



Carbonaceous materials–20: electrochemical determination of viloxazine on a novel carbonaceous template

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Abstract

Since viloxazine is used as an antidepressant and approximately 15% of it is excreted from the body unchanged, detecting viloxazine in both bodily fluids and environmental samples is important. There are a limited number of studies in the literature on the detection of viloxazine by electrochemical methods. However, in recent years, there has been an increasing utilization of natural carbonaceous materials as electrodes due to their low cost, easy accessibility, and high electrical conductivity. This study explores the applicability of carbonaceous electrodes, derived from the pyrolysis of pine (*Pinus sylvestris*) pollen powder, coal tar, and their mixture, for the determination of viloxazine. The obtained carbonaceous material was characterized using scanning electron microscopy and electrochemical methods. The detection limit of the developed method was 0.02 μM with a detection range of 0.1–20 μM . The developed method was selective against various interferents, including dopamine, ascorbic acid, uric acid, and nitrite. Finally, the developed method was successfully applied to tap water and human serum samples for the detection of viloxazine.

Keywords Viloxazine · Differential pulse voltammetry · Carbonaceous electrodes · Coal tar

Introduction

The consumption of antidepressants has increased rapidly especially in developed countries. It has been reported that approximately 121 million people worldwide are affected by depression due to a chronic or recurrent mood disorder (Rani et al. 2016). In a report published ten years ago, approximately 7.5% of the population in Europe regularly use antidepressants (Lewer et al. 2018). Viloxazine (VX), a morpholine derivative (2-[(2-ethoxyphenoxy) methyl] morpholine), is a selective norepinephrine reuptake inhibitor with minimal inhibitory effect on the reuptake of serotonin

receptors (Madej et al. 2019). Approximately 12–15% of VX is eliminated without being metabolized and excreted in the urine (Finder et al. 1977). Also, VX is reported to be used for attention-deficit hyperactivity disorder (ADHD), a prevalent neurological disorder among children and adolescents aged 2 to 17 years (Singh et al. 2022). Consequently, the widespread use of VX also raises concerns about the presence of this active substance in the environment (Madej et al. 2019). On the other hand, in vivo microdialysis in a rodent model has also showed that therapeutic concentrations of VX potentiate fivefold to sixfold increases in norepinephrine and dopamine levels in the prefrontal cortex (Yu and Downard 2005), a region strongly implicated in ADHD pathophysiology (Levy 2008). Therefore, monitoring of viloxazine, especially in ADHD patients, may be considered important.

VX was determined usually by chromatography methods in the literature (Fazio et al. 1984; Gillilan and Mason 1981; Groppi and Papa 1985; Norman et al. 1979; Thomare et al. 1990). Electroanalytical techniques have started to be preferred more than other techniques with their simplicity, low cost, and relatively short response times (Üstündağ and Şahin 2023). However, there are limited studies regarding the detection of viloxazine in other sensor platforms. For

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instance, as electrochemical sensor platforms, a method for determining VX from human plasma using square wave voltammetry (SWV) has been reported (Garrido et al. 2008). In this study, the sensor's operating range was 7–45 μM , with a detection limit of 0.8 μM . In another study, Madej and co-workers proposed a method using differential pulse voltammetry (DPV) (Madej et al. 2019). Their method has been employed for determination of VX in spiked tap and river water samples with recovery between 95–99%.

Pyrolyzed carbonaceous materials with low cost, high electrical conductivity, and stable physical and chemical properties (Alcántara et al. 2000) have been used as electrode material in electrochemical studies as an alternative to other expensive carbon materials such as carbon nanotubes, graphene, and fullerenes (Küçükayar et al. 2023). In the literature, there are many electrodes prepared from various carbon sources including various animal tissue-derived (Chen and Shen 2022; Xue et al. 2021) and plant-derived biomass (Yanjia Zhang et al. 2018). Such electrodes have been used as a component in various applications including electrochemical biosensing (Erkal et al. 2016; Küçükayar et al. 2023), supercapacitor (Saini et al. 2021), catalysis (Shetty et al. 2023), solar cell (Liu et al. 2014), fuel cell (Peppley 2006), battery (Yang et al. 2022) and water purification (Bhatnagar et al. 2015). The use of pollen as biomass is preferred because it naturally serves as a carbon source with a diameter of 2 μm –100 μm (Mampage et al. 2022; Lu Zhang et al. 2019).

We report a new method for the determination of VX by electrochemical technique using electrodes made by green chemistry, first time. Low-cost, environmentally friendly electrodes based on green chemistry synthesis (Güleşen et al. 2019; Küçükayar et al. 2023; Üstündağ 2023; Üstündağ and Erkal 2017) have been reported before by our group. Here, a novel sensitive electrode was prepared by pyrolysis of a mixture of pine pollen dust (PPD), a biomass waste, raw-coal tar pitch (r-CTP), and a carcinogenic waste (Erkal et al. 2016). Then, this electrode was successfully used for the determination of VX in human serum and tap water.

Experimental section

Chemicals and equipment

r-CTP was purchased from CSense Chemical Co. (Turkey). Pine pollen dust (PPD) was obtained from pine trees located in the Kütahya Dumlupınar University campus. All chemicals used in this study were of analytical purity and were purchased from the local representatives of Merck and Sigma-Aldrich company (USA). The carbonized pine pollen (CPP) was prepared as follows: First, PPD was carbonized in tube furnace (Protherm, USA) at 1000 °C for

1 h under argon atmosphere, with a temperature increase rate of 5 °C/min (Yeter et al. 2021). The carbonized material was ground using a planetary ball-mill (Retsch, PMI, Germany) for 4 h under 400 rpm (Erkal et al. 2016) and then dissolved in 0.5 M sulfuric acid solution to remove organic impurities. Finally, the product was washed to a neutral pH value, and dried in a vacuum furnace at 55 °C for 6 h. Then, 5 g of CPP was added in viscose suspension of 5 g of r-CTP in acetone. The mixture was carbonized in tube furnace at the same condition with first carbonization. The carbonaceous material was denoted as CTP-CPP. For control experiments, r-CTP were carbonized under the same conditions and then ground. The carbonaceous product was denoted as CTP.

Electrochemical measurements were carried out with using an Ivium CompactStat (Netherland) with triple electrode system. The reference electrode and auxiliary electrode were Ag/AgCl/KCl(sat) and platinum wire, respectively. Carbonaceous material, CTP-CPP, attached on the glassy carbon electrode (GC) was used as working electrode. The bare GC electrodes were cleaned as described elsewhere (Mohammed Albayati et al. 2023; Saygılı-Canlıdınç et al. 2023). The electrode was cleaned and polished on microcloth (Buehler, Lake Bluff, IL, USA) with 100 nM and 50 nM of the aluminum oxide suspension (Baikowski, USA) (Erkal et al. 2014). The polished electrodes were sonicated with ultra-pure water (UPW, 18.2 M Ω cm, Human Power 1+, S. Korea) mixture of 1:1 (v/v) isopropyl alcohol (IPA)/acetonitrile (MeCN) in an ultrasonic bath (SK1200H, China) for approximately 5 min (Yavuz et al. 2016).

Electrode modification

Electrode modification using CTP-CPP on the GC was implemented according to following procedure: 100 mg of CPP and CTP-CPP were diluted in 10 mL of acetone, and then, 20 μL of each suspension was dropped onto bare GC electrodes. The electrodes were then dried under the IR lamp (75 W) for 5 min (Yılmaz-Alhan et al. 2022). CPP-, CTP- and CTP-CPP-modified GC electrodes were denoted as GC/CPP, GC/CTP and GC/CTP-CPP, respectively.

Characterization of materials

The CTP-CPP was characterized with scanning electron microscopy (SEM, Nova, NanoSEM-650, Belgium). Electrochemical characterization was done using linear sweep voltammetry (LSV) in 0.1 mM VX in acetate buffer solution (ABS, pH 5.0) at a scan rate of 200 mV/s versus Ag/AgCl_(sat).

Optimization of carbonaceous electrode

GC/CTP-CPP electrode was used for determination of 0.1 mM VX in 0.1 M ABS by cyclic voltammetry (CV) versus Ag/AgCl_(sat) at different scan rates (10–300 mV/s).

Before proceeding with the calibration study, the working pH and the concentration of the carbonized material used for modification on the electrode surface were optimized. After pH optimization was carried out at 3.5, 4.0, 4.5, 5.0, and 5.5 pHs in ABS, optimization of the suspension volume was performed at 5, 10, 15, 20, and 25 μ L of 100 mg CTP-CPP in acetone at the optimized pH. In these studies, characterization was performed with DPV using 20 μ M VX on CTP-CPP in ABS. DPV measurements were recorded under 25 mV of pulse amplitude, 10 mV of step potential with 0.1 s pulse time.

Electroanalytical application of viloxazine

DPV measurements of VX at different concentrations (0.1–20 μ M) were performed at the optimum parameters, such as pH and CTP-CPP suspension volume. The reaction mechanism of VX on modified electrode is presented as Figure S1 (Madej et al. 2019).

After the calibration curve was obtained, regression analysis of the developed method was performed. The limit of detection (LOD) was calculated by taking into account the 3σ noise level using calibration curves (Saadati et al. 2013). The quantification limit (LOQ) was calculated as three times the LOD value. Intraday and interday accuracy and repeatability values of the developed method were also investigated. Then, ascorbic acid (AA, 500 μ M), dopamine (DA, 100 μ M), uric acid (UA, 100 μ M), and nitrite (NO₂⁻, 100 μ M) in ABS, which are potential interfering agents found in biological samples, were used for the interference studies.

Real sample analyses were conducted by detecting VX spiked to commercially obtained human serum (Cat No: H4522, Sigma-Aldrich, USA) and also tap water exposed to a potential contaminant. Standard addition to human serum samples was performed according to the literature (Garrido et al. 2008). In this analysis, the added VX concentrations were 5 and 10 μ M for the serum, and 1 and 10 μ M for tap water.

In the studies, analyses and measurements were conducted with a minimum of five repetitions unless otherwise stated due to analytical requirements. The results were represented as the mean \pm standard deviation (σ) of these analyses/measurements.

Results and discussion

Characterization studies

The carbonaceous electrode and its constituents have been characterized using SEM and linear sweep voltammetry

techniques. In Fig. 1, SEM images of CPP, the carbonized form of PPD, are presented at 500X (top) and 5000X (bottom) magnifications. On the right panel, SEM images of CTP/CPP obtained by doping PPD into coal tar are displayed. The size of CPP is in between 8–32 μ m, and the CPP incorporation with the bulk-structure of CTP/CPP is distinguishable in the SEM image. At higher magnification (bottom panel), a detailed microstructure is observed. Particularly in the CTP/CPP form, CPP appears to be distributed relatively uniformly within the CTP.

The voltammetric performance of carbonaceous electrodes for the detection of 0.1 mM VX was determined using the LSV technique (Fig. 2). The peak current values for 0.1 mM VX on GC, GC/CPP, GC/CTP, and GC/CTP-CPP electrodes were found to be 0.86 ± 0.01 , 2.46 ± 0.04 , 2.81 ± 0.04 , 3.02 ± 0.06 μ A respectively. The peak current value of VX on the GC/CTP-CPP electrode is higher compared to the others. This increase may be due to both the change in the active surface area of the electrode and the material forming the surface film (Üstündağ and Şahin 2023). Therefore, it was decided that the most suitable electrode for VX detection is GC/CTP-CPP.

To understand whether the oxidation of VX on the GC/CTP-CPP electrode is diffusion-controlled, an analysis was conducted by varying the scan rate using the CV technique in the range of 10–300 mV/s. The peak current $-i_p^{1/2}$ graph exhibits a linear change with a determination coefficient of 99% (Fig. 3). According to Randles–Sevcik equation, it was understood that diffusion-controlled voltammetric determination of VX could be made (Üstündağ and Şahin 2023).

Optimization studies

Voltammograms of 20 μ M VX on the GC/CTP-CPP electrode were obtained at various pH levels (pH 3.5–5.5 in ABS) using the DPV technique. In Fig. 4, the peak current of 20 μ M VX vs pH change on the electrode surface prepared by modifying 20 μ L of CTP-CPP suspension onto the GC surface are provided using the DPV technique. The highest peak current was achieved at pH 5.

After determining the optimum pH value (pH 5), the amount of CTP-CPP used in the formation of the GC/CTP-CPP electrode was optimized. For this purpose, 100mg of CTP-CPP was added to 10 mL of acetone, and sonicated for 10 min to prepare the suspension. Afterward, 5, 10, 15, 20 and 25 μ L of this suspension were taken with a micropipette, respectively, and dropped onto the glassy carbon (GC) electrode. Then, drop-casting was completed by drying the suspension using an IR lamp (75 W) for 10 min. The modified electrodes were sonicated in pure water for 2 min to ensure that stable modified surfaces were formed. Peak current values were determined by taking measurements of 20 μ M VX in ABS buffer (pH 5) with

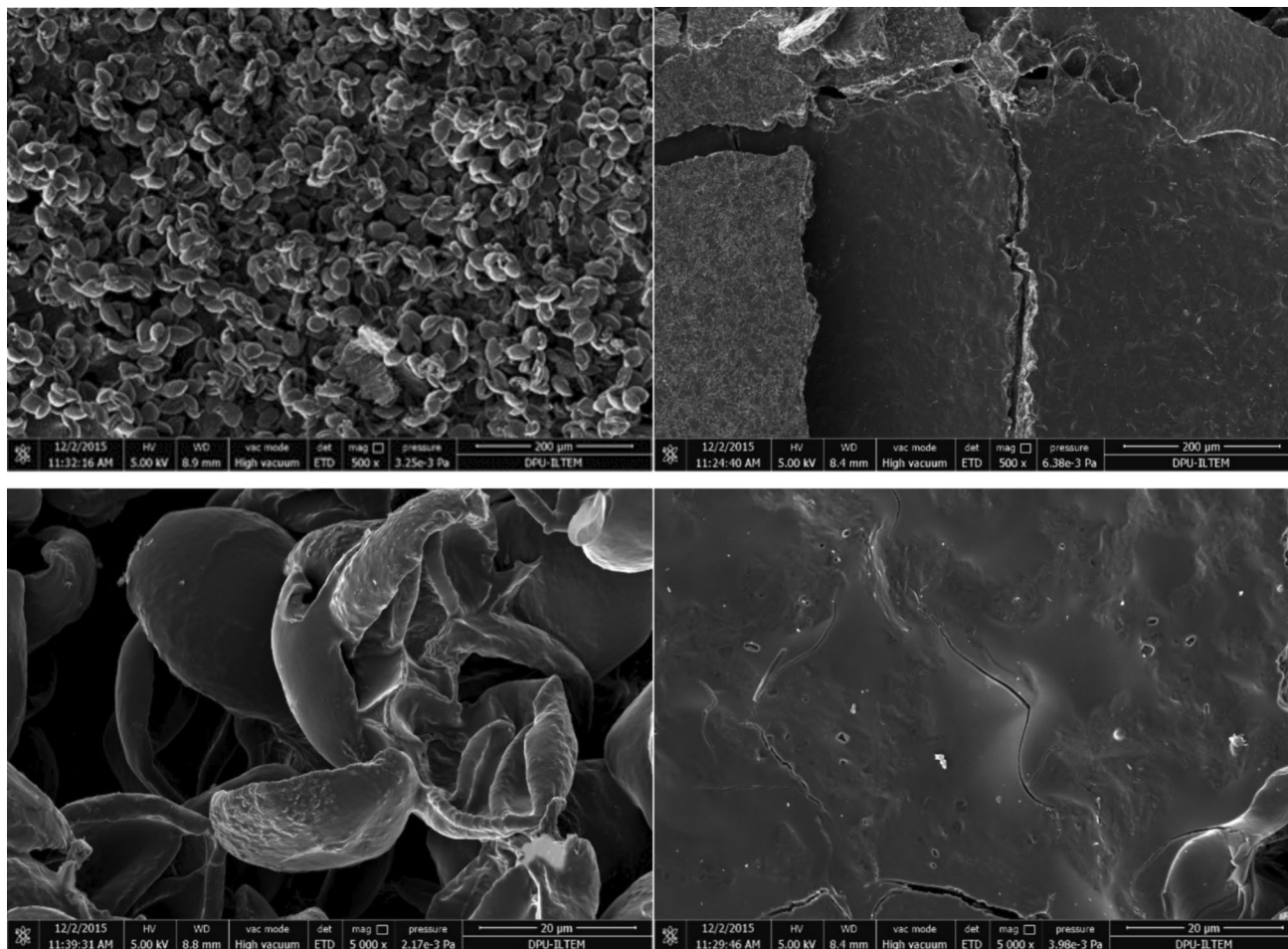


Fig. 1 SEM images of CPP (left) and CTP/CPP (right) with 500X (top) and 5000X (bottom) magnifications

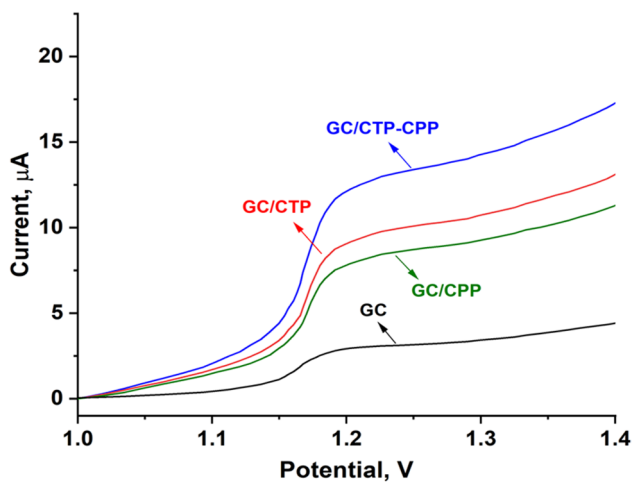


Fig. 2 LSVs of 0.1 mM VX on GC, GC/CTP, GC/CTP-CPP, and GC/CTP-CPP versus $\text{Ag}/\text{AgCl}_{(\text{sat})}$ (scan rate is 200 mV/s)

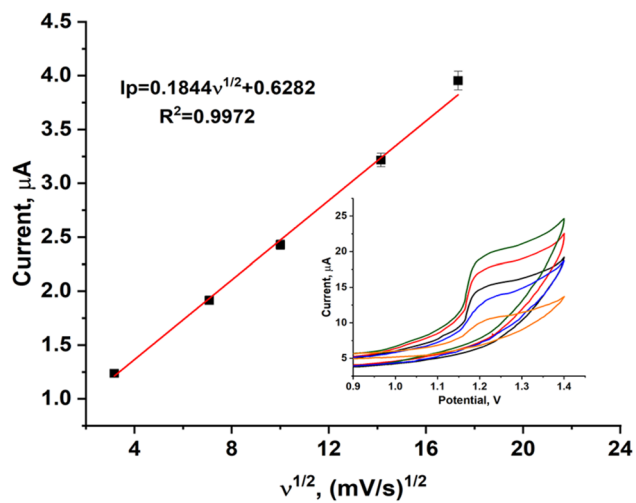


Fig. 3 CVs of 0.1 mM VX on GC/CTP-CPP versus $\text{Ag}/\text{AgCl}_{(\text{sat})}$ (scan rate range is 10–300 mV/s), and their anodic peak current i_p versus $v^{1/2}$ graph

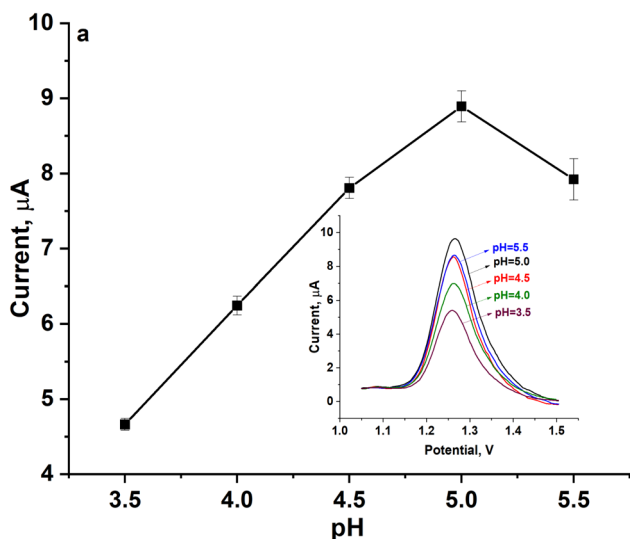


Fig. 4 DPV of 20 μM VX on GC/CTP-CPP versus $\text{Ag}/\text{AgCl}_{(\text{sat})}$ at different pHs (Pulse amplitude 25 mV, step potential 10 mV, pulse time 0.1 s)

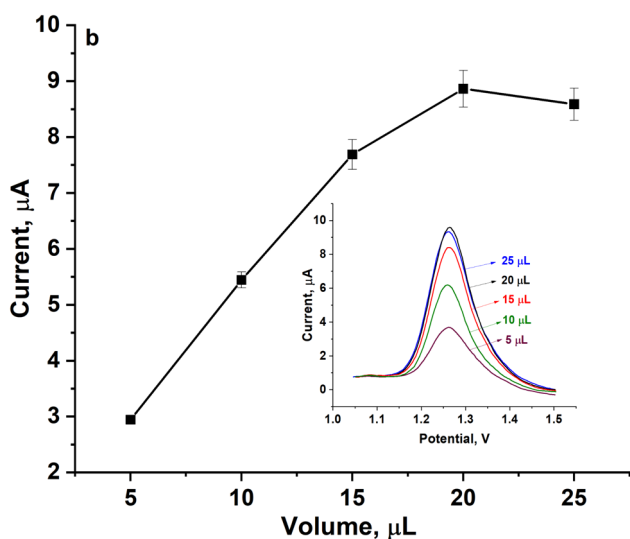


Fig. 5 DPV of 20 μM VX on GC/CTP-CPP for different suspension volume of CTP-CPP (Pulse amplitude 25 mV, step potential 10 mV, pulse time 0.1 s)

these electrodes by DPV. In Fig. 5, as the highest peak current value is 8.863 μA , the optimum suspension volume was selected as 20 μL .

The calibration curve of VX in ABS was obtained from DPV voltammograms using GC/CTP-CPP electrodes modified with 20 μL CTP-CPP suspension, and VX concentration in the range of 0.1–20 μM prepared in ABS buffer (Fig. 6). Voltammogram measurements were recorded as five replicates, and then, the average of these data is used to draw the calibration curve. Analytical parameters of the developed method are given in Table 1.

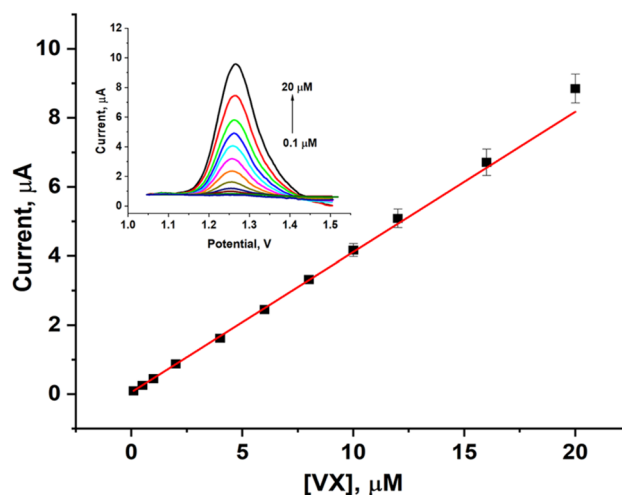


Fig. 6 DPVs of different concentration of VX in ABS and the calibration curve (Pulse amplitude 25 mV, step potential 10 mV, pulse time 0.1 s)

Table 1 Analytical parameters of the developed method

Analytical parameter	Value
Linear range	0.1–20 μM
Linear equation ($n=5$) (peak current (I_p), μA - viloxazine concentration [VX], μM)	$I_p = 0.4061[\text{VX}] + 0.0534$
Standard error of slope, \pm	0.0046
Standard error of intercept, \pm	0.0022
R^2	0.9987
LOD (S/N ratio of 3)	0.02 μM
LOQ	0.06 μM

The accuracy and precision of the developed method were determined by making intraday and interday measurements for five consecutive days. In Table S1, the relative error (RE) and the relative standard deviation (RSD) values, which is a measure of accuracy and precision, respectively, are given as the average of five measurements for 1.0 and 10 μM VX standard solution in ABS (pH 5). Below $\pm 5\%$ RE, and below 5% RSD results show the reliability of the developed method.

To determine the possible interference effects, changes in the peak current were determined by adding the species given in Table S2 to the solution containing 1.0 μM VX (in ABS, pH 5). The effects of interferent species added, i.e., AA (500 μM in ABS), and DA, UA and NO_2^- (100 μM in ABS) on the peak current of VX only data were below $\pm 5\%$. The highest interference effect obtained was 3.76% for the NO_2^- ion, but this interference effect is within accuracy limits.

Following the interference experiments, real sample tests were carried out for the developed VX sensor. Recovery

Table 2 Recovery values obtained from standard additions of VX to real samples

Sample	Amount added, μM	Found \pm SD, μM	Average recovery % ($n=3$)
Human serum	5	4.77 ± 0.11	95.4
	10	10.08 ± 0.32	100.8
Tap water	1	1.03 ± 0.03	103.0
	10	10.32 ± 0.47	103.2

Table 3 Comparison of electrochemical VX detection methods

Technique	Electrode	Linear Range, μM	LOD, μM	References
DPV	CTP-CPP	0.1–20.0	0.02	This study
SWV	GC	7.0–45.0	0.80	(Garrido et al. 2008)
DPV	BDD	0.1–20.0	0.04	(Madej et al. 2019)

BDD Boron-doped diamond, *SWV* Square wave voltammetry

values for 5 μM and 10 μM VX standards spiked human serum samples and 1 μM and 10 μM VX standards spiked to tap water samples are given in Table 2. According to the obtained data, the recoveries for human serum and tap water samples ranged from 95.4% to 103.2%. These recoveries are well within the analytical performance limits, and the real sample tests for the developed method were deemed as quite satisfactory.

A comparison of the proposed method with the electrochemical VX detection methods reported in the literature is given in Table 3. The LOD and detection ranges of the reported sensors and the developed method in this study are quite comparable. On the other hand, the limited number of studies in the literature for electrochemical detection of VX makes this study important in terms of filling the gap in the literature.

Conclusions

In this study, a novel electrode for the electrochemical detection of VX was developed using low-cost and environmentally friendly materials prepared through a green chemistry approach. To achieve this, an electrode was prepared by pyrolysis of a mixture of pine pollen powder and coal tar pitch as abundant carbon source. The analytical performance of the developed method was quite well within the limits of the other electrochemical methods reported in the literature for VX. The linear detection range was 0.1–20 μM VX, while LOD and LOQ values were 0.02 μM VX and 0.06 μM VX, respectively. The accuracy and the precision of the method were high, i.e., as maximum relative error of 4.3%, and as maximum RSD% of 4.55, and within acceptable limits of the analytical requirements. Moreover, the maximum interference effect as signal change %, i.e., 3.76, for interferent species (DA, AA, and UA molecules and NO_2^- ion) was within the accuracy limit. Finally, analytical performance for real sample tests as percentage of recoveries

of spiked standards was satisfactory as it is between 95.4% and 103.2% in human serum and tap water samples. These results show that the developed electrochemical method is reliable and sensitive for VX detection in various media. Furthermore, as the green-synthesized electrode material, it can be a cost-effective alternative for graphene/graphite like carbon nanomaterials, since direct pyrolysis and grinding is adequate to produce.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11696-024-03586-0>.

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