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Simultenous Voltammetric Detection of Acetaminophen and Tramadol using Molybdenum Tungsten Disulfide-Modified Graphite Screen-Printed Electrode

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Nanopetal-structured molybdenum tungsten disulfide (MTD) have been successfully synthesized and applied as a very good modifier for constructing a new and strongly sensitive electro-chemical sensing ground for concurrent detection of two crucial pain reliever medicines, acetaminophen and tramadol. XRD, EDX and SEM have been employed for characterizing synthesized nanoparticles. A comprehensive examination by electro-chemical techniques, including differential pulse voltammetry (DPV) and cyclic voltammetry (CV) has been conducted for clarifying electro-catalytic features of nanopetals by modify graphite screen printed electrode (SPE). Under the optimal condition, the MTD/SPE provided linearly responses in range between 0.04-600.0 and little determination limit of 12.0 nM for acetaminophen. Accurate and sensitive concurrent detection of acetaminophen and tramadol in biological samples and medicine formulations validated the suggested sensor reliability.

Keywords: Acetaminophen**,** Tramadol**,** Nanopetal-structured $MoWS_2$**,** Graphite screen printed electrode

1. INTRODUCTION

Acetaminophen (N- acetyl -p-aminophenol: paracetamol, AC) is commonly employed in the world, which is widely applied to relieve headache, fever, over the counter analgesic (pain reliever), additional partial pains. It is one of the main ingredients in several colds and flu cures [1-3]. It can be provided with no prescription and is often suggested while aspirin has issues for patients, especially in pediatrics or after surgeries. Also when it is combined with opioid analgesics and nonsteroidal antiinflammatory drugs (NSAIDs), acetaminophen is employed in managing more serious pains like cancers or post-operative pain. A single-dose of acetaminophen exhibit analgesic action various severe pain syndromes without any unpleasant impacts. Yet, ingestion of excessively high doses (more than 1000 milligram per single dose and more than 4000 milligrams per day for adult people, more than 2000 milligram per day if a person drinks alcohol) or long term consumption of acetaminophen may result in accumulating poisonous metabolites, declining liver's capability for treatment, and creating some health issues, including deadly nephrotoxicity and hepatotoxicity [4-9].

Tramadol, (1R, 2R)-2 -[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol, is an l -opioid recipient agonist that acts on analgesic centrally, is applied initially for treating modest to severe pains [10,11]. It causes analgesia in the transient and persistent pains via synergistically combining weak-opioid and mono-aminergically (serotonin & noradrenaline) intervened mechanisms. Severe analgesic opioid impacts of tramadol are moderated by its major metabolite O -desmethyltramadol (ODMT). Tramadol alone does not possess opioid activity; however, it has anti-nociceptive impacts via inhibiting norepinephrine re-absorption, which causes its' effectiveness for treating neuropathic pain. An overdose of tramadol has a risk of mortality and unpleasant consequences, including slowness or ceased breath, hazardous alterations in heart beats and even death [12-16].

With regard to the supplementary mechanisms of two analgesics, if we combine these medicines as a established pill; that is, acetaminophen 325 mg and tramadol 37.5 mg, it will increase analgesic efficacy, decrease unpleasant consequences, enhance the medicine action, and create efficient analgesia in patients who have modest to very serious pain and individuals who have long-term tender states described by sporadic aggravations of pain. Nevertheless, overdosing the medicines are poisonous to the body and inappropriate or unrestricted consumption may cause severe health side effects. Determining the amounts of the presence of the compounds in biological fluids and drugs is crucial for preventing overdose that leads to unpleasant impacts or drunkenness. Thus, sensitive, easy, selective, and precise detection of acetaminophen and tramadol will be one of the significant steps for efficient therapy and control of the medicines [17-19]. Numerous procedures were applied for concurrent determination of the two analytes. The procedures contain high efficiency liquid chromatography [20], LC–MS [21] and second derivative spectroscopy [22]. Yet, their analysis can be done in a long-term process, may be costly, and mostly requires a pre-treatment phase of sampling. Acetaminophen and tramadol are electro-active complexes that may be oxidized electro-chemically. Electro-chemical techniques were commonly known as strong means for determining electroactive biological compound because of their small cost, simple operational processes, acceptable specificity, very good stability, great sensitivity, and little limit of determination [23-34]. Nevertheless, such

substance systems are inhibited because of low speed electrode kinetics and great over-potential that result in lower charge transport and higher interference from another electro-active specimen, which coexist with the sample, which finally reduce devices' sensing capacities according to the substances [35-37]. The use of modifier material caused possible improvement of sensitivity and selectivity in determining the target [38-42]. Therefore, an acceptable well-modified electrode substance is essential for selective and sensitive diagnosis [43-55].

Screen printed electrodes are often employed in analytical uses due to their specific characteristics, including little dimensions, small determination limits, quick response duration, great reproducibility, and so forth. As well as the selectivity of such tools may be increased by rational modification of the electrode substrate with electron mediators [56-64].

Currently, researchers have been extensively attracted by nanomaterials because of the enhanced requirement for controlling various analytes found in environmental relevance samples. Electrodes are changed by nano-materials that may increase electro-chemical transduction with the following methods: improving surface to volume ratio, which leads to the augmented bio-chemical interplay between recognition layer and analyte, and increased transfer of electron [65-74]. Integration of highly versatile nano-materials with electrode based system may dramatically increase sensors features, which allows for reliable and sensitive quantification of different clinical significant bio-molecules [75-84].

Molybdenum disulfide (MoS_2), which is a graphene- like 2D layered TMD, has been greatly attracted by different areas. With regard to the previous studies, MoS_2 consists of 3 atomic layers of S-Mo-S piled together by vanderWaals interplays between inter-layers. Tungsten incorporation onto MoS_2 sheets may increase electrical features for better catalytic use. Nevertheless, because of the layered nature and great surface energy of 2D-structure, MoS_2 is subject to reinforce together via π-π interplays during the process of preparation that can result in the blockage of the catalytic edge locations on MoS_2. Numerous procedures were designed for preparing MoS_2 with acceptable catalytic characteristc, including variable morphologies, exfoliation, and composition with another substance [85-87].

Therefore, this research aimed to develop a modified electrode for sensitive detection of acetaminophen and tramadol, using MTD nanostructure in order to augment characteristics of screen-printed electrodes. The final electrode has been successfully used to determine acetaminophen and tramadol in real specimens.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

In this stage, we utilized the Auto-lab potentiostat/galvanostat (PGSTAT 302N; Eco Chemie:the Netherlands) for electro-chemical experimentations and monitored the system with the general-purpose electrochemical system software.

In addition, SPE (DropSens; DRP-110: Spain) had 3 conventional electrodes of the graphite counter electrode, unmodified graphite working electrode as well as a silver pseudo-reference electrode. In addition, Metrohm 710 pH meter has been used to measure pH.

Tramadol, acetaminophen, as well as all the remaining reagents had analytical grade, obtained via Merck (Darmstadt: Germany). Finally, we utilized the ortho-phosphoric acid and its salts to reach a pH range from 2.0 to 9.0. The nanocomposite was synthesized according to the literatures [88].

2.3. Preparing Electrode

The bare screen-printed electrode was coated by MTD composite. To prepare the MTD composite stock solution in 1 mL of aqueous solution, the MTD composite (1 mg) was distributed by 30-minute ultra-sonication, whereas the $MoWS_2$ suspension aliquots (5 µl) were cast on carbon working electrodes. Then, the solvent was left to be evaporated at an ambient temperature.

2.4. Production of Real Samples

To this end, 10 ml of directly refrigerated urine samples was centrifuged at 2,000 rpm for 15 minutes, followed by filtering the supernatant using a 0.45 µm filter. Then, different volumes of solution were distributed into a 25-ml volumetric flask and diluted with PBS (pH = 7.0) until the mark, which were anaesthetized by various doses of tramadol and acetaminophen. The standard addition method was used to determine the tramadol and acetaminophen concentrations.

First, 5 100-mg acetaminophen pill (Tehran Chemie Pharmaceutical Co., Iran) were powdered to prepare a solution by dissolving the powder (500 mg) in water (25 ml) in exposure to ultra-sonication. Then, different dilutions were poured in 25-ml volumetric flasks and reached final volume with PBS at pH of 7.0. The standard addition method was used to measure the acetaminophen concentrations.

Second, 5 100-mg tramadol pill (Tehran Chemie Pharmaceutical Co., Iran) were also powdered to prepare the solution by dissolving the powdered drug (500 mg) in water (25 ml) using ultra-sonication, followed by preparing different dilutions of the drug in 25-ml volumetric flask and the diluting to the final volume with PBS at pH of 7.0). The tramadol concentrations were measured in accordance with the standard addition method.

3. RESULT AND DISCUSSION

3.1. Electrochemical Profile of the Acetaminophen on the MTD/SPE

For studying electro-chemical behaviours of acetaminophen that has been considered to be depended on pH, obtaining an optimal pH-value would be of high importance for achieving acceptable outputs. Therefore, we used the modified electrode to run experimentations at different pH values in a range from 2.0 to 9.0. Finally, the most acceptable outputs have been observed for electro-oxidation of acetaminophen at thepH of 7.0.

Figure 1 represents cyclic voltammograms in the presence of 100.0 µM acetaminophen with the bare SPGE (Curve b) and MTD/SPE (Curve a). Based on the CV outputs, the greatest oxidation of acetaminophen on the MTD/SPE occured at 300 mV that has been ~200 mV more negative than the bare SPGE.

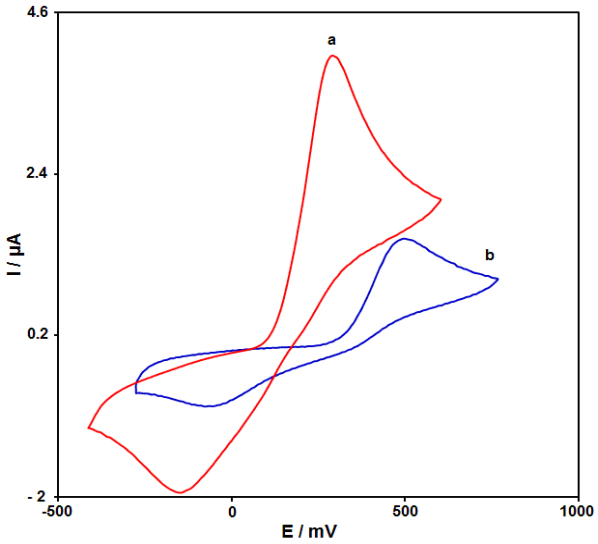

Figure 1. The CVs of a) MTD/SPE, (b) unmodified SPE in 0.1 M PBS at pH of 7.0 in the presence of 100.0 µM acetaminophen at 50 mVs^{-1} scan rate.

3.3. Impacts of Scan Rates on the Results

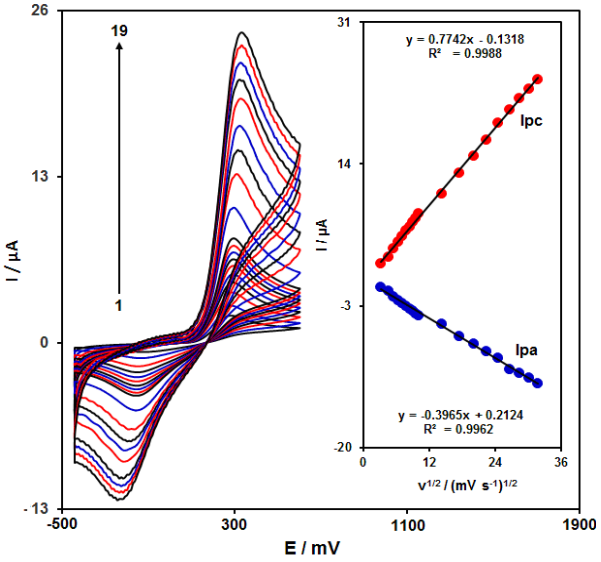

Figure 2. CVs of MTD/SPE in 0.1 M PBS at pH of 7.0 consisting of 150.0 µm acetaminophen at distinct scan rates; 1-9 corresponded to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mV s^{-1}. Inset; variations in the anodic peak currents vs. $v^{1/2}$.

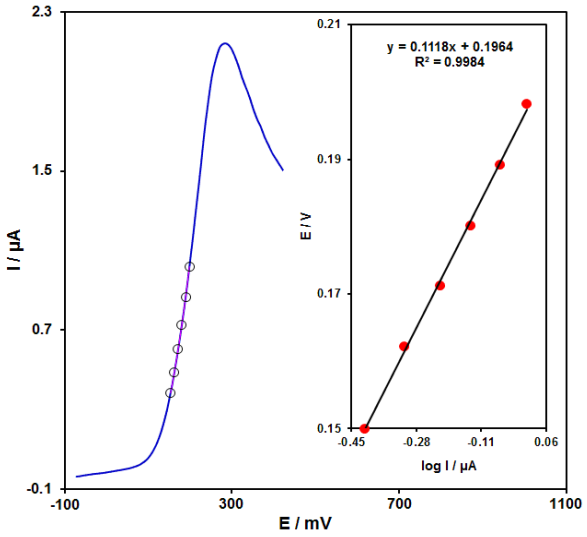

Figure 3. The linear sweep voltammograms at 10 mV s^{-1} of the electrodes in 0.1 M PBS at pH equal to 7.0) with 150.0 µM acetaminophen. These points stand for the output utilized in Tafel plot. As seen, inset represnts Tafel plot derived from the linear sweep voltammogram.

The enhanced scan rates resulted in the augmented oxidation peak current based on the outputs shown on the effectiveness of the potential scan rates on acetaminophen oxidation current (Figure2). Additionally, it has been found that Ip linearly related to the square root of the potential scan rate ($\nu^{1/2}$), demonstrating acetaminophen oxidation procedure has been diffusion-controlled [75,80].

In this stage, Tafel plot has been drawn from data obtained from the ascending part of current voltage curve recorded at the scan rate equal of 10 mVs^{-1} for acetaminophen (Figure3). It is notable that this piece of voltammogram, which has been named the Tafel region has been influenced by the electron transfer kinetics between the substrate (acetaminophen) and MTD/SPE. The, the Tafel slope of 0.1118 V has been observed in complete agreement with contribution of 1 electrons in the rate determining phase of the electrode [80,83,84] supposing the charge transfer coefficient, α=0.47 for acetaminophen.

3.4. Chronoamperometric Analyses

The analysis of chronoamperometry for acetaminophen specimens has been done with $MoWS_2$ /SPE at 0.35 V. Figure 4 displays Chronoamperometric outputs of diverse concentrations of the acetaminophen sample in PBS at pH equal to 7.0).

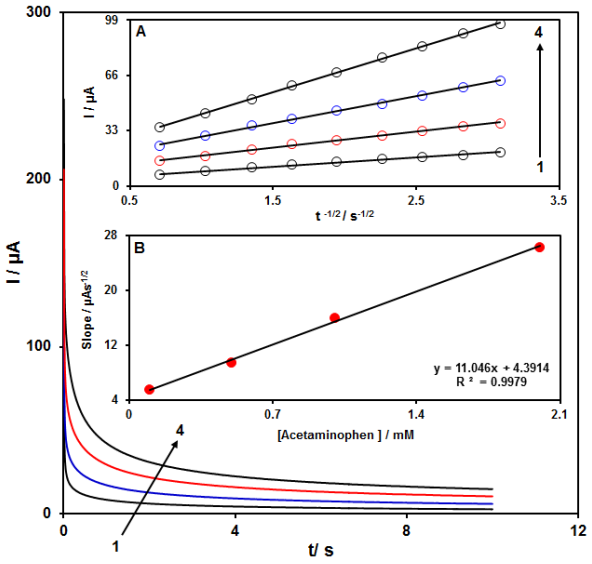

Figure 4. The chrono-amperograms obtained at MTD/SPE in 0.1 M PBS at pH of 7.0 for different concentrations of acetaminophen. It is notable that 1–4 correspond to 0.1, 0.4, 0.8, 1.3 and 2.0 mM of acetaminophen. Inset A. the I plot versus $t^{-1/2}$ observed by chrono-amperograms 1 to 4. B. Slope plot of the straight line vs. concentration of acetaminophen.

In addition, Cottrell equation has been offered for the chronoamperometric analyses of electroactive moiety based on the mass transfer restricted conditions [83,84,89]:

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

So that D implies the diffusion coefficient (cm^2 s^{-1}). C_b stands for the exerted bulk concentration (mol cm^{-3}). Figure 4A shows experimental results of I vs. $t^{-1/2}$, reflecting the best fit for distinct concentrations of acetaminophen. Afterwards, final slopes relative to the straight lines in Figure 4A have been drawn versus acetaminophen concentration (Figure 4B). Therefore, D mean-value equaled to 1.0×10^{-5} cm^2/s with regard to Cottrell equation and resultant slopes.

3.5. Calibration Curve

Considering the finalpeak current of acetaminophen with the MTD/SPE, acetaminophen has been quantitatively analyzed in the water solution (Figure 5). Therefore, we utilized the modified electrode (MTD/SPE) as a working electrode in a concentration range of acetaminophen in 0.1 M PBS in DPV based on the DPV merits like more reasonable sensitivity and more acceptable performance in the analytical utilizations.

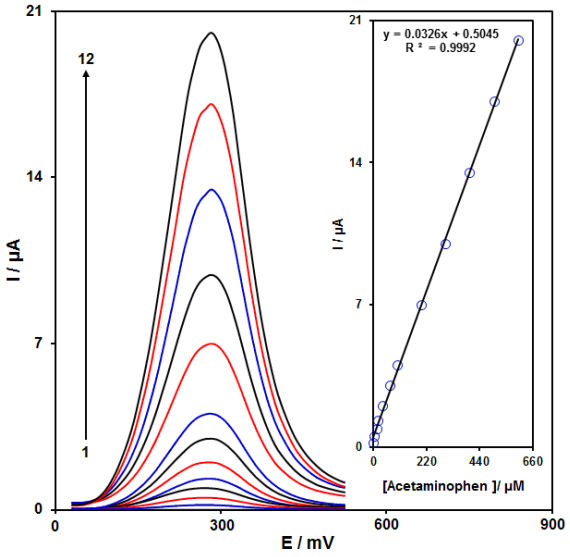

Figure 5. DPVs of MTD/SPE in 0.1 M PBS (pH 7.0) containing different concentrations of acetaminophen. Numbers 1–12 correspond to 0.04, 5.0, 15.0, 20.0, 40.0, 70.0, 100.0, 200.0, 300.0, 400.0, 500.0 and 600.0 µM of acetaminophen. The inset shows the plot of the peak current as a function of the acetaminophen concentration in the range of 0.040-600.0 µM.

Hence, the peak current linearly related to acetaminophen concentration in a concentration ranging from 0.04-600.0 µM with a correlation coefficient equal to 0.9992. Finally, LOD equalled 12.0 nM.

Table 1 shows a comparison of analytical properties for the detection of acetaminophen at the prepared electrode in this work and some other works.

Table 1. A comparison of the efficiency of various modified electrodes reported for the detection of acetaminophen.

Sensor	Analytical methods	Linear range (µM)	LOD	Charge transfer coefficient (α)	Diffusion coefficient (D)	Ref
Pt/NGr/GCE	SWV	0.05-90.0	0.008 µM	0.54	-	90
CPE/GO- Y_2O_3	DPV	7.0-400.0	1.45 µM	0.46	-	91
$BaFe_{12}O_{19}$/GSPE	DPV	5.0-500.0	1.5 µM	-	-	92
p-PhR/GCE	DPV	0.4-1.8 mM	6.8 µM	0.30	3.59×10^{-6} cm ^{2} s $^{-1}$	93
Yb_2O_3-SPEs	DPV	0.25-654.0	55.0nM	0.5	7.2×10^{-6} cm^2 s^{-1}	94
MTD/SPE	DPV	0.04-600.0	12.0 nM	0.47	1.0×10^{-5} $cm^2.s^{-1}$	This Work

3.6. Concurrent Detection of Acetaminophen and Tramadol

No study has been reported on the use of the SPE modified with MTD for detection of acetaminophen and tramadol. In addition, because the electrochemical detection of acetaminophen in the presence of tramadol through the unmodified electrodes would have the drawback of interferences of tramadol a result of comparative oxidation capacity of both samples, we can regard it as an essential stage.

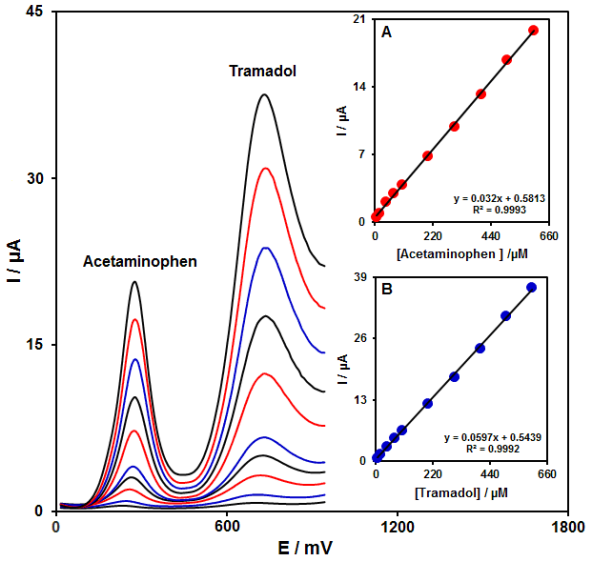

Figure 6. The DPV of the MTD/SPE in 0.1 M PBS at pH of 7.0 with various concentrations of acetaminophen + tramadol. Notably, 1–7 corresponded to 5.0+5.0, 15.0+15.0, 40.0+40.0, 70.0+70.0, 100.0+100.0, 200.0+200.0, 300.0+300.0, 400.0+400.0, 500.0+500.0 and 600.0+600.0 µM of acetaminophen and tramadol. Inset A. The Ip plot vs. acetaminophen concentration. B. The Ip plot vs. tramadol concentration.

This stage has been proceeded by concurrent changes in the analyte concentration and achievement of DPV (Figure 6). However, specific anodic has been found at 280 and 740 mV for oxidizing acetaminophen and tramadol that confirms the use of MTD/SPE. Finally, it is possible to detect the analytes with no interference from either of them (Figure 6).

3.7. Analyzing the Real Samples

For assessing the modified electrode usability for determination of acetaminophen as well as tramadol in the real samples, this new technique has been utilized to detect acetaminophen and tramadol in the tramadol and acetaminophen pills, and urine samples. Consequently, a standard addition procedure has been employed and Table 2 presents the outputs. As seen, reasonable recovery of acetaminophen and tramadol as well as reproducible outcomes have been seen based on the mean relative standard deviation (RSD).

Table 2. Determination of acetaminophen and tramadol in acetaminophen and tramadol tablet and urine samples. All the concentrations are expressed in μM (n = 5).

Sample	Spiked		Found		Recovery (%)		R.S.D. (%)	
	Acetaminophen	Tramadol	Acetaminophen	Tramadol	Acetaminophen	Tramadol	Acetaminophen	Tramadol
Acetaminophen Tabet	0	0	7.0	-	-	-	3.2	-
	2.0	5.0	8.8	5.1	97.8	102.0	1.8	3.1
	4.0	10.0	11.2	9.8	101.8	98.0	2.4	2.1
	6.0	15.0	13.5	14.8	103.8	98.7	2.7	2.5
	8.0	20.0	14.9	20.3	99.3	101.5	2.3	2.9
Tramadol Tablet	0	0	-	5.0	-	-	-	2.8
	7.5	2.5	7.6	7.3	101.3	97.3	1.9	3.5
	12.5	5.0	12.3	10.1	98.4	101.0	2.8	1.9
	17.5	7.5	18.1	12.4	103.4	99.2	2.4	2.7
	22.5	10.0	22.4	15.5	99.5	103.3	2.9	2.3
Urine	0	0	-	-	-	-	-	-
	5.0	7.5	4.9	7.6	98.0	101.3	3.4	1.8
	10.0	12.5	10.1	12.2	101.0	97.6	2.4	2.8
	15.0	17.5	14.6	17.9	97.3	102.3	2.7	3.3
	20.0	22.5	20.4	22.0	102.0	97.8	1.7	2.1

4. CONCLUSION

To sum up, this study efficiently dealt with sensing efficacy for acetaminophen and tramadol via MTD/SPE PE electrode. The detection limit 12.0 nM for the acetaminophen at MTD/SPE was obtained. The suggested electrode showed ultrahigh sensitivity, reproducibility, durability, and selectivity toward concurrent selective detection of acetaminophen and tramadol. Furthermore, usability of MTD/SPE has been acceptable with real specimens and the resulting findings have been verified.

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