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Original scientific paper

A sensitive sensor based on graphitic carbon nitride/1-butyl-3-methylimidazolium hexafluorophosphate ionic liquids modified carbon paste electrode for sensing of carmoisine dye in food samples

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Abstract

The electrochemical sensors for carmoisine detection were explored and applied in the field of food safety because of the striking merits of fast detection speed, high sensitivity and easy operation. Herein, we developed a high-performance electrochemical carmoisine sensor based on g-C_3N_4 and ionic liquids (ILs), prepared by a one-pot hydrothermal synthesis method. Most importantly, the sensing performances of electrochemical carmoisine sensors based on g-C_3N_4 (graphitic carbon nitride)/IL (1-butyl-3-methylimidazolium hexafluorophosphate) are better than the bare carbon paste electrode (CPE) sensors. The detection limit of this sensor (g-C_3N_4/ILs/CPE) determined via differential pulse voltammetry was 0.1 µM over a wide linear range of 0.4–125.0 µM.

Keywords
Electrochemical sensors; differential pulse voltammetry; food analysis; chemically modified electrodes

Introduction

Color additives were used by the ancient Egyptians. Historians estimated that colored food appeared by 1500 B.C. Now, food manufacturers deem color as a vital criterion for food choice; thus, synthetic colorants are now being widely used due to their coloring properties, uniformity, stability, low cost, and availability in many hues, which overall increase the esthetic quality (Mpountoukas Below the acceptable daily intake (ADI) limit, the intake of artificial colorants seems to be safe. However, consumption of high concentrations of these artificial colorants may result in many ailments, especially in children, due to their low body weights. Children are the major consumers of colored food in developing countries, and thus, they are at high risk because they often exceed prescribed ADIs [1,2].

Carmoisine dye is a synthetic azo food dye broadly used in food industries to get on red color products. Because this dye is not carcinogenic and toxic, it is utilized as a coloring agent in confectionery, ice cream, gelatin desserts drinks, medicine and cosmetics. However, at high doses of carmoisine, the vital organs in the human body, such as the liver and kidneys, can be damaged and the gens can be changed from the mediated reaction of this dye with DNA. In Europe, carmoisine is authorized for use in certain foods and beverages, such as cheeses, dried fruit, and some alcoholic beverages, and is permitted for use as an excipient in medications. Therefore, the determination of this type of food additive is very important in food products [3-5].

Until now, different methods such as chromatography, spectroscopy and liquid-liquid microextraction have been reported for the determination of carmoisine dye [6-10]. In addition, voltammetry was also used to determine carmoisine dye based on its electrochemical activity [11,12].

Electrochemical sensors showed many advantages for the analysis of food compounds due to their fast response. High oxidation/reduction over-potential and low sensitivity of electroactive compounds at the surface of unmodified electrodes are major problems for trace level analysis [13-20]. Therefore, modified electrodes were suggested as highly powerful tools for trace-level analysis of electroactive compounds. According to the previously published papers, room temperature ionic liquids and nanomaterials can improve the sensitivity of electrochemical sensors for the analysis of electroactive materials [21-35].

Room temperature ionic liquids (RTILs) are an exciting class of solvent, receiving much attention in recent years as a replacement for conventional solvents in many applications. As their name suggests, ionic liquids are salts that melt below 100 °C and room temperature ionic liquids exist in the liquid state at room temperature (25 °C). They are typically comprised of a bulky organic cation (*e.g.,* imidazolium, tetraalkylammonium or pyrrolidinium) paired with an inorganic/organic anion (*e.g.,* tetrafluoroborate, hexafluorophosphate, bis(triflurormethylsulfonyl)imide). RTILs possess several archetypal properties such as low volatility, high physical and chemical stability, wide electrochemical windows, intrinsic conductivity, wide liquid range, high polarity and the ability to dissolve a wide range of compounds. In addition to the use of RTILs in applications such as catalysis, 'green' chemistry, organic reactivity, analytical methods, biocatalysis and enzymes and applications in the chemical industry, they have also become recognized as ideal alternative electrolytes for use in many electrochemical devices, such as actuators, capacitors, batteries, fuel cells, solar cells and sensors [36-38].

Nanomaterials have become the most popular material due to their unique properties of low cost, large surface area, good biocompatibility and distinct catalytic activity [39]. Many researchers have been studying nanomaterials for electrochemical sensors [40-47]. Intensive research on g-C_3N_4 has been conducted since its appearance. With amazing properties of high chemical and thermal stability, medium energy gap (2.7 eV), ideal electronic structure, non-toxic, low density and high conductivity, g-C_3N_4 has become one of the hottest materials for modifying electrochemical sensors [48-50].

Therefore, in the present study, we fabricated a highly sensitive voltammetric sensor based on a carbon paste electrode modified with g-C_3N_4 and ionic liquids for the analysis of carmoisine in food samples. The fabricated sensor showed good electrical conductivity compared to the unmodified electrode and improved the quality of the carbon paste electrode for analysis of carmoisine in food samples.

Experimental

Apparatus and chemicals

All the electrochemical measurements were carried out on a PGSTAT302N potentiostat/galvanostat Autolab consisting of a traditional three-electrode system: a bare or modified CPE as the working electrode, an Ag/AgCl as the reference electrode and a Pt wire as a counter electrode. Solution pH values were determined using a 713 pH meter combined with a glass electrode (Metrohm, Switzerland). Carmoisine and other chemicals used were analytical grade and were purchased from Merck. Ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate) was purchased from Sigma Aldrich co. Graphitic carbon nitride nanostructures were synthesized in our laboratory and a typical SEM is shown in Figure 1.

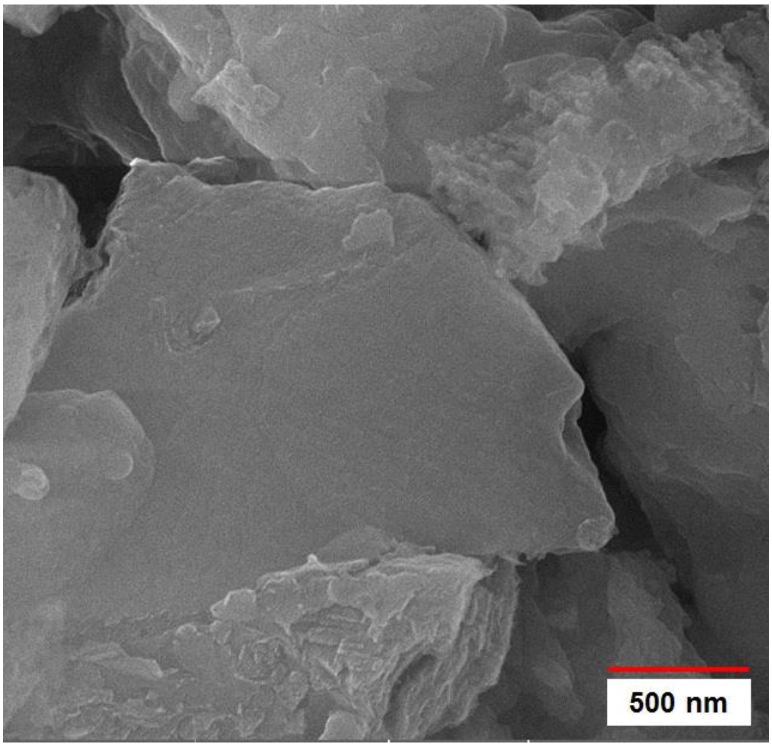

Figure 1. *FE-SEM image of g-C_3N_4*

Preparation of g-C_3N_4/ILs/CPE

g-C_3N_4/ILs/CPE was prepared by mixing 0.04 g of g-C_3N_4 with 0.96 g graphite powder and approximately ~0.8 mL of ionic liquids with a mortar and pestle. The paste was then packed into the end of a glass tube (3.4 mm internal diameter and 15 cm long). A copper wire inserted into the carbon paste provided the electrical contact. For comparison, ionic liquids/carbon paste electrode (IL/CPE) in the absence of g-C_3N_4, (g-C_3N_4/CPE) consistent with g-C_3N_4 powder and paraffin oil, and bare carbon paste electrode consisting of graphite powder and paraffin oil were also prepared in the same way.

The surface area of g-C_3N_4/ILs/CPE and the bare CPE were obtained by CV using 1 mM $K_3Fe(CN)_6$ at different scan rates. Using the Randles-Sevcik formula for g-C_3N_4/ILs/CPE, the electrode surface was found 0.288 cm^2 which was about 3.2 times greater than bare CPE.

Results and discussion

Electrochemical behavior of carmoisine at the surface of various electrodes

The effect of the electrolyte pH on the oxidation of 35.0 µM carmoisine was investigated at g-C_3N_4/ILs/CPE using differential pulse voltammetry (DPV) measurements in the phosphate-buffered solution (PBS) in the pH range from 2.0 to 9.0. According to the results, the oxidation peak current of carmoisine depends on the pH value and increases with increasing pH until it reaches the maximum at pH 7.0, and then decreases with higher pH values. The optimized pH corresponding to the higher peak current was 7.0, indicating that protons are involved in the reaction of carmoisine oxidation.

The electrochemical behavior of carmoisine was investigated by differential pulse voltammetry. The differential pulse voltammograms obtained using the bare CPE (curve d), ILs/CPE (curve c), g-C_3N_4/CPE (curve b) and g-C_3N_4/ILs/CPE (curve a) in 0.1 M PBS (pH 7.0) in the presence of 60.0 µM carmoisine are shown in Figure 2. On a bare CPE, an irreversible signal with a low oxidation current of ~2.5 µA was obtained with a peak potential of ~740 mV. After modifying the electrode with ILs/CPE, and g-C_3N_4/CPE, the peak current increased up to ~6.06 and ~8.93 µA, respectively. In contrast, g-C_3N_4/ILs/CPE exhibited an enhanced sharp anodic peak current (I_{pa} = 12.0 µA) at a much lower overpotential E_p = 450 mV. These results confirmed that the g-C_3N_4/ILs improved the sensitivity of the modified electrode by enhancing peak current and decreasing the overpotential of the oxidation of carmoisine.

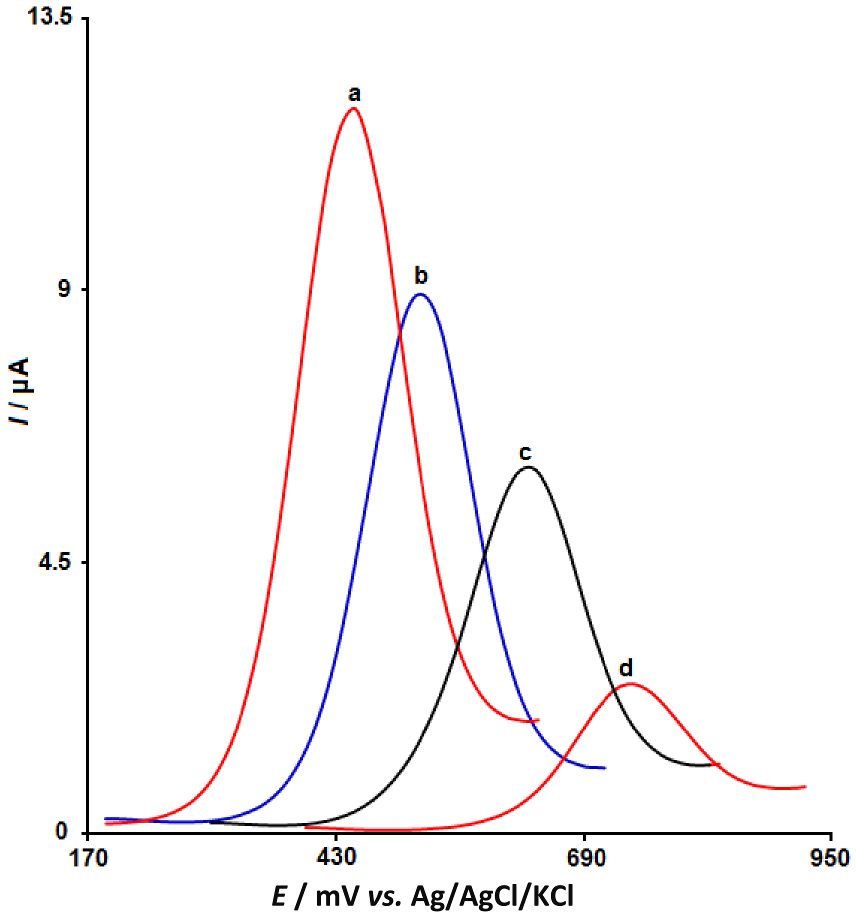

Figure 2. *Differential pulse voltammograms of (a) g-C_3N_4/ILs/CPE, (b) g-C_3N_4 /CPE, (c) ILs/CPE and (d) bare CPE in 0.1 M PBS (pH 7.0) in the presence of 60.0 µM carmoisine at the scan rate 50 mV s^{-1}*

Effect of scan rate on the determination of carmoisine at g-C_3N_4/ILs/CPE

The influence of the scan rate (ν) on the peak currents (I_{pa}) of carmoisine at g-C_3N_4/ILs/CPE was investigated by linear sweep voltammetry (LSV). Figure 3 shows the voltammetric response of 35.0 µM carmoisine at g-C_3N_4/ILs/CPE at different scan rates in the range of 5 to 400 mV s^{-1}. The oxidation peak current of carmoisine increases linearly with increasing scan rate. A linear regression equation was obtained from the plot Ipa and *vs.* $\nu^{1/2}$ (square root of scan rate) as follows; $I_{pa} = 1.5769\,\nu^{½} - 1.4736$ (R^2 = 0.9995) for the oxidation process, which indicates that the reaction of carmoisine at g-C_3N_4/ILs/CPE is diffusion controlled.

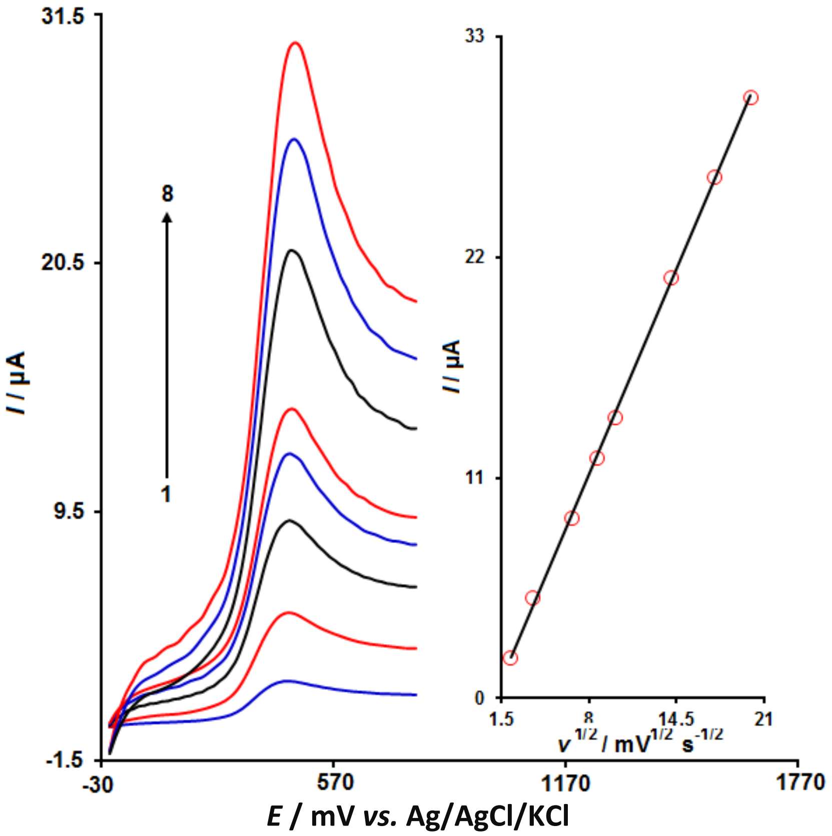

Figure 3. *Linear sweep voltammograms of g-*C_3N_4*/ILs/CPE in 0.1 M PBS (pH 7.0) containing 35.0 µM carmoisine at various scan rates; 1-8 correspond to 5, 15, 45, 75, 100, 200, 300 and 400 mV* s^{-1}*, respectively. Inset: variation of anodic peak current* vs. $v^{1/2}$

In addition, the plot of log *I vs. E* (Tafel plot) is linear, having the following regression equation: log $I = 0.1743E + 0.3498$ (R^2=0.9945) (Figure 4). Assuming that n_α=1, the value of α was calculated as ~0.71.

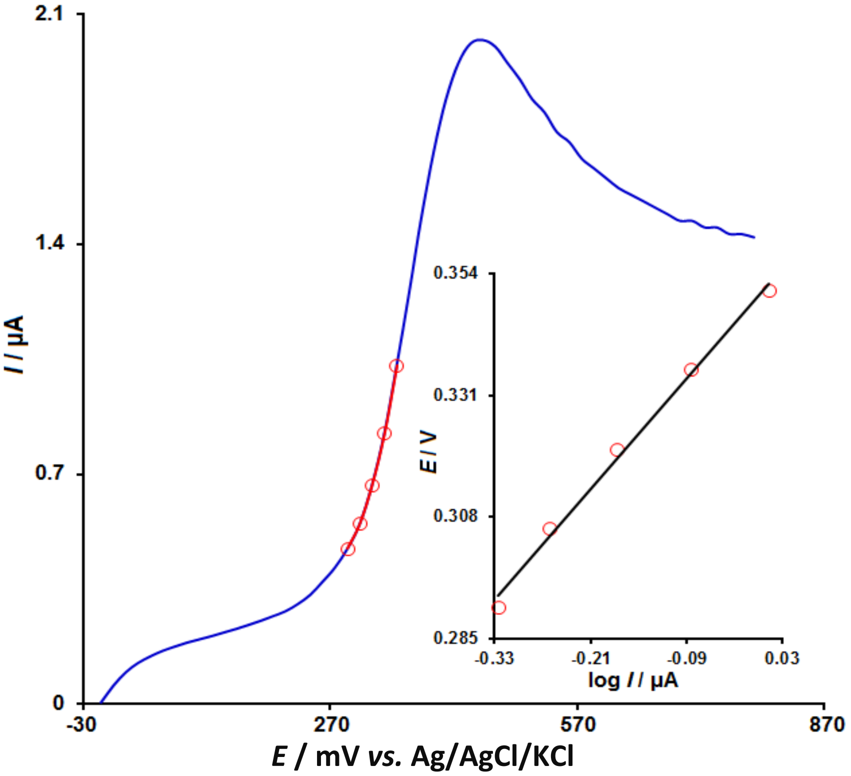

Figure 4. *Tafel plot for g-*C_3N_4*/ILs/CPE in 0.1 M PBS (pH 7.0) in the presence of 35.0 µM carmoisine and scan rate 10 mV* s^{-1}

Chronoamperometric analysis

The analysis of chronoamperometry for carmoisine samples was performed using g-C_3N_4/ILs/ /CPE *vs.* Ag/AgCl/KCl (3.0 M) at 0.5 V. The chronoamperometric results of different concentrations of carmoisine in PBS (pH 7.0) are demonstrated in Figure 5.

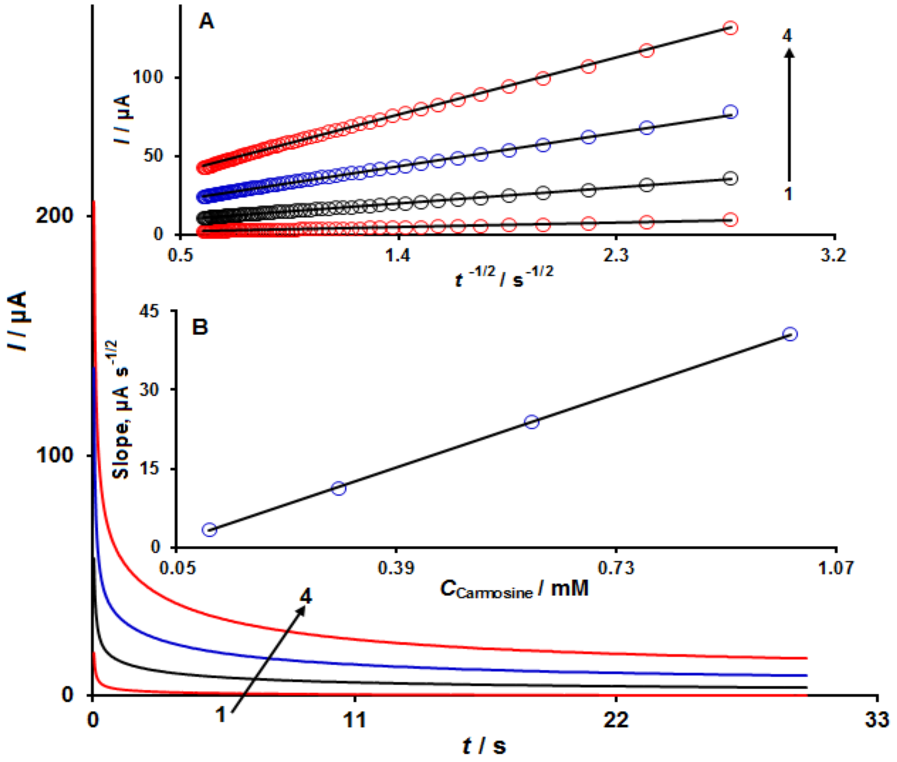

Figure 5. *Chronoamperograms obtained at g-C_3N_4/ILs/CPE in 0.1 M PBS (pH 7.0) for different concentration of carmoisine. The 1-4 correspond to 0.1, 0.3, 0.6 and 1.0 mM of carmoisine. Insets: (A) Plots of* I vs. $t^{-1/2}$ *obtained from chronoamperograms 1-4. (B) A plot of the slope of the straight lines against carmoisine concentration*

The Cottrell equation for chronoamperemetric analysis of electroactive moieties under mass transfer limited conditions is as in equation (1):

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \tag{1}$$

where D represents the diffusion coefficient (cm^2 s^{-1}), and C_b is the applied bulk concentration (mol cm^{-3}). Experimental results of I *vs.* $t^{-1/2}$ were plotted in Figure 5A, with the best fits for different concentrations of carmoisine. The resulting slopes corresponding to straight lines in Figure 5A, were then plotted against the concentration of carmoisine (Figure 5B). The mean value of D was determined to be 7.2×10^{-5} cm^2/s according to the resulting slope and Cottrell equation.

Calibration curve

Because DPV commonly has a higher sensitivity than cyclic voltammetry technology, the DPV technique was applied for the quantitative detection of carmoisine. Figure 6 shows the differential pulse voltammograms of carmoisine at various concentrations using g-C_3N_4/ILs/CPE.

As seen, the oxidation peak currents of carmoisine enhance gradually by increasing its concentration. The oxidation peak currents (I_{pa}) show a good linear relationship with the concentrations of carmoisine ranging from 0.4 M to 125.0 μM. The linear equation is $I_{pa} = 0.1914C_{Carmoisine} + 0.8746$ ($R = 0.9992$) (Fig. 6 (inset)). Also, the detection limit, C_m, of carmoisine was obtained using the equation (2):

$$C_m = 3s_b / m \tag{2}$$

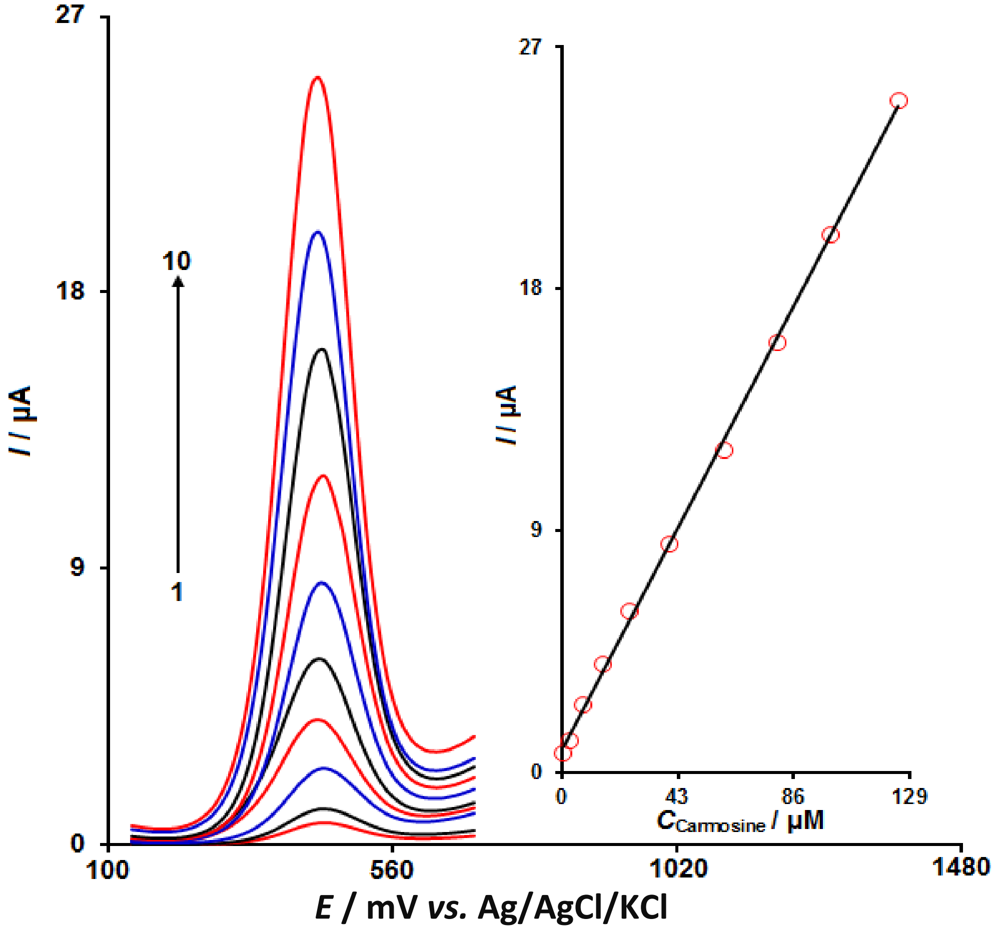

Figure 6. *DPVs of g-C_3N_4/ILs/CPE in 0.1 M (pH 7.0) containing different concentrations of carmoisine. Numbers 1–10 correspond to 0.4, 2.5, 7.5, 15.0, 25.0, 40.0, 60.0, 80.0, 100.0 and 125.0 µM of carmoisine. Inset: plot of the electrocatalytic peak current as a function of carmoisine concentration in the range of 0.4-125.0 µM*

In the above equation, m is the slope of the calibration plot (0.1914 µA.µM^{-1}) and s_b is the standard deviation of the blank response obtained from 20 replicate measurements of the blank solution. The detection limit is 0.1 µM.

Stability, repeatability, and reproducibility

The long-term stability test of the g-C_3N_4/ILs/CPE using DPV was performed at room temperature. The results exhibited that the peak current of 50.0 µM carmoisine at the g-C_3N_4/ILs/CPE stayed at 93.7 % of its primary current after 7 days, 92.9 % after 14 days, and 89.1 % after 21 days, indicating the superior long-term stability of the proposed sensor.

The oxidation of carmoisine (50.0 µM) on the same g-C_3N_4/ILs/CPE electrode was performed by ten repeated voltammetric measurements, the results of which confirmed the superior repeatability of a fabricated sensor with the relative standard deviation of 3.2 %.

Similar conditions were provided for the carmoisine of the response currents of carmoisine (50.0 µM) on five g-C_3N_4/ILs/CPE, the results of which confirmed the excellent reproducibility of a fabricated sensor with the RSD of value 3.8 %.

Analysis of real samples

The real samples for the analysis were prepared and quantified by the DPV method. The developed sensor was applied to detect carmoisine in lemon juice and powdered juice samples. The results are summarized in Table 1. Each measurement was repeated three times. The recovery and relative standard deviation (RSD) values confirmed that the g-C_3N_4/ILs/CPE sensor has a great potential for analytical application.

Table 1. *The application of g-C_3N_4/ILs/CPE for determination of carmoisine in real samples (n=3)*

Sample	Concentretion, µM		Recovery, %	RSD, %
	Spiked	Found		
Lemon juice	5.0	4.9	98.0	3.1
	7.0	7.2	102.9	1.9
Powdered juice	5.5	5.6	101.8	2.2
	7.5	7.3	97.3	3.0

Conclusion

A facile electrochemical sensor for carmoisine detection was designed and constructed by using g-C_3N_4/ILs modified carbon paste electrode. The electrochemical sensor possessed excellent performance with wide linear range (0.4-125.0 µM) and a low limit of detection (0.1 µM), which is due to the good rich accessible active sites. Additionally, the as-prepared electrochemical sensor has good selectivity, reproducibility, repeatability, stability, and potential application in real samples.

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