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Short Communication

Fabrication of an electrochemical sensor based on a nanocomposite of CoO@f-CNTs for determination of tramadol narcotic drug in urine of athlete volunteers

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The current research is focused on the development of a stable, sensitive, and selective electrochemical sensor made up of CoO nanoparticles and functionalized carbon nanotubes (CoO@f-CNTs) for the detection of tramadol as a narcotic in urine for doping analysis. The CoO@f-CNTs nanocomposite modified glassy carbon electrode was made using an electrodeposition approach (GCE). The electrodeposition of a well-crystalline CoO@f-CNTs nanocomposite on GCE was confirmed by structural studies utilizing XRD and SEM analysis. Electrochemical studies using DPV and amperometry revealed that f-CNTs and CoO nanoparticles had a synergistic electrocatalytic effect in promoting charge transfer in the oxidation of tramadol as a sensitive and selective sensor with a linear range of 1 to 300 µM. The detection limit and sensitivity were calculated to be 0.44971µA/µM and 6 nM, respectively. The usefulness and precision of CoO@f-CNTs/GCE for determining tramadol in prepared real samples from urine samples of athlete volunteers were explored. The results showed that the ELISA and amperometric analyses had a high level of agreement, and the recovery (98.50% to 100.50%) and RSD (3.33% to 4.18%) values were acceptable. The findings showed that the suggested approach has adequate validity, precision, and high promise for practical urine sample analysis.

Keywords: CoO nanoparticles; Functionalized CNTs; Nanocomposite; Tramadol; Urine samples; Amperometry

1. INTRODUCTION

Tramadol (2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol) is an opioid-containing centrally acting oral analgesic approved for the treatment of moderate to moderately severe pain in adults [1, 2]. Tramadol alleviates pain by connecting to opioid receptors in the brain [3, 4]. It is

one of the least strong painkillers available, although it is beneficial in treating mild to moderate acute or chronic pain [5-7].

Tramadol, being a narcotic pain reliever, has the potential for abuse and is harmful in high dosages [8, 9]. Tramadol, for example, produced considerable liver and renal damage, as seen by significant increases in serum spartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase activity, as well as creatinine levels [10, 11]. It also has the potential to cause long-term brain damage, such as an increased risk of mental health disorders, particularly depression and anxiety [12, 13]. Constipation, discouragement, drowsiness, warmth, unexpected cold, itching or skin rash, loss of strength or weakness, muscle aches and pains are some of the most common tramadol adverse effects [14-16].

Tramadol's success in the treatment of musculoskeletal pain may be connected to its analgesic and mood-enhancing properties, and the reinforcement of euphoric emotions may lead to the development of addiction. Cycling (65%), triathlons (8%), and rowing (6%) are the sports with the highest consumption [17-19]. Tramadol was found to increase 20-minute cycling time trial performance by 5% in studies [20, 21]. Thus, tramadol has been banned in cycling competitions in 2019 [17]. Therefore, determination of tramadol in clinical samples is very important, and much research has been conducted on optimization of the sensing performance using HPLC [22], spectrophotometry [23, 24], gas chromatography–mass spectrometry [25], electrochemiluminescence [26], capillary electrophoresis [27], colorimetry [28, 29] and electrochemical methods [30-37].
However, many of these approaches' precision and applicability have been hampered due to time-consuming, expensive, and intricate procedures, as well as the presence of interfering compounds in clinical and biological fluids [38]. Electrochemical approaches have demonstrated acceptable accuracy and selectivity for tramadol determination in clinical and biological fluid samples among these methods. However, more research is needed to improve tramadol electrochemical sensor sensing performance [39-43]. As a result, the current research is focused on the development of a CoO@f-CNTs nanocomposite as a stable, sensitive, and selective electrochemical sensor for the detection of tramadol as a narcotic in urine relevant to doping analysis.

2. EXPERIMENT

2.1. Synthesis CoO@f-CNTs nanocomposite

Electrodeposition method was employed for preparation the CoO@f-CNTs nanocomposite modified GCE [44, 45]. Briefly, 1.5g of CNTs (Jiaxing Guohe Technology Co., Ltd., China) were functionalized (f-CNTs) by dispersing in 300 mL of 4.5 M HNO_3 (70%, Sigma-Aldrich) for 20 minutes under magnetic stirring. The mixture was then ultrasonically dispersed for 20 minutes before being placed in an oil bath at 95 °C for 5 hours with magnetic stirring. After cooling, the f-CNTs were centrifuged for 5 minutes at 1000 rpm before being ultrasonically washed with deionized water for 20 minutes. The f-CNTs were then dried for 10 hours at 95°C in an oven. Prior to electrodeposition, the GCE surface was polished with alumina powder (99.99 %, Sigma-Aldrich) for

15 minutes on a polishing cloth (LAM PLAN S.A., Gaillard, France), and then rinsed with a mixture of water and ethanol for 10 minutes by ultrasonication. For preparation the electrodeposition electrolyte, 100 mg of f-CNTs were ultrasonically dispersed in 100 ml of 30 mM $CoSO_4 \cdot 7H_2O$ (≥ 99 %, Merck, Germany) solution. Next, the resulted suspension was mixed with 100 ml of 40 mM Na_2SO_4 (≥ 99 %, Merck, Germany) solution and stirred for 20 minutes to obtain a black flocs suspension. Electrodeposition of CoO@f-CNTs on GCE was performed in an electrochemical workstation potentiostat (CS150, Xian Yima Optoelec Co., Ltd., China) using a three-electrode electrochemical cell setup which contained Ag/AgCl as reference, graphite rod as counter, and GCE as working electrode at a potential window from -0.7 V to 0.7 for 50 cycles at a scan rate of 15 mV/s. For electrodeposition f-CNTs on GCE, the procedure was accomplished using electrolyte without $CoSO_4 \cdot 7H_2O$ and Na_2SO_4 at a potential window from -0.7 V to 0.7 for 50 cycles at a scan rate of 15mV/s. For electrodeposition of CoO on GCE, the procedure was carried out in electrolyte without f-CNTs at potential of -0.65 V for 25 minutes.

2.2. Characterization

The electrochemical workstation potentiostat galvanostat (CYKY, Zhengzhou CY Scientific Instrument Co., Ltd., China) was fitted with a three-electrode electrochemical cell containing Ag/AgCl, Pt mesh, and bare or modified GCE as reference, counter electrode, and working electrodes, respectively. All electrochemical tests were carried out in a 0.1M phosphate buffer solution (PBS) electrolyte (pH 7.4) with an equal volume ratio of 0.1M NaH_2PO_4 (99%, Sigma-Aldrich) and 0.1M Na_2HPO_4 (99%, Merck, Germany). The crystal structure of the produced nanostructures was determined using an X-ray diffractometer (XRD, 38066 Riva, d/G. via M. Misone, 11/D (TN) Italy). For morphological analyses of modified electrode surfaces, a FE-SEM (JEOL JSM-6500F, Japan) was used.

2.3. Study the actual sample

The accuracy and applicability of CoO@f-CNTs/GCE was used to identify tramadol in urine samples from athlete volunteers. The urine samples were centrifuged for 12 minutes at 1000 rpm. The supernatant was filtered after phase separation and used to make 0.1 M PBS (pH=7.4). The solution was then put into the electrochemical cell for analysis without any extra preparation. Analytical experiments were conducted using the standard addition method. The genuine samples were also analyzed using Tramadol ELISA Kits from Neogen Corporation (Michigan, United States).

3. RESULTS AND DISCUSSION

3.1. XRD and SEM studies

XRD was used to examine the structural properties of powders of f-CNTs, CoO, and CoO@f-CNTs nanocomposite, as revealed in Fig. 1. The diffraction peak in XRDs of f-CNTs lies at 26.46°,

which corresponds to the (002) reflection of the hexagonal graphitic structure of f-CNTs (JCPDS card No. 41–1487) [46-48]. There are diffraction peaks at 36.65°, 42.50°, 61.68°, 73.95°, and 77.76° in the XRD pattern of CoO, which correspond to the (111), (200), (220), (311), and (222) crystallographic planes of face-centered cubic (fcc) CoO, respectively (JCPDS card No. 78-043) [49-51]. The XRD pattern of CoO@f-CNTs nanocomposite shows diffraction peak of (111), (200), (220), (311) and (222), indicating to successful electrodeposition well-crystalline CoO@f-CNTs nanocomposite on GCE [52].

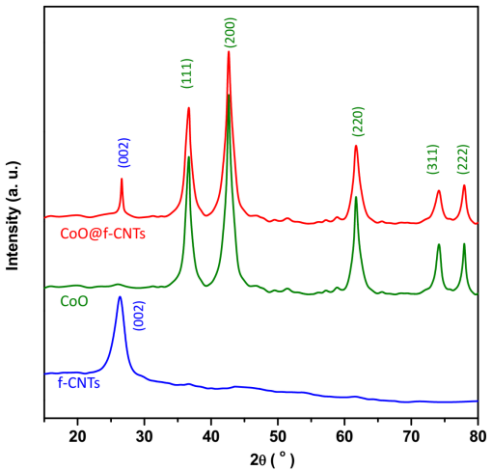

Figure 1. XRD patterns of powders of electrodeposited f-CNTs, CoO and CoO@f-CNTs nanocomposite.

Figure 2 shows FE-SEM of f-CNTs and CoO@f-CNTs nanocomposite improved GCE. The f-CNTs were electrodeposited on GCE with an average diameter of 30nm and a length of several micrometers, resulting in a randomly oriented spaghetti-like morphology with a high aspect ratio and specific surface area, as shown in the FE-SEM image. The simultaneous electrodeposition of f-CNTs and CoO nanoparticles on the electrode surface is shown in this FE-SEM picture of CoO@f-CNTs nanocomposite modified GCE. The chemically functionalized CNTs can produce strong interfacial covalent bonds with CoO nanoparticles [53-56]. Moreover, the CoO nanoparticles are randomly decorated on the f-CNTs surface which creates high specific surface area and porous structures.

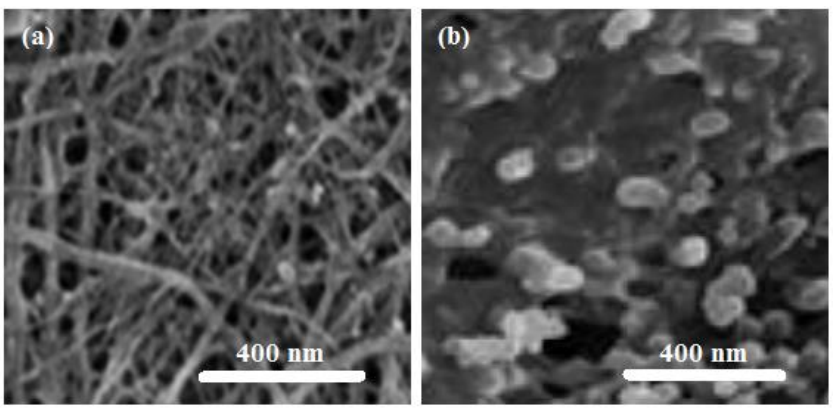

Figure 2. FE-SEM of (a)f-CNTs and(b) CoO@f-CNTs nanocomposites modified GCE.

3.2. Electrochemical studies

Figure 3 shows the DPV responses of GCE, f-CNTs/GCE, CoO/GCE, and CoO@f-CNTs/GCE into 0.1M PBS in the absence and presence of 100 M tramadol in the potential window from 0.0V to 1.2 V at a scanning rate of 30 mV/s. In the presence of 100 M tramadol, GCE, f-CNTs/GCE, CoO/GCE, and CoO@f-CNTs/GCE show anodic peaks at 0.75 V, 0.73 V, 0.62 V, and 0.62V, respectively, which are associated with tramadol oxidation and could include equal electrons and protons transferrs in the electrochemical process [35], as shown in Figure 4. The DPV curves indicate that the peak current of GCE is extremely poor at 0.75 V, but CoO@f-CNTs/GCE shows an extremely great peak current at a lower potential of 0.62 V that is about 1.4-fold, and 2-fold higher than the peak currents of f-CNTs/GCE and CoO/GCE, respectively [57]. The oxidation potential of the CoO nanoparticles modified electrode decreases toward the GCE and f-CNTs/GCE [58-60]. In addition, the DPV curve of CoO@f-CNTs/GCE illustrates that f-CNTs and CoO nanoparticles present synergistic electrocatalytic effects to promote charge transfer processes in the oxidation of tramadol [61, 62]. The large surface area, higher conductivity of CNTs, and presence of oxygen functional groups and defect sites on the outer walls of f-CNTs not only provide a favorable matrix for nucleating and anchoring CoO nanocrystals to improve the conductivity and stability of nanocomposite modified electrodes, but also provide electroactive sites for absorption analytes and easy electron transfer to boost catalytic activity [63-66]. Only CoO@f-CNTs/GCE data will be given in the following electrochemical investigations due to the synergistic effects of f-CNTs and CoO nanoparticles in catalytic reactions for tramadol determination.

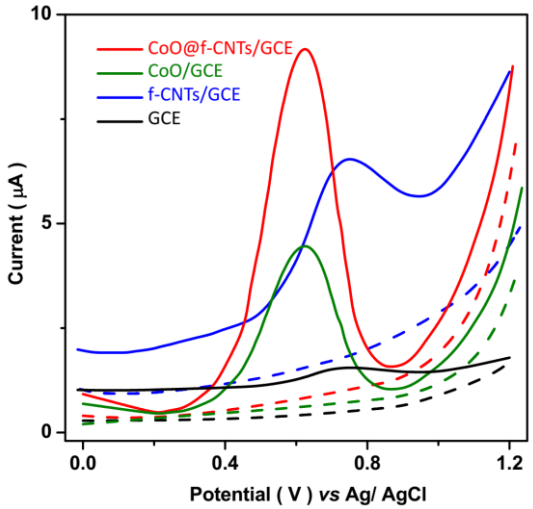

Figure 3. DPV responses of GCE, f-CNTs/GCE, CoO/GCE and CoO@f-CNTs/GCE at the potential window from 0.0V to 1.2 V with a scanning rate of 30 mV/s in 0.1 M PBS (pH 7.4) in absence (dashed line) and presence (solid line) of 100 µM tramadol.

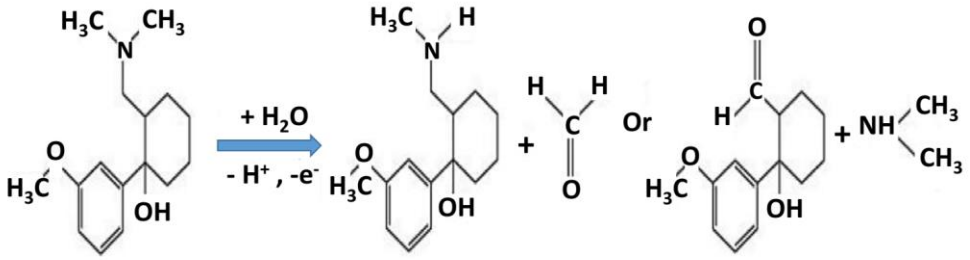

Figure 4. Schematic image of oxidation process of tramadol.

Figure 5 shows amperometry observations and a calibration plot of CoO@f-CNTs/GCE after adding 10 µM tramadol solution to 0.1 M PBS (pH 7.4) at a potential of 0.62 V. With each addition of 10 mM tramadol solution in the range of 1 to 300 mM, the amperometric current of CoO@f-CNTs/GCE increases linearly. The electrocatalytic peak current (IP) and tramadol concentration (C) are found to have a linear relationship as follows [67-69]:

I_P (µA) = 0.44971 C (µA/µM) + 0.02465 R^2=0.99980 (1)

Where R^2 is correlation coefficient. From the obtained linear relationship, the sensitivity can be determined to be 0.44971 µA/µM, and the detection limit (S/N=3) can be calculated to be 6 nM. Table 1 shows a comparison of the findings of the proposed tramadol sensing method with those of other tramadol electrochemical sensors that have been described in the literature. It is shown that the proposed tramadol sensing method based on CoO@f-CNTs/GCE has a comparable or even better sensing performance than other reported tramadol sensors, owing to the improved electronic structure, increased number of catalytic active sites, and abundance of oxygen vacancies and defects on the coupled interfacial nanostructure between CoO nanoparticles and f-CNTs [70-72].

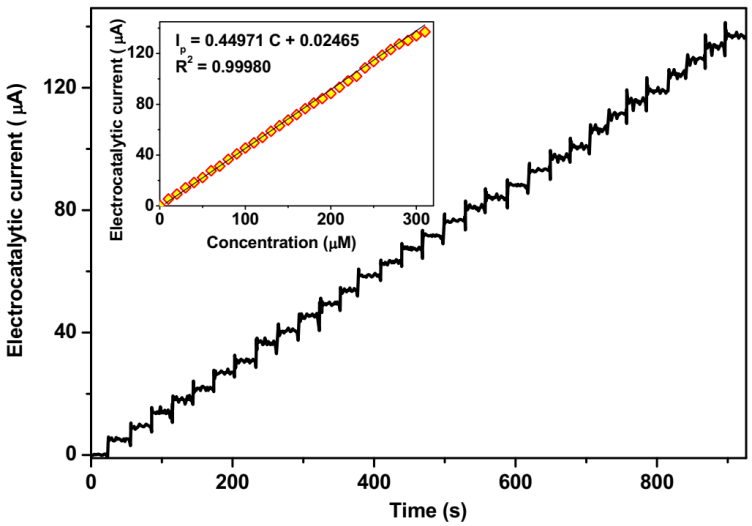

Figure 5. Amperometry measurements and corresponded calibration plot of CoO@f-CNTs/GCE after adding 10 µM tramadol solution into 0.1 M PBS (pH 7.4) at potential of 0.62 V.

Table 1. Performance of tramadol proposed sensing method in present study and other reported sensor in literatures.

Electrode	**Technique**	**LOD (nM)**	**Linear range (µM)**	**Ref.**
CoO@f-CNTs/GCE	Amperomertry	6	1 to 300	Present study
Magneto layer double hydroxide/Fe_3O_4@GCE	DPV	300	1.0 to 200	[30]
Pencil graphite electrode	DPV	3.8	0.1 to 1.5	[36]
Au/CNTs/pencil graphite electrode	DPV	130	0.1 to 3	[34]
molecularly imprinted polymer/CNTs	SWV	4	0.01 to 20	[31]
CuO/polypyrrole/pencil graphite electrode	SWV	1	0.005 to 380	[32]
$NiFe_2O_4$/graphene/carbon paste electrode	SWV	3	0.01 to 9	[33]
Carbon nanofibers/screen printed electrode	CV	0.016	5×10^{-5} to 0.1	[35]

The effect of possibly interfering chemicals on the detection of tramadol was explored in this work to evaluate the interference influence and selectivity of the proposed tramadol sensor. Table 2 shows the results of amperomertic electrocatalytic currents of CoO@f-CNTs/GCE at 0.62 V in 0.1 M PBS (pH 7.4) with 5µM tramadol and 30µM interfering compounds, which showed an extremely high response of CoO@f-CNTs/GCE to tramadol and poor electrocatalytic currents to a 6-fold excess of interfering compounds. As a result, the suggested tramadol sensor can be deduced to have a high selectivity for tramadol determination in clinical samples.

Table 2. Results of amperomertic electrocatalytic currents of CoO@f-CNTs/GCE at 0.62 *V* into 0.1M PBS with addition of 5µM tramadol and 30 µM of interfering compounds

Substance	**Added(µM)**	**Amperomertic current(µA) at 0.62 *V***	**RSD**
Tramadol	5	2.2486	±0.02811
L-Cystine	30	0.0731	±0.0078
Morphine	30	0.0915	±0.0089
Glucose	30	0.0290	±0.0015
Asparagine	30	0.0833	±0.0049
Cysteine	30	0.0419	±0.0019
Ascorbic acid	30	0.0324	±0.0015
Folic acid	30	0.0734	±0.0033
Uric acid	30	0.0723	±0.0029
Glutamine	30	0.0820	±0.0024
Citric acid	30	0.0367	±0.0012
Urea	30	0.0663	±0.0022
Acetaminophen	30	0.0649	±0.0019
Codeine	30	0.0582	±0.0025
Tyrosine	30	0.0455	±0.0014

Ibuprofen	30	0.0713	±0.0019
Dopamine	30	0.0301	±0.0022
NH_4^+	30	0.0301	±0.0024
NO_3^-	30	0.0277	±0.0022
Mg^{2+}	30	0.0546	±0.0020
K^+	30	0.0320	±0.0019
Fe^{3+}	30	0.0289	±0.0015
SO_4^{2-}	30	0.0365	±0.0014
Cl^-	30	0.0423	±0.0017
Ce^{2+}	30	0.0229	±0.0011

RSD: Relative Standard Deviation

The accuracy and utility of CoO@f-CNTs/GCE for identifying tramadol in prepared actual samples from urine samples collected from athlete volunteers were investigated. The RSD and recovery values were obtained using the findings of amperometric experiments at 0.62 V and an ELISA kit used for tramadol determination in prepared genuine samples before and after tramadol administration. Table 3 presents a summary of the findings. As can be observed, the ELISA and amperometric studies have a high level of agreement. The recovery (98.50% to 100.50%) and RSD (3.33% to 4.18%) values in Table 3 are both satisfactory. The findings show that the suggested approach has adequate validity, precision, and high promise for practical urine sample analysis.

Table 2. The analytical findings of determination of tramadol in the prepared real samples of urine samples collected from athlete volunteers.

Amperometry				**ELISA**		
spiked (µM)	**detected (µM)**	**Recovery (%)**	**RSD (%)**	**detected (µM)**	**Recovery (%)**	**RSD (%)**
0.00	0.00	---	3.33	0.00	---	3.53
2.00	2.01	100.50	4.18	1.98	99.00	4.08
4.00	3.96	99.00	3.87	3.97	99.25	3.68
6.00	5.91	98.50	4.12	5.97	99.50	4.09

4. CONCLUSION

The goal of this study was to create a CoO@f-CNT nanocomposite utilizing an electrodeposition approach on GCE as a stable, sensitive, and selective electrochemical sensor for detecting tramadol as a narcotic in urine for doping investigation. The results of structural tests suggested that a well-crystalline CoO@f-CNTs nanocomposite was successfully electrodeplated on GCE. Electrochemical investigations revealed that CoO@f-CNTs/GCE f-CNTs had a sensitive and selective performance, with a linear range of 1 to 300 µM for tramadol detection. The detection limit and sensitivity were calculated to be 0.44971 µA/µM and 6 nM, respectively. The usefulness and

precision of CoO@f-CNTs/GCE for determining tramadol in prepared real samples from urine samples of athlete volunteers were explored. The results showed that the ELISA and amperometric analyses had a high level of agreement, and the recovery and RSD values were acceptable. The findings showed that the suggested approach has adequate validity, precision, and high promise for practical urine sample analysis.

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