*Anal. Bioanal. Electrochem., Vol. 16, No. 12, 2024, 1098-1108*
*https://www.doi.org/10.22034/abec.2024.719413*

*Analytical &*
*Bioanalytical*
*Electrochemistry*

2024 by CEE
www.abechem.com

*Full Paper*

# A New Sensor Electrode for Caffeine Determination Using Square Wave Voltammetry: Cytosine Modified Glassy Carbon Electrode

**Şeyma Korkmaz,[1] Aysel Alovset Rustamova,[1,2] Shabnam Jamaladdin Aliyeva,[1,2] Hafizullah Sharifi,[1] İbrahim Ender Mülazımoğlu,[3,]* and Ayşen Demir Mülazımoğlu[3]**

[1]*Necmettin Erbakan University, Institute of Science, Chemistry Department, Konya, Turkey*
[2]*Azerbaijan State Agricultural University, Azerbaijan*
[3]*Necmettin Erbakan University, Ahmet Keleşoğlu Education Faculty, Chemistry Department, Konya, Turkey*

*Corresponding Author, Tel.: +905061064651
E-Mail: iemulazimoglu@erbakan.edu.tr

*Received: 12 November 2024 / Received in revised form: 19 December 2024 /*
*Accepted: 24 December 2024 / Published online: 31 December 2024*

---

**Abstract**- In this study, cytosine (CT) modified glassy carbon (GC) electrode was used to investigate the detectability of caffeine (CAF) found in foods such as tea, coffee and soft drinks. Using the cyclic voltammetry (CV) technique, 1 mM CT was prepared in pH 7.2 phosphate buffer solution (PBS) and modified at a scanning rate of 100 mV $s^{-1}$ in the potential range of 1.0 V to 2.1 V. CT/GC electrode was characterized by CV, scanning electron microscope (SEM) and electrochemical impedance spectroscopy (EIS) techniques. 1 mM CAF solutions were prepared in different supporting electrolytes such as $H_2SO_4$, HCl, $HNO_3$, and $CH_3COOH$ within the potential range of +0.5 V to +1.7 V using square wave voltammetry (SWV) technique. Effect of the supporting electrolyte concentrations were investigated. CAF solutions in the concentration ranges of 0.75 to 0.05 mM and 0.025 to 0.0025 mM were prepared in 100 mM $H_2SO_4$ supporting electrolyte and calibration graphs were drawn. Line equations were created using the values read from the drawn graphs. Correlation coefficient ($R^2$) values were calculated as 0.9947 and 0.9961, respectively. Using the calibration curves, limit of detection (LOD) and limit of quantification (LOQ) were found as 0.93 µM and 2.79 µM.

**Keywords-** Cytosine; Caffeine; Glassy Carbon Electrode; Cyclic Voltammetry; Square Wave Voltammetry

---

## 1. INTRODUCTION

Caffeine (CAF), also known as 1,3,7-trimethylxanthine with the chemical formula $C_8H_{10}N_4O_2$, is a plant-derived purine alkaloid. Its other name is 3,7-dihydro-1,3,7-trimethyl-H-purine-2,6-dione, and it is also referred to as mateine or guaranine [1]. The chemical structure of CAF is shown in Figure 1. CAF is produced by plants as a means of self-defense and is generally found in seeds; it is known for its psychoactive stimulant and diuretic effects [2]. CAF, which is crystalline, has a bitter taste and is known for its white color, and it is found in various medications [3]. Additionally, tea, coffee, energy drinks, some drugs, some soft drinks and cocoa are known as primary sources of CAF, and it naturally occurs in the fruits, leaves, and seeds of more than 63 plant species worldwide, including Yaupon Holly, guarana, and yerba mate. According to research conducted by the U.S. Food and Drug Administration, the maximum amount of CAF in beverages is typically below 200 mg/L, and the lethal dose is reported to be only 10 g of CAF (approximately 170 mg/kg body weight) per person [4].

CAF, in moderate doses (200-300 mg), elevates mood, improves psychomotor performance, and enhances intellectual performance and electroencephalographic stimulation. However, at higher doses, symptoms such as increased anxiety, headaches, tremors, irritability, insomnia, and nausea may be observed [5]. When daily CAF intake is excessive, it is known to lead to side effects such as anxiety, increased blood pressure, and cardiovascular diseases, as well as health issues like heart failure, hypertension, and arrhythmias [6-8].

Considering the physiological effects of CAF on metabolism, it is important to determine the amount of CAF in beverages and foods using a more sensitive, simple, rapid, and cost-effective analytical method. Therefore, numerous analyses have been conducted aimed at developing reliable methods for CAF determination. These methods include spectrophotometry, high-performance liquid chromatography [9], UV-Vis spectrophotometry [10], capillary electrophoresis [11], Fourier-transform infrared spectroscopy [12], near-infrared spectroscopy [13], fluorescence polarization immunoassays [14], and voltammetry [15,16].

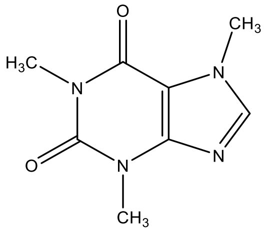

**Figure 1.** Chemical structure of CAF

Electrochemical sensors have become significant in environmental, biological, and medical analyses due to their simplicity, sensitivity, and cost-effectiveness. In this study; (I) Modification of CT by GC electrode, (II) Characterization of CT/GC electrode surface with CV, EIS and SEM, (III) Effect of scan rate at CT/GC electrode, (IV) Optimization of the supporting electrolyte, (V) Effect of the supporting electrolyte concentrations and (VI) Standard calibration curve for CAF were investigated.

## 2. EXPERIMENTAL SECTION

### 2.1. Equipment and chemicals

For electrochemical measurements, a Reference 600+ potentiostat/galvanostat/ZRE from Gamry Instruments (USA) electrochemical analyzer was used. The cell system employed a BAS model C3 cell stand. The working electrode was a GC electrode (0.071 cm²) (BAS Model MF-2012, USA). The reference electrode was $Ag/Ag^+$ electrode (BASi, MF-2056, USA) for non-aqueous media and Ag/AgCl 3M KCl electrode (BASi MF-2056, USA) for aqueous media. A platinum electrode (BASi MW-1032, USA) was used as the counter electrode. The calibration of the reference electrodes was carried out using the half-wave potentials ($E_{1/2}$) of ferrocene for non-aqueous and $Fe(CN)_6^{3-}$ for aqueous medias. The chemicals used were purchased from Sigma-Aldrich and Riedel, ensuring they were of high purity by preparing their solutions. For the preparation of aqueous solutions, as well as for cleaning glassware and electrodes, 18.2 MΩ cm ultra-pure water (MP MINIPURE Purification System, DEST UP, USA) was used.

### 2.2. Preparation of the working electrode

GC electrode used as the working electrode in this study was subjected to a cleaning and polishing procedure prior to use. Initially, GC electrode surface was gently rubbed with circular motions on slightly moistened sandpaper (4000 grit) without applying excessive pressure. It was then polished with alumina suspensions of 1.0, 0.3, and 0.05 µm sizes by rubbing in circular motions on a polishing cloth (Buehler, USA). Subsequently, the electrode was sonicated in an ultrasonic bath, first in water and then in a 1:1 mixture of isopropyl alcohol/acetonitrile ($CH_3CN$) for 10 minutes [17].

## 3. RESULTS AND DISCUSSION

### 3.1. Modification of CT by GC electrode

In electroanalytical studies, modifying electrode surfaces either as a single or multilayer is a commonly used method to obtain new surfaces that are selective and specific to different species. In this study, similar modification pathways were followed by using CT as a modifier for GC electrode surface, and thus the determination of CAF using the single-layer CT modified electrode surface was investigated.

The modification of GC electrode surface was executed using a 1 mM CT solution prepared in PBS 7.2, with an Ag/AgCl 3M KCl electrode, applying a potential range of 1.0 V to 2.1 V for 10 cycles. Although the modification was observed starting from the second cycle, the procedure was continued for 10 cycles to ensure complete coverage of the electrode surface with no pinholes.

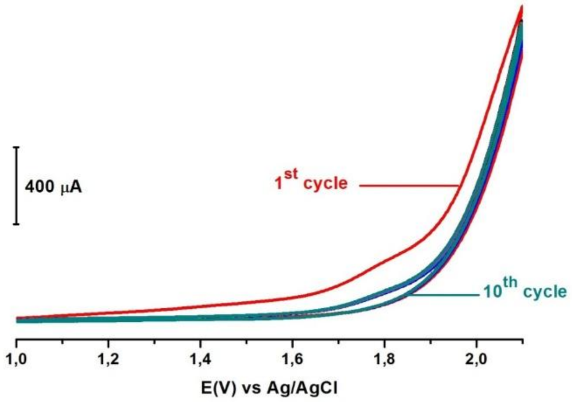

**Figure 2.** CT modification voltammogram on bare GC electrode surface at potential range +1.0 V and +2.1 V using 100 mV $s^{-1}$ scanning rate for 10 cycles

It is understood from the modification voltammogram given in Figure 2 that the modification processes using the amine oxidation method were successfully applied due to the $–NH_2$ functional groups found in the CT structure. As seen in the modification voltammogram, a 10-cycle process was sufficient to ensure the electrode surface was totally covered by CT without any small gaps known as pinholes. Since no polymerization was observed in the voltammogram, CT was evaluated as being modified onto GC electrode surface in a single layer. The obtained voltammogram is presented in Figure 2.

### 3.2. Characterization of CT/GC electrode surface with CV, EIS and SEM

One of the most important stages in electroanalytical studies is the ability to perform surface characterization processes in a way that supports modification processes. For this purpose, various techniques such as electrochemical, spectroscopic, microscopic, etc., can be employed. In this study, both electrochemical and microscopic techniques were used for surface characterization. The electrochemical characterization processes utilized voltammetry in a non-aqueous media with a ferrocene redox probe and in an aqueous media with a ferri-cyanide redox probe. When examining the voltammograms obtained from both characterization processes, it was observed GC electrode surface was fully covered by the CT, preventing electron transfer. Additionally, the EIS technique, using a ferri/ferrocyanide redox probe in an aqueous media, further supported the voltammetric surface characterization processes. Here, it was also observed that the CT/GC electrode surface did not facilitate electron transfer when compared to bare GC electrode surface, thus transforming into an electroactive surface that supports voltammetric surface characterization studies.

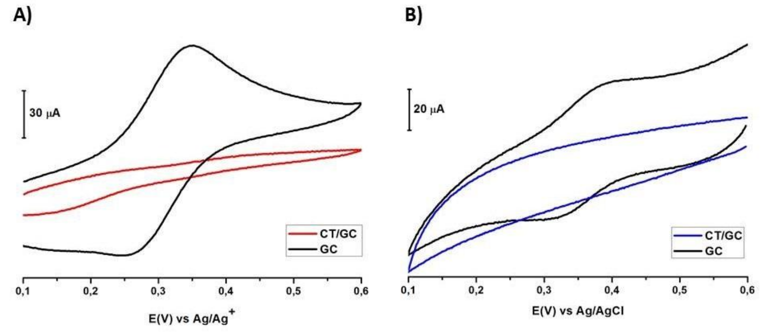

**Figure 3.** Overlaying cyclic voltammograms for A) CT/GC and bare GC electrode surface in 1 mM ferrocene redox probe solution vs. $Ag/Ag^+$ (10 mM) at potential range +0.1 V and +0.6 V using 100 mV $s^{-1}$ scanning rate; B) CT/GC and bare GC electrode surface in 1 mM $Fe(CN)_6^{3-}$ redox probe solution vs. Ag/AgCl/3 M KCl reference electrode at potential range +0.6 V and +0.1 V using 100 mV $s^{-1}$ scanning rate

In the voltammetric surface characterization processes, a solution of 1 mM ferrocene was prepared in a 100 mM $NBu_4BF_4$ supporting electrolyte solution dissolved in $CH_3CN$ for the non-aqueous media, which contains $Fe^{2+}$ ions. In contrast, a solution of 1 mM $Fe(CN)_6^{3-}$ was prepared in a 100 mM $H_2SO_4$ supporting electrolyte solution for the aqueous media, which contains $Fe^{3+}$ ions. This setup allows the observation of oxidation events in the non-aqueous media through anodic scanning and reduction events in the aqueous media through cathodic scanning. The results were compared with those from bare GC electrode surface, providing insights into the status of the molecule on the electrode surface.

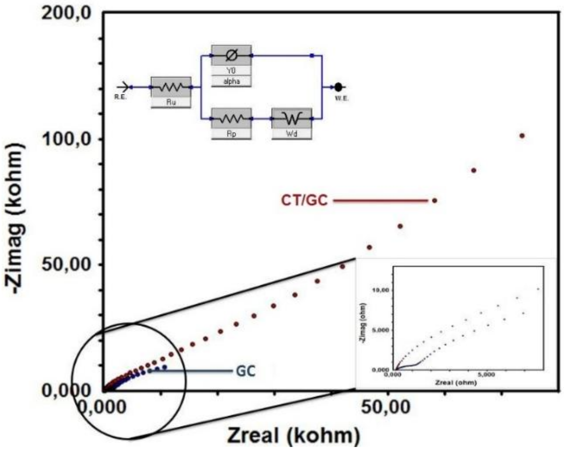

**Figure 4.** Nyquist plots of CT/GC and bare GC electrode surface at frequency range 100.000–0.05 Hz and 10 mV wave amplitude

In Figure 3A, the voltammogram obtained using the ferrocene redox probe in the non-aqueous media showed oxidation and reduction peaks on the bare GC surface, while these peaks were absent on the CT modified GC electrode surface. A similar situation was observed when using the ferricyanide redox probe in the aqueous media, as shown in Figure 3B. Here, the bare GC electrode surface displays reduction peaks followed by oxidation peaks, whereas the cytosine-modified GC electrode surface shows no peaks. This observation in both the non-aqueous and aqueous mediums indicates the electrode surface was coated with CT and that the surface was electro-inactive.

Another characterization process that supports the voltammetric surface characterization studies is the one performed using the EIS technique. In this case, a redox probe solution consisting of a 1 mM $Fe(CN)_6^{3-/4-}$ redox probe mixture was prepared in a 100 mM KCl solution. Since the EIS technique is applied by applying a low potential during the process, it does not damage the surface. Figure 4 showed the Nyquist plots obtained for both bare GC electrode and CT/GC electrode surface.

When examining the Nyquist plots in the graph, it was observed that there was almost no resistance to electron transfer on bare GC electrode surface, while there was a resistance of approximately 50 kohms on CT/GC electrode. According to the results of the voltammetric surface characterization processes, this was an expected outcome, and the EIS technique also confirmed that bare GC electrode surface was electroactive, whereas CT/GC electrode surface was electro-inactive.

One of the commonly used methods for analyzing electrode surfaces is the use of microscopic techniques. Additionally electrochemical surface characterization process, characterization was performed using the SEM technique. As shown in Figure 5, the morphological differences between bare GC electrode surface and the CT modified GC electrode surface demonstrated, similar to the electrochemical techniques, that GC electrode surface was completely covered by CT molecule and that a new surface was obtained.

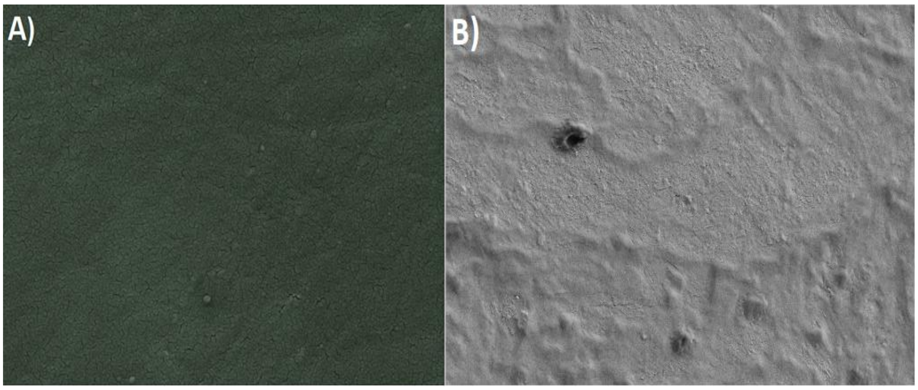

**Figure 5.** SEM morphologies of A) Bare GC and B) CT/GC electrode surface

### 3.3. Effect of scan rate at CT/GC electrode

In most electroanalytical studies, it is desired for the modifier or the species to be determined to reach the electrode surface through diffusion control. With this aim, a scan rate study can be conducted. The evaluation of the voltammograms obtained from these studies, based on the Randles-Sevcik equation, shows that a plot of the peak current against the square root of the scan rate is linear, and the slope of the plot of the logarithm of the peak current against the logarithm of the scan rate is approximately 0.5. This indicates that the species present in the solution reaches the electrode surface under diffusion control. This situation was demonstrated in Figure 6 through both voltammograms and graphs.

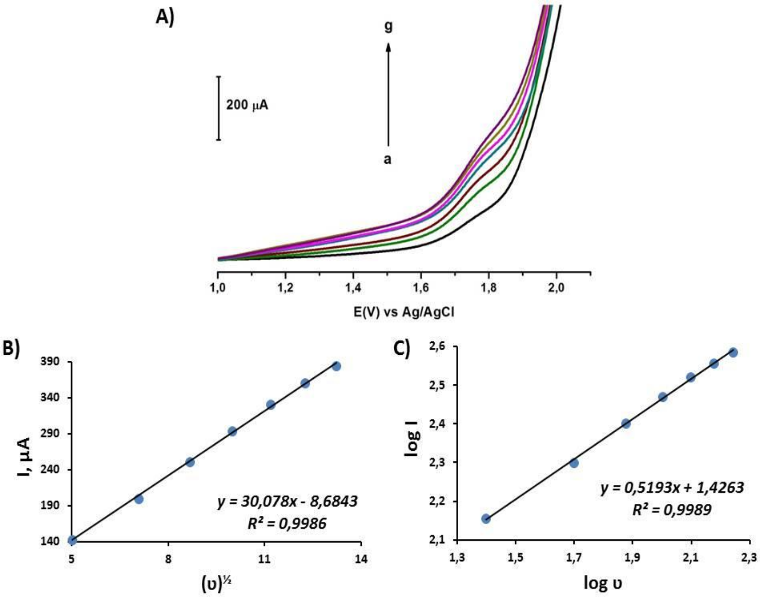

**Figure 6.** A) LS voltammograms obtained on bare GC electrode surface in PBS (pH 7,2) including 1 mM CT in the potential range of +1.0 and +2.1 V at scan rates (a) 25, (b) 50, (c) 75, (d) 100, (e) 125, (f) 150, (g) 175 mV $s^{-1}$; B) Plot of peak current versus square root of scan rate; C) Relation between log scan rate and log peak current

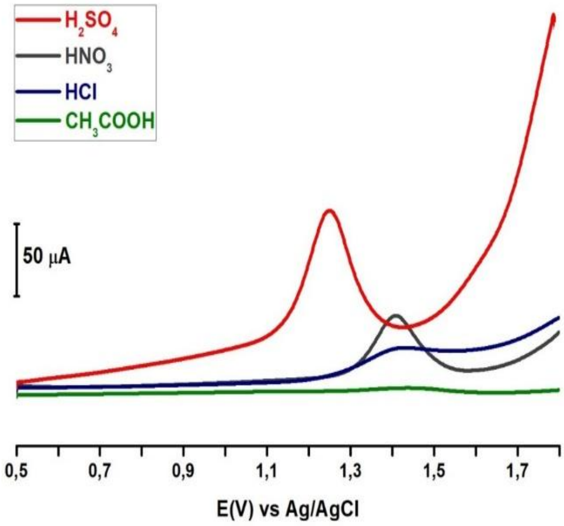

**Figure 7.** SW voltammograms taken on CT/GC electrode surface using different supporting electrolyte containing 1 mM CAF in the potential range between +0.5 and +1.8 V

### 3.4. Selection of the optimum supporting electrolyte

After characterizing CT/GC electrode, 1 mM CAF solutions were prepared in different supporting electrolytes. SW voltammograms were obtained for 1 mM CAF prepared separately in $H_2SO_4$, HCl, $HNO_3$, and $CH_3COOH$ within the potential range of +0.5 V to +1.8 V, and the results were overlayed using SWV technique. Upon examining the SW voltammograms in Figure 7, it was determined $H_2SO_4$ provided the highest peak current.

### 3.5. Effect of the supporting electrolyte concentrations

The selection of pH is important for the determination of different species. In this study, 100, 10, 1, and 0.1 mM solutions of $H_2SO_4$, selected as supporting electrolyte, were prepared, and SW voltammograms were carried out the potential range between 0.5 V and 1.7 V and overlayed in Figure 8.

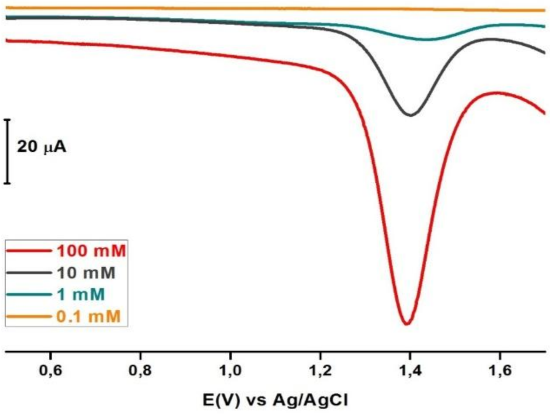

**Figure 8.** SW voltammograms taken on CT/GC electrode surface at different concentrations of $H_2SO_4$ including 1 mM CAF in the potential range of +0.5 and +1.7 V

### 3.6. Standard calibration curve for CAF

After selecting 100 mM $H_2SO_4$ as the supporting electrolyte, CAF solutions were prepared in different concentrations. The SW voltammograms for CAF determined on CT/GC electrode surface were demonstrated in Figure 9A for the concentration range of 0.75 to 0.05 mM and in Figure 9C for the range of 0.025 to 0.0025 mM, respectively. Upon examining the calibration plots drawn from the voltammograms, linear relationships were observed with $R^2$ of 0.9947 and 0.9961, respectively (Figures 9B, 9D), and the following equations were obtained:

$$I_{pa}\ (\mu A) = 73.038\ mM \times [CAF] + 5.2767\ R^2 = 0.9947$$

$$I_{pa}\ (\mu A) = 191.36\ mM \times [CAF] + 0.9084\ R^2 = 0.9961$$

To calculate the limit of detection (LOD) and the limit of quantification (LOQ), based on the calculations, LOD was found to be 0.93 µM and LOQ was determined to be 2.79 µM.

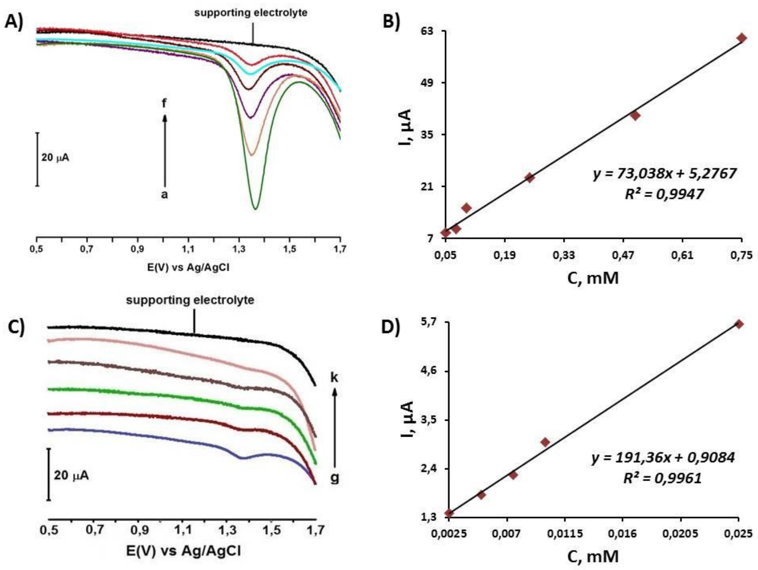

**Figure 9.** A) SW voltammograms for different concentration of CAF in 100 mM $H_2SO_4$ at CT/GC electrode (a) 0.75, b) 0.5, c) 0.25, d) 0.1, e) 0.075, f) 0.05 mM); B) Graph of peak current vs CAF concentration; C) SW voltammograms for different concentration of CAF in 100 mM $H_2SO_4$ at CT/GC electrode (g) 0.025, h) 0.01, i) 0.0075, j) 0.005, k) 0.0025 mM); D) Graph of peak current vs CAF concentration

### 3.7. Reaction mechanism

In the reaction mechanism given in Figure 10, firstly, the modification of CT with CV technique using the amine oxidation method on the GC electrode surface is seen. Then, CAF molecules were determined on the CT modified GC electrode surface. CAF molecules are adsorbed on the CT/GCE surface.

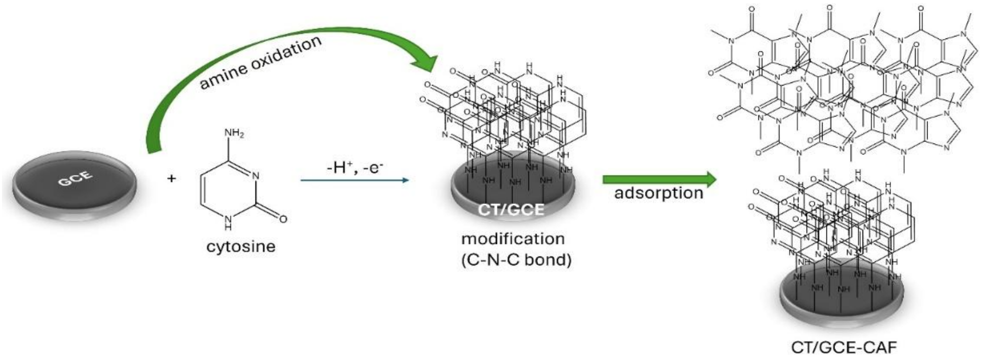

**Figure 10.** The reaction mechanism involving the modification of CT on the GC electrode surface and the adsorption of CAF

### 3.8. Investigation of interference effect

The suitability of the analytical method for CAF samples was assessed by evaluating its robustness through the introduction of a 10-fold concentration of various species, including ascorbic acid, dopamine, uric acid, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $PO_4^-$, $NO_3^-$, and $Cl^-$, into the supporting electrolyte ($H_2SO_4$) containing 100 µM CAF. Upon examination of the results, no significant change in peak current was observed upon the addition of these interfering substances to the analyte solution. The introduction of these interfering agents did not cause any detectable alteration in the peak current.

## 4. CONCLUSION

In this research, the electrochemical detectability of CAF was examined using the CT/GC electrode. After modification and characterization, CT/GC electrode was utilized for the determination of CAF for the first time after determining the optimum conditions. 0.75 – 0.0025 mM linear range and based on the calculations, the LOD was found to be 0.93 µM and the LOQ was determined to be 2.79 µM. Our ongoing studies using this sensor electrode aim to determine the amount of CAF in different samples.

## Acknowledgments

We would like to thank to the Research Foundation of Necmettin Erbakan University, Konya-TURKEY (BAP-23DR10002) for financial support of this work.

## Declarations of interest

The authors declare no conflict of interest in this reported work.

## REFERENCES

[1] B. Zereykaya, D. Eskiköy Bayraktepe, and Z. Yazan, Cumhuriyet Sci. J. 41 (2020) 680.

[2] K.W. Andrews, A. Schweitzer, C. Zhao, J.M. Holden, J.M. Roseland, M. Brandt, J.T. Dwyer, M.F. Picciano, L.G. Saldanha, K.D. Fisher, E. Yetley, J.M. Betz, and L. Douglass, Anal. Bioanal. Electrochem. 389 (2007) 231.

[3] A. Nehlig, J.L. Daval, and G. Debry, Brain Res. Rev. 17 (1992) 139.

[4] P.D. Tzanavaras, and D.G. Themelis, Anal. Chim. Acta 581 (2007) 89.

[5] C.H. Ashton, Br. Med. J. (Clin Res Ed) 295 (1987) 1293.

[6] D.C. Mitchell, C.A. Knight, J. Hockenberry, R. Teplansky, and T.J. Hartman, Food Chem. Toxicol. 63 (2014) 136.

[7] G. Faudone, S. Arifi, and D. Merk, J. Med. Chem. 64 (2021) 7156.

[8] J. Monteiro, M.G. Alves, P.F. Oliveira, and B.M. Silva, Crit. Rev. Food Sci. Nutr. 59 (2019) 2597.

[9] M.A. Rostagno, N. Manchón, M. D'Arrigo, E. Guillamón, A. Villares, A. García-Lafuente, A. Ramos, and J.A. Martínez, Anal. Chim. Acta 685 (2011) 204.

[10] E.G. Demissie, G.W. Woyessa, and A. Abebe, Food Ind. 17 (2016) 109.

[11] A.D. Meinhart, C.S. Bizzotto, C.A. Ballus, M.A. Prado, R.E. Bruns, J.T. Filho, and H.T. Godoy, Food Chem. 120 (2010) 1155.

[12] M.M. Paradkar, and J. Irudayaraj, Food Chem. 78 (2002) 261.

[13] X. Zhang, W. Li, B. Yin, W. Chen, D.P. Kelly, X. Wang, K. Zheng, and Y. Du, Spectrochim. Acta A Mol. Biomol. Spectrosc. 114 (2013) 350.

[14] L. Oberleitner, J. Grandke, F, Mallwitz, U. Reschgenger, L.A. Garbe, R.J. Schneider, J. Agric. Food Chem. 62 (2014) 2337.

[15] L.G. Shaidarovaa, I.A. Chelnokovaa, D.A. Koryakovtsevaa, D.A. Kirilenkoa, and H.C. Budnikov, J. Anal. Chem. 79 (2024) 721.

[16] Ş. Korkmaz, W. Bosnali, H. Sharifi, İ.E. Mülazımoğlu, A. Demir Mülazımoğlu, Curr. Anal. Chem. In press (2024) DOI: 10.2174/0115734110324545240725114425.

[17] H. Sharifi, Ş. Korkmaz, İ.E. Mulazimoglu, İ. Yılmaz, and E. Yılmaz, Anal. Bioanal. Electrochem. 16 (2024) 659.

***Copyright © 2024 by CEE (Center of Excellence in Electrochemistry)***

**ANALYTICAL & BIOANALYTICAL ELECTROCHEMISTRY (http://www.abechem.com)**

***Reproduction is permitted for noncommercial purposes.***