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PREPARATION OF VOLTAMMETRIC SENSORS FOR TAURINE DETECTION FROM Cu(II)-LOADED CARBON MATERIALS

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ABSTRACT. In this study, for the detection of taurine in philological fluids, modified electrodes were prepared from glassy carbon electrodes with MWCNT (multi-wall carbon nanotube) + $Cu(NO_3)_2$ + carbon-based material. A carbon-based material was obtained by carbonization of *Pinus sylvestris* branch needles (CPS). $Cu(NO_3)_2$ and MWCNT structure ($Cu(NO_3)_2$/MWCNT) were added to the carbon-based material and the obtained mixture was used in the modification of the glassy carbon electrode surface. The surface morphology and structure of the prepared electrodes were characterized by electron microscopy scanning (SEM) and atomic force microscopy (AFM). Taurine detection with a modified electrode was made by cyclic voltammetry (CV) in 0.1 M KCl (vs. Ag/AgCl). The calibration curve of the modified electrode for taurine in the 10-150 µM range showed a linear response, and R^2 value was 0.976. Limit of detection (LOD) and limit of quantification (LOQ) of the modified electrode were found 19 µM and 9.75 µM, respectively. The application of the electrode as a chemical sensor was carried out with commercially available energy drink samples. Satisfactory results have been obtained from the samples. The sensor can be used for routine taurine analysis. This electrode can quickly determine the amount of taurine in real samples with 90% accuracy in two minutes.

KEY WORDS: Taurine detection, Modified electrode, MWCNT, Sensor, Cyclic voltammetry

INTRODUCTION

With the molecular formula $C_2H_7NO_3S$, taurine, one of the most prevalent free amines in the cell, is a semi-essential antioxidant amino acid (Figure 1). About 150 years ago, it was removed from cattle bile. Biochemical and medicinal interest in taurine increased following Hayes *et al.* research's on it in 1975 [1]. In order to maintain calcium homeostasis, it also functions as an antioxidant, osmoregulatory, neurotransmitter, growth regulator, cell membrane protector, a component of bile salt, and anticonvulsant and insulinogenic agent [2]. Taurine is a water-soluble colorless molecule that plays a significant function in the brain, liver, muscle, blood, cardiovascular, reproductive systems, and retina [3]. Both the brain and liver contain large amounts of endogenous taurine. In addition, it plays a significant role in maintaining the proper amounts of sodium, potassium, magnesium, and calcium during nerve transmissions [4, 5]. Cysteine sulfinic acid decarboxylase (CSAD) catalyzes the synthesis of taurine from methionine and cysteine [6].

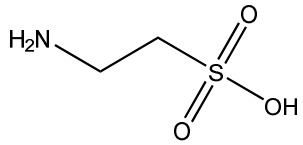

Figure 1. Taurine (2-aminoethanesulfonic acid).

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Taurine reduces the risk of heart disease and infarction due to its antihypertensive properties [7, 8]. Taurine is frequently used by athletes due to its capacity to enhance performance [9, 11]. Taurine has some negative effects associated with its high concentration in energy drinks [11, 12]. Due to these negative effects, its determination and detection are very important. Taurine content in food supplements is determined by analytical techniques such as sensors (electrochemical, optical, thermal, etc.), chromatographic analysis, and other techniques [13-17].

Compared to conventional methods, the electrochemical sensor is very advantageous in many applications due to its short detection time, no pre-treatment, no need for high-cost instruments and less need for competent personnel [18, 19]. For this reason, it is increasingly in demand, especially in biomedical analysis and food control applications. In particular, easy and powerful analytical approaches desired for food preservation and quality control can be easily achieved with accurate and sensitive sensors. In the preparation of such sensors, especially conductive carbon structures [20-22] and nanoparticles [23-25] are of great importance. Today, the importance and success of electrochemical sensors reinforced with carbon-based nanomaterials are increasing, as well as the number and variety of commercial electrodes used in the clinic and the field [26-29].

In this study, the glassy carbon electrode surface was modified by adding MWCNT and $Cu(NO_3)_2$ to the functional carbon structure obtained from *Pinus sylvestris* (yellow pine) leaves by dry carbonization method in a nitrogen atmosphere for the determination and detection of taurine, which is widely used especially in energy drinks, was tested with electrochemical techniques such as CV. Within the scope of the study, the modification of the GC electrode was carried out using a three-component coating. Cu(II) ions from these components were preferred for the accumulation of the analyte on the electrode surface. The MWCNT structure was used to increase the electron transfer rate and electrode sensitivity. The carbon-based structure obtained from *Pinus sylvestris* needles was carried out for a porous and high surface area modification. In this way, taurine concentration was obtained sensitive and reproducible measurement results by modified GCE. Thanks to this method, a high-sensitivity taurine sensor was prepared using a simple and modified electrode.

EXPERIMENTAL

Materials

Taurine (2-aminoethanesulfonic acid), copper(II) nitrate trihydrate ($Cu(NO_3)_2.3H_2O$), potassium dihydrogen phosphate (KH_2PO_4), sodium mono hydrogen phosphate (Na_2HPO_4), and *N*,*N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich chemical company. Sodium acetate (CH_3COONa), acetic acid (CH_3COOH), potassium chloride (KCl), sodium chloride (NaCl), hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Merck. Multi-Wall Carbon Nano Tube (MWCNT) structure was purchased from the Graphene Company. All aqueous stock solutions and electrolyte solutions were produced with ultra-pure water. All electroanalytical studies were performed at room temperature. All the solutions were stored at room temperature.

Methods

Voltammetric studies were performed using a BAS 100BW (Bioanalytical Systems, Inc., USA) potentiostat device equipped with a C3 electrochemical cell with a Faraday cage. The triple electrode system (BASi C3 cell stand) consisted of a glassy carbon study electrode (GCE), Ag/AgCl in saturated KCl solution (Ag/AgCl/sat. KCl) as a reference electrode and a platinum wire counter electrode. For the cleaning of the electrodes and dissolve the solutions, A Bandelin Sonorex brand RK 100 ultrasonic bath was used. The ultrapure water was obtained from a Millipore Milli-Q water system. The surface morphology of the modified electrode was

investigated by scanning electron microscopy (SEM) technique. The surface properties and morphologies of MWCNT-$Cu(NO_3)_2$ material, MWCNT+$Cu(NO_3)_2$-CPS structures were obtained with LEO EVO-40xVP scanning electron microscope. The surface of the samples to be examined was coated with 20 nm Au-Pd with BALTECK brand sputter, before scanning electron microscope (SEM) analysis. Atomic force microscopy (AFM) images and surface roughness of the modified electrode were determined with the park system XE100 AFM device. AFM measurements were performed at room temperature using the noncontact mode.

Preparation of the carbon-based materials (CPS) from the Pinus sylvestris

The raw materials of *Pinus sylvestris* were ground to the appropriate size at room temperature. The dried samples are carbonized in a high-temperature furnace with three heating zones at a maximum temperature of 500 ºC for 1 hour at a temperature of 10 ºC/min under a nitrogen atmosphere. After the carbonization samples are removed from the furnace and cooled to room temperature, they are stored for characterization. FT-IR was used for the structural characterization of the carbonized *Pinus sylvestris* (CPS).

Preparation of glassy carbon electrode

Before modification, the bare GCE was cleaned mechanically on cleaning pads (BAS, MF-1040) dripping 1.0, 0.3, and 0.05 µm, alumina (Al_2O_3) slurries, respectively. The polished electrode was rinsed successively with ethanol/water, HNO_3/H_2O (1:3 v/v), and sonicated in ultrapure water for 5 min. After the electrode was cleaned, the bare GCE was electrochemically cleaned by applying cyclic voltammetry (CV) in the range of -1200 to + 1200 mV in 0.5 M H_2SO_4 and scanning it 20 times [30]. Finally, to remove impurities, the working electrode surface was cleaned with ultrapure water. Before each modification, GCEs were cleaned mentioned above.

Cu(II)/MWCNT doping and preparation of modified electrodes

After obtaining *Pinus sylvestris*-based carbon material, a series of electrodes were prepared. For this purpose, firstly, mixtures consisting of a certain amount of *Pinus sylvestris*-based carbon material (50%, 60%, 70%) and (50%, 40%, 30%) $Cu(NO_3)_2$ were added 2 mL each of DMF and stirred in an ultrasonic bath for about 30 min. Then 3% MWCNT was added to these mixtures and stirred for another 1 hour. After the optimum ratio was determined, modified electrodes were prepared by dropping different amounts (2, 3, 4, and 5 µL) of the solution consisting of *Pinus sylvestris* based carbonic material, $Cu(NO_3)_2$, and MWCNT onto the GCE surface. These modified electrodes were dried at room temperature for the determination and optimization of taurine.

RESULTS AND DISCUSSION

FT-IR, SEM, and AFM analysis of the modified electrode

The characterization of carbonized *Pinus sylvestris* (CPS), MWCNT - $Cu(NO_3)_2$ structures, and MWCNT + $Cu(NO_3)_2$ + CPS structures prepared for the modification of the electrodes were first determined by FTIR spectroscopy. The FTIR spectrum of the carbonized *Pinus sylvestris* (CPS). MWCNT-CPS structures and MWCNT + $Cu(NO_3)_2$ + CPS structures are given in Figure 2. In the spectrum of the MWCNT structure, aromatic ring C=C and aromatic ring backbone –C–H bending vibration were observed at a lower frequency around 1610 cm^{-1} and 1405 cm^{-1} [31]. In the FTIR spectrum of the $Cu(NO_3)_2$ structure, the H bond stretching vibration of the –OH groups on the surface structure of the nanoparticle is observed between 3539 cm^{-1} and 3412 cm^{-1} [32]. The peaks at 1420 and 1347 cm^{-1} are asymmetric and symmetric stretching peaks of O-NO_2,

respectively [33]. The peak at 1013 cm^{-1} is connected to N-O stretching pulsation of monodentate O-NO groups. The peaks at 865, 701, and 660 cm^{-1} are due to bending vibrations of Cu-OH groups with different hydrogen bonds [34]. The peaks of both structures confirm that the desired structure was obtained.

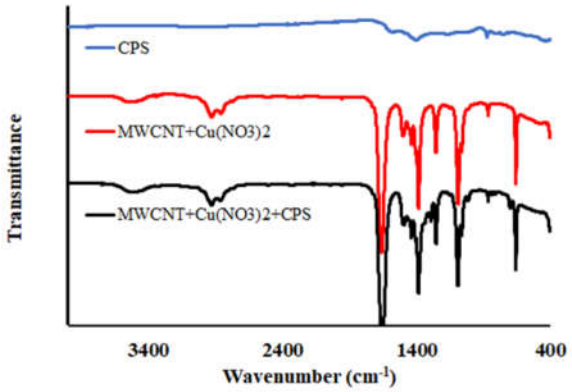

Figure 2. FT-IR images of CPS, MWCNT+$Cu(NO_3)_2$, and MWCNT+$Cu(NO_3)_2$+CPS.

Surface cavities of MWCNT + $Cu(NO_3)_2$ + CPS structures produced are very important for optimum electrode surface modification within the scope of the study. Because with the application of such structures-doped carbon based- on the electrode surface, the surface roughness of the electrode increases, and accordingly the sensitivity of the active electrode increases. The modified electrodes obtained with this method can successfully detect lower concentrations. For this reason, SEM analyses of the MWCNT - $Cu(NO_3)_2$ and obtained MWCNT + $Cu(NO_3)_2$ + CPS material were performed at different magnifications which showed in Figure 3 (A). The roughness of MWCNT + $Cu(NO_3)_2$ + CPS material was also examined by AFM analysis. In Figure 3 (B), the AFM images of the MWCNT + $Cu(NO_3)_2$ + CPS material and MWCNT + $Cu(NO_3)_2$ were compared. In these images, the surface of the MWCNT + $Cu(NO_3)_2$ + CPS modified sensor (lower panel) was found to be fractured and rough concerning Cu(II) free sample, thereby Cu(II) ions were visible in the carbon-based material. Surface roughness increased compared to MWCNT-$Cu(NO_3)_2$. This rough image confirms the increased surface area. As the roughness increased, the surface area grew and the sensitivity of the sensor increased.

Optimization studies of GC-modified electrode

In this investigation, taurine was measured on MWCNT + $Cu(NO_3)_2$ and the MWCNT + $Cu(NO_3)_2$ + CPS (MWCNT + $Cu(NO_3)_2$ + *Pinus sylvestris*) using four different electrolytes (0.1 M PBS pH 7, 0.1 M KCl, 1 M KCl, and NaOAc (pH = 5)) containing 100 µM taurine by CV technique vs. Ag/AgCl) at -1.0 to +1.0 V at a scan rate of 100 mV/s. A calibration curve was created for the best ion-loaded species concerning its surroundings. After assessing stability, reproducibility, and the impact of scanning speed, the LOD, and LOQ values of the prepared modified electrode were established. According to the Turkish Food Codex Energy Drinks Declaration, which is available on the market, no one type of energy drink could contain more than 800 mg/L. The energy content's taurine was determined and calculated.

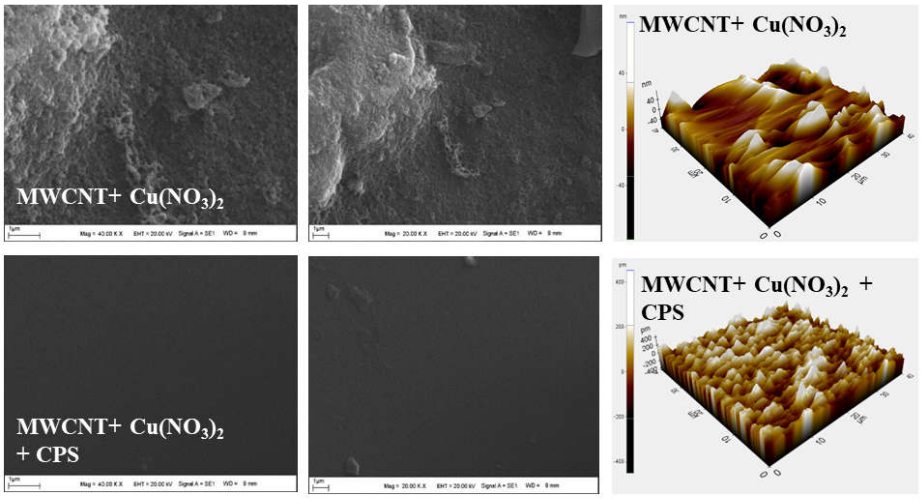

Figure 3. (A) SEM images of MWCNT - $Cu(NO_3)_2$ and MWCNT + $Cu(NO_3)_2$ + CPS, (B) AFM images of MWCNT + $Cu(NO_3)_2$ and MWCNT + $Cu(NO_3)_2$ + CPS material.

Effect of support electrolyte

The behavior of MWCNT + CPS coated GCE, $Cu(NO_3)_2$ + MWCNT + CPS coated GCE electrodes which were developed for taurine determination by the voltammetric method was investigated in four different electrolytes, 0.1 M KCl, 1.0 M KCl, 0.1 M PBS (pH 7) and 0.05 M NaOAc (pH 5), respectively. Figure 4A shows no cathodic peak expected from the MWCNT + CPS-coated GCE electrode. In Figure 4B, it is seen that $Cu(NO_3)_2$ + MWCNT + CPS coated GCE electrode gave a cathodic peak in 0.1 M KCl electrolyte. It was determined that 0.1 M KCl electrolyte should be used in the rest of the study.

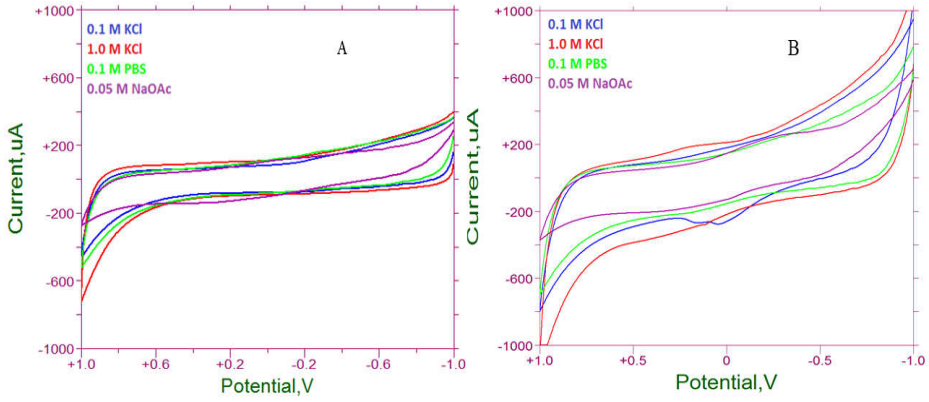

Figure 4. Effect of the supporting electrolyte on the (A) MWCNT + CPS, B) $Cu(NO_3)_2$ + MWCNT + CPS (100 µM taurine CV technique (vs. Ag/AgCl) at -1.0 to +1.0 V at a scan rate of 100 mV/s).

Voltammetric measurements were carried out through the addition of taurine into a continuously stirred 0.1 M KCl (vs. Ag/AgCl) at an applied potential of -1.0 to +1.0 V.

Calibration curve

Figure 5 illustrates the calibration of the multi-walled carbon nanotubes (MWCNT) + $Cu(NO_3)_2$ + cross-linked poly(sulfosalicylic acid) (CPS) modified glassy carbon electrode (GCE) for the detection of taurine. The voltammetric measurements were performed in a 0.1 M potassium chloride (KCl) solution (vs. Ag/AgCl), and the obtained results are presented over a concentration range of 10 to 260 µM.

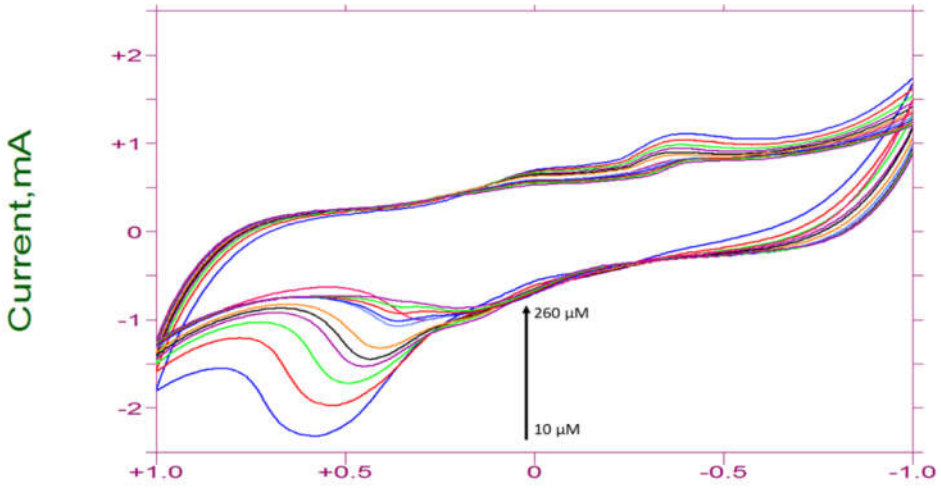

Figure 5. MWCNT + $Cu(NO_3)_2$ + CPS modified GCE electrode calibration plot in 0.1 M KCl.

The calibration curve obtained from the experimental data serves as a crucial tool for quantifying the concentration of taurine in unknown samples based on their respective current responses. Ideally, a linear relationship between taurine concentration and current response is expected in this concentration range. However, it is essential to verify the linearity of the curve within this range to ensure the accuracy and reliability of the sensor's performance.

The calibration graph is given in Figure 6. From this graph, the R^2 value was determined to be 0.976 between 10 µM and 150 µM taurines. It was established that the LOD and LOQ values of the modified electrode were 19 µM and 9.75 µM, respectively. Cathodic response to 60 µM taurine in 0.1 M KCl at a potential range of -1.0 to +1.0 V and a scan rate of 100 mV/s is achieved in just two minutes.

The calibration curve data presented in Figure 5 and Figure 6 is a result of meticulous experimental work and provides valuable insights into the analytical capabilities of the MWCNT + $Cu(NO_3)_2$ + CPS modified GCE electrode. It enables researchers and analysts to quantitatively determine taurine concentrations in unknown samples by comparing their respective current responses to the calibration curve. Moreover, this information contributes to the understanding of the sensor's performance and its potential applicability in various fields, such as biomedical research, environmental monitoring, and food analysis.

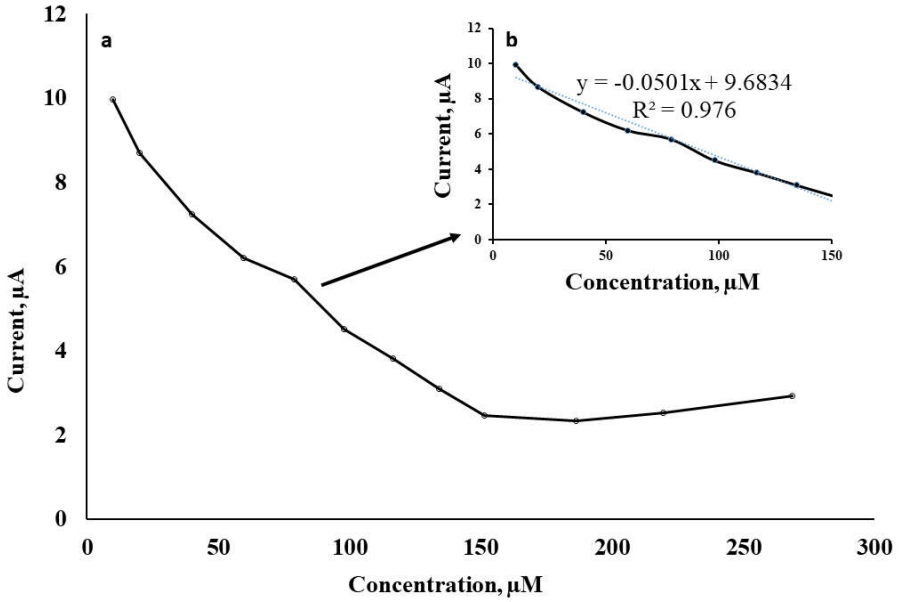

Figure 6. The calibration curve for the MWCNT + $Cu(NO_3)_2$ + CPS modified GCE electrode for increased concentration of taurine (a; 10-260 µM taurine concentration range and b; 10-150 µM taurine concentration range in 0.1 M KCl solution (vs Ag/AgCl)).

Analytical performance was evaluated by comparing this method with other similar electrochemical methods for determining taurine. Taurine determination methods reported in the literature, are summarised in Table 1.

Table 1. Comparative analytical performance of the MWCNT/$Cu(NO_3)_2$/CPS modified GCE with other electrodes previously reported in the literature for the determination of taurine.

Electrode type	Technique	Linear range (M)	LOD (M)	Ref
α-CD-AgNPs-GCE	DPV detection	7.0×10^{-10} - 1.0×10^{-4}	-	[35]
L-methionine-modified gold electrode	DPV detection	Not reported	1.2×10^{-6}	[36]
LSPR biosensor	Optic fiber	Not reported	5.3×10^{-5}	[37]
GCE	Voltammetric determination	6.8×10^{-6} - 1.0×10^{-4}	2.1×10^{-6}	[38]
MWCNT/$Cu(NO_3)_2$/CPS-GCE	CV	1.0×10^{-5} - 1.50×10^{-4}	1.9×10^{-5}	This work

α-CD-AgNPs composite material of α-cyclodextrin and silver nanoparticles, *LSPR* localized surface plasmon resonance, *GCE* glassy carbon electrode, MIECS molecularly imprinted electrochemical sensor, *MWCNT* multi-wall carbon nanotube, *CPS Pinus sylvestris* branch needles.

To summarize the present sensor showed acceptable performance than the previously reported electrochemical methods in terms of achieving a lower limit of detection and acceptable linear dynamic range.

Effect of scan rate

From the calibration curve graph of the MWCNT + $Cu(NO_3)_2$ + CPS modified GCE electrode with significant current responses in 0.1 M KCl (vs. Ag/AgCl) solution, containing 60 µM taurine by CV technique at -1.0 to +1.0 V. Alternate CV voltammogram of the modified GCE electrode using MWCNT + $Cu(NO_3)_2$ + CPS in 0.1 M KCl (vs. Ag/AgCl) solution containing 60 µM taurine at -1.0 to +1.0 V are shown in Figure 7 for various scan rates (10, 25, 50, 75, 100, 150, 200 and 250 mV/s). The anodic peak current (Ipa) and the cathodic peak currents (Ipc) values increase linearly with the scanning rate. The anodic and cathodic peak currents were found to increase linearly with scanning speed, with the clearest peak currents being produced at 100 mV/min.

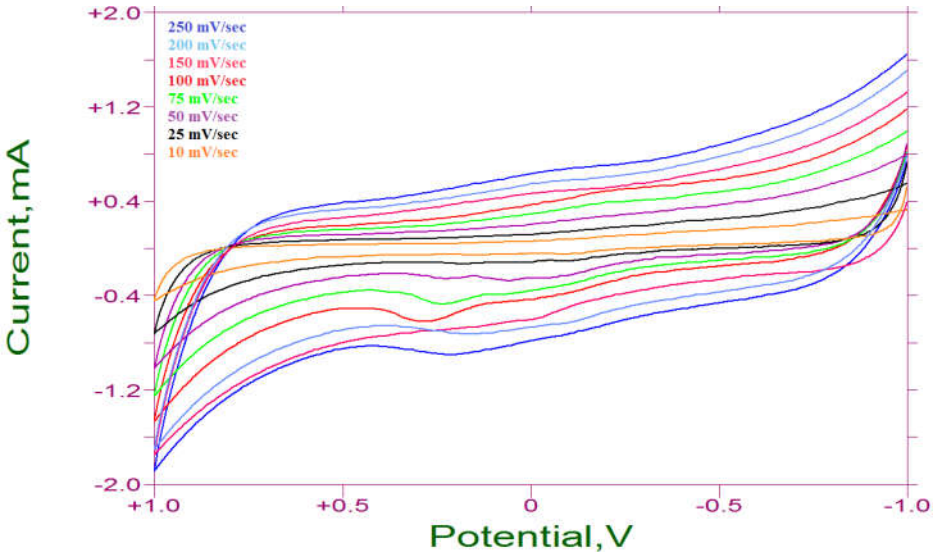

Figure 7. MWCNT + $Cu(NO_3)_2$ + CPS modified electrode CVs respond to 60 µM taurine in 0.1 M KCl (vs. Ag/AgCl) at different scan rates (10, 25, 50, 75, 100, 150, 200 and 250 mV/s).

Stability and reproducibility of MWCNT+$Cu(NO_3)_2$+CPS modified electrode

Repeatability studies were carried out on the MWCNT + $Cu(NO_3)_2$ + CPS modified GCE electrode against 60 µM taurine at a scanning rate of 100 mV/s in a 0.1 M KCl solution. The response of the modified electrode to taurine decreased by almost 50% after the fourth experiment, as shown in Figure 8 (A and B).

When the modified sensor (GCE/MWCNT/Cu(II)/CPS) was stored at room temperature, it was observed that there was a decrease of only about 0.5–1.0% in peak current density after two weeks. It was understood that the prepared modified sensor was stable, repeatable, and reproducible, also repeated analyses were performed with the modified electrode.

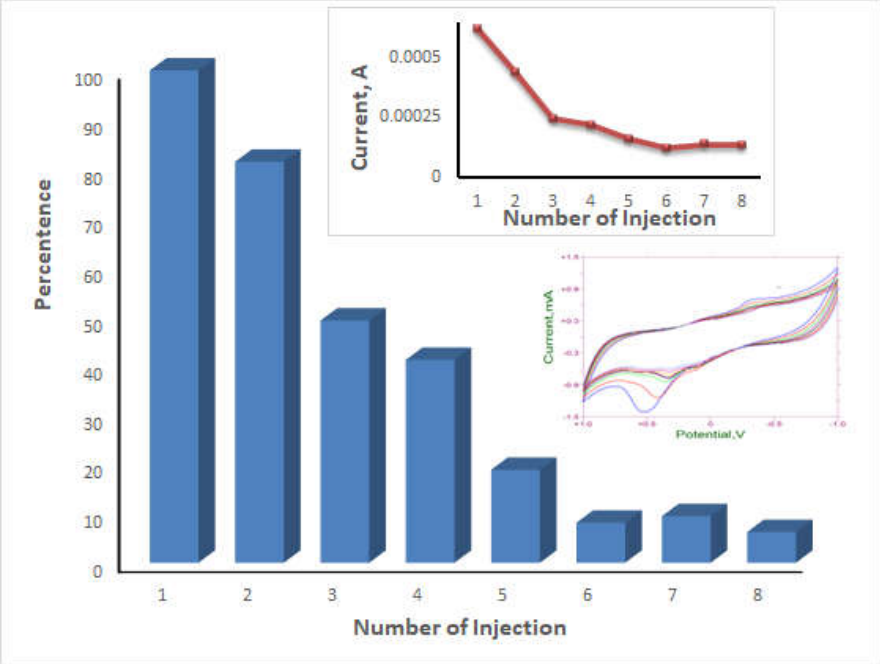

Figure 8. Reproducibility studies of MWCNT + $Cu(NO_3)_2$ + CPS modified GCE (0.1 M KCl (vs. Ag/AgCl) containing 60 µM taurine by CV technique at -1.0 to +1.0 V).

Determination of taurine in energy drinks samples

The applicability of the developed method for taurine determination in real samples was investigated using commercially available energy drink samples. The MWCNT + $Cu(NO_3)_2$ + CPS modified GC electrode was used for the quantification of taurine in an energy drink. Known amounts of taurine in energy drinks, as shown in Table 2, were taken into account to study the accuracy. According to the results obtained in an energy drink sample, the developed modified electrode could be used in real sample analysis (Table 2).

Table 2. Determination of taurine in energy drink samples.

Sample number	Concentrations ppm	Energy (kcal)	Founded with MWCNT+$Cu(NO_3)_2$+CPS (ppm)	Recovery (%)
1	800	37	688	86.00
2	800	46	675	84.38
3	800	56	725	90.63
4	600	54	550	91.67

CONCLUSION

In this study, electrodes from carbon-based materials loaded with MWCNT and Cu(II) were developed to be used in the detection of taurine in food samples and physiological fluids. Pine tree needles were carbonized as a carbon-based material. Due to the basicity of the amino group on taurine, it forms less stable metal complexes to Cu(II) than its carboxylic analog, β-alanine.

The complexion behavior of taurine strengthens the accumulation of taurine on the electrode surface and increases the electrode sensitivity. In conclusion, the development and preparation of voltammetric sensors for taurine detection from Cu(II)-loaded carbon materials represent a significant advancement in analytical chemistry and sensor technology. The study demonstrated the potential of using carbon materials as the sensing platform, incorporating Cu(II) ions as a crucial mediator for enhancing taurine detection sensitivity and selectivity.

The synthesis and modification of carbon materials with Cu(II) ions exhibited promising results in terms of sensor performance. The Cu(II) ions acted as effective catalysts, facilitating the electrochemical oxidation of taurine and leading to improved current responses. This achievement is particularly noteworthy as taurine detection is of great importance due to its involvement in various physiological processes and potential implications in disease diagnosis and monitoring.

Furthermore, the voltammetric approach utilized in this research offers numerous advantages, including rapid response times, simplicity, and cost-effectiveness. The sensitivity of the sensors achieved in this study demonstrates their potential for detecting taurine at low concentrations, which is vital for applications in real-world scenarios where taurine levels may be present in trace amounts.

Nevertheless, while this study presents promising results, further investigations are warranted to fully explore and optimize the sensor's performance. Parameters such as the type and structure of carbon materials, Cu(II) loading, and the design of the sensing interface should be systematically explored to enhance the sensor's efficiency and sensitivity further.

The research method presented here was carried out using simple, selective, sensitive, low-cost, and easily prepared GC electrodes that require only a short analysis time to determine taurine using MWCNT + $Cu(NO_3)_2$ + CPS. There is interest in the deposition of taurine with a fast and easily-prepared MWCNT + $Cu(NO_3)_2$ + CPS modified GCE in 0.1 M KCl. The optimum conditions for the determination of taurine were as follows: 0.1 M KCl, 0.5 V, and a scan rate of 100 mV/s. The developed sensor showed, under optimum conditions, sensitivity and selectivity towards taurine with linearity in the range of 10-150 µM. The quantitation (LOQ) and detection limits (LOD) of the modified electrode were 9.75 and 19 µM, respectively. As a result, the prepared metal ion-loaded carbon-based electrodes, namely Cu(II) doped electrodes, exhibited good reproducibility, wide linear range, and selectivity for taurine determination. Furthermore, the applicability of the proposed electrode in energy drink samples gives promising results. Therefore, it is thought that such carbon-based structures may increase the selectivity and sensitivity of taurine quantification in the food industry. MWCNT + $Cu(NO_3)_2$ + CPS modified GC electrode can determine the amount of taurine in real samples with 90% accuracy.

In conclusion, the preparation of voltammetric sensors for taurine detection from Cu(II)-loaded carbon materials opens up new possibilities in the field of electrochemical sensing. These sensors hold significant potential for future applications in various fields, including biomedical and environmental monitoring, contributing to advancements in healthcare, food safety, and environmental protection. With continued research and refinement, these sensors could become valuable tools for accurate and reliable taurine detection, benefiting society as a whole.

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