 μM, 100 μM, 150 μM and 200 μM) is described by the Cottrell equation (Fig. 5). We drew the experimental plots of I *vs.* $t^{-1/2}$ with four different concentrations of Xt (Fig. 6). According to the results, the slope of it should be equal to the Cottrell equation, and D value was calculated 2.7×10^{-5} cm^2/s.

3.3. LDR and LOD determination

We use another electrochemical technique named differential pulse voltammetry (DPV) to determine Xt and caffeine concentrations.

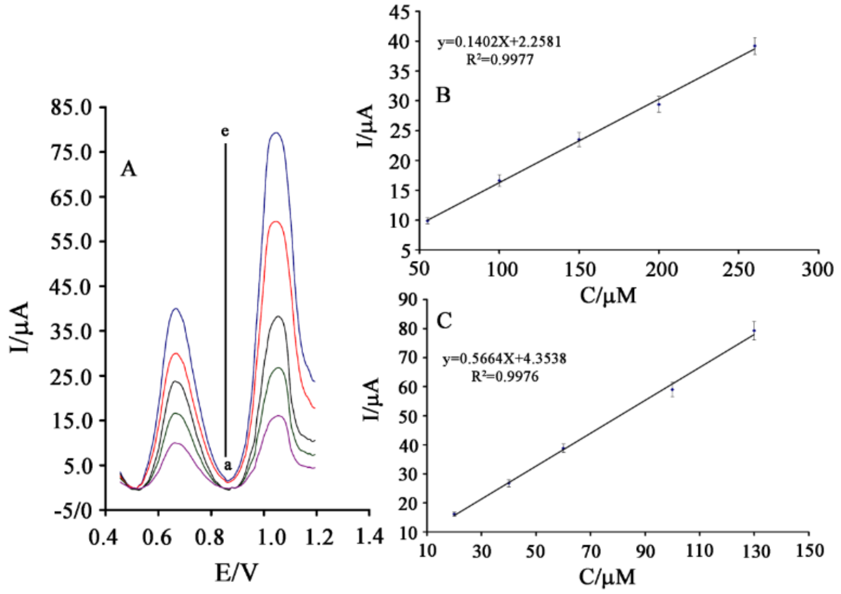

Fig. 6. The differential pulse voltammograms of $CoFe_2O_4$/NP/IL/CPE in pH 6.0 containing different concentrations of Xt and caffeine (from inner to outer) mixed solutions of: a) 55+20.0; b) 100.0+ 40.0; c) 150.0+60; d) 200.0+ 80.0; and e) 260.0+ 130.0 μM Xt and caffeine, respectively. B and C plots of the peak currents as a function of Xt and caffeine concentrations, respectively (n=4)

The voltammograms show that the plot of peak current *vs*. Xt and caffeine concentration individually. A linear dynamic range 8.0 nM-500μM with a good detection limit of 3.0 nM for Xt and wide range between 0.01-300 μM with detection limit of 0.05 μM for caffeine obtained.

3.4. Simultaneous determination of xanthine and caffeine

The ability of the modified sensor ($CoFe_2O_4$/NP/IL/CPE) in the simultaneous determination of Xt and caffeine was studied by changing the concentration of Xt and caffeine simultaneously (Fig. 6 A).

The DPV results show two good oxidation peaks at potentials of 700 and 1100 mV corresponding to the oxidation of Xt and caffeine separately. Plot of current *vs.* concentration Xt and caffeine was down. The slope of current *vs*. concentration of simultaneously Xt is similar to slope of Xt in absence of caffeine (Fig. 6 B) and slope of simultaneously caffeine is similar to slope of caffeine in absence of Xt (Fig. 6 C). This similarity confirms, this is possible for novel sensor to determine Xt and caffeine simultaneously.

3.5. Interference study

In order to assess the selectivity of the suggested sensor (CoFe2O4/NP/IL/CPE) in determination of Xt and caffeine in presence of 15.0 μm Xt, the effect of different foreign species found in real samples was investigated. We took the tolerance limit as the total foreign substance concentration resulting in an estimated relative error of ±5 percent. The outcomes are provided in Table 1.

Table 1. Interference study for the determination of 15.0 μM Xt

Species	**Tolerant limits** **($W_{Substance}/W_{Analytes}$)**
K^+, Br^-, F^-, Ca^{2+}, NO_3^-, Cl^-	100
Vitamin B_2, Glucose, Vitamin B12, ascorbic acid*,	700
Pehnyl-Alanine, glycine, Alucine	300
Uric acid	50
Starch	Saturation

*Ascorbic acid interference can be minimize using ascorbic oxidase

3.6. Real sample analysis

To study the application of $CoFe_2O_4$/NP/IL/CPE, it was applied for the analysis of additives in real samples such as canned tuna fish and fruit juice. The measurements data (Table 2) confirm the good ability and accuracy of the modified electrode for determination of xanthine in presence of caffeine in food sample.

Table 2. Determination of Xt and Caffeine in commercial samples (n=4)

Sample	Added Xt (mg/L)	Founded Xt (mg/L)	Recovery (%)	Added Caffeine (mg/L)	Founded Caffeine (mg/L)	Recovery (%)
Fruit juice drink	---	<LOD	---	---	<LOD	---
	10.00	9.75±0.44	97.5	10.00	10.34±0.43	103.4
fish meat	---	10.33±0.66	---	---	<LOD	---
	5.00	15.76±0.95	102.8	---	<LOD	---

4. CONCLUSION

In this project, $CoFe_2O_4$ nanoparticles and 1-buthyl3-methyl imidazolium bromide as the binder were fabricated to design a rapid and sensitive electrode for determination of Xt in presence of caffeine using many different electrochemical parameters. Differential pulse voltammetry results showed a good linear dynamic ranging range 8.0 nM-500 µM with a good detection limit of 3.0 nµ for Xt and wide range between 0.01-300 µM with detection limit of 0.05 µM for caffeine at the surface of the proposed electrode. Application of $CoFe_2O_4$/NP/IL/CPE was acceptable in real samples and the statistic calculations confirmed the accuracy and validity of the sensor.

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