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Simultaneous Detection of Morphine and Diclofenac Using Graphene Nanoribbon Modified Screen-Printed Electrode

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The graphene nanoribbon modified screen printed electrode (G/SPE) has been produced to determine morphine and diclofenac via differential pulse voltammetric, cyclic voltammetric, and chronoamperometry. Graphene nanoribbon shows great selectivity and sensitivity in determining morphine and diclofenac. The impact of scan rates has been explored. Findings showed that it is diffusion-controlled. The impact of morphine concentrations has been investigated in ranges of 0.07-600.0 μM. Determination limit for morphine has been 20.0 nM. In general, an easy experimental method for manufacturing graphene oxide nanoribbon has been suggested that takes advantage of selectivity, reproducibility, and sensitivity toward electro-active specimens, as well as biological matrices.

Keywords: Morphine,Diclofenac**,** Graphite Screen Printed Electrode, Graphene nanoribbon.

1. INTRODUCTION

Morphine [(5α, 6α)-7,8- didehydro-4,5- epoxy-17-methyl-morphine-an-3,6 -diol] is a phenolic complex and an alkaloid that is able to disrupt central nervous system. It is often applied to alleviate very bad pains in patient, particularly individuals who undergo a surgical operation. Morphine has been the initial active principle constituent filtered from a plant source. It is also one of almost 50 alkaloids of numerous various kinds found in opium, Poppy Straw and the remaining poppy derivatives [1-3]. Morphine is one of the commonly employed and strongly efficient analgesic factor for treating severe and transient pains related to cancers, which has been advised by the World Health Organization. Nevertheless, it is poisonous if used excessively or abusedly. As another opioids, morphine functions immediately on central nervous system (CNS) in order to mitigate pains. It enjoys a great capacity to cause addiction in people. In addition, it quickly develops psychological dependence and tolerance [4-8]. A variety of techniques were applied to determine morphine, including liquid chromatography (LC) [9], gas chromatography (GC) [10], electrochemical methods [11,12], ultraviolet (UV) spectroscopy [13], and high performance liquid chromatography (HPLC) [14].

Diclofenac [o- [(2,6 - dichlorophenyl)amino] phenyl] acetic acid has been well recognized for over 30 years. It is a member of a well-known group of medicines known as non-steroidal antiinflammatory (NSAIDs) with powerful anti-pyretic, and antiinflammatory, and analgesic features. It is used to relieve symptoms of a lot of illnesses, including non-articular rheumatism, osteoarthritis, sport injuries, and rheumatoid arthritis. The proposed daily dose (50-150 mg), diclofenac is completely tolerated. For this reason, it is a nonsteroidalantiinflammatory medicine that has been the initial selection for consumption in treating chronic and inflammatory conditions. Nevertheless, if it is overdosed, numerous unpleasant consequences may happen within therapy that include non-steroidal drug colitis, changes in inflammatory and degenerative and liver, practical and morphological renal alterations, and gastropathy [15-19]. A few methods including gas chromatography–mass spectrometry [20], liquid chromatography [21], spectrophotometry [22], spectrofluorimetry [23], and voltammetry [24,25] have already been used for the detection of diclofenac in pharmaceutical as well as in biological fluids.

Researchers found that combining diclofenac and morphine in little doses is synergistic in serious inflammatory pains. Actually, using the combination declines consuming morphine necessary for sufficient analgesia in comparison to morphine itself [26]. Thus, concurrent detection of the two medicines is of high significance.

Electrochemical-based sensors are one of the analytical procedures, which may be helpful for concurrent analysis of medicines because of their capability for modifying by various moderators (in order to enhance sensitivity & selectivity), lower cost, quick responses, good portability and ease in automation [27-43]. Nevertheless, one of the main problems is that at bare electrodes, potentials of anodic peak for morphine and diclofenac are nearly similar that leads to the overlapping current response, and causes many difficulties in their differentiation. So those electordes that have been chemically modified are too attractive means for analyzing by sensitive electro-analytical methods [44-52]. Chemically modified electrodes take advantages of speciation works for choosing the easiest

modifier for each analyte, since selectivity and sensitivity of electro-analytical responses are dependent on the modifier properties [53-59]. Various conductive mediators, including nano-materials have enhanced the compounds ranges for electrochemical analyses and provided a novel capability for simultaneously determining electroactive complexes in biological specimens [60-72].

Nanotechnology has made considerable progress in recent decades. The use of them is accompanied by improvement of sensitivity and accuracy of sensor largely because of higher levels of ratio surface, acceptable bio-compatibility and desirable electro-conductivity that are of high significance for analytical chemistry [73-82].

Graphenenano-materials are one of the most commonly investigated ones that have a lot of prominent features, including great surface area, higher mechanical strength, catalytic electron transport features, and very good thermal and electrical conductivity [83,84]. The reactivity of graphene oxide nanoribbons is active due to the existing of open-ended graphene sheet. And the versatile dimension and form which may be adjusted for imparting them from semi-conductor to semi-metal nature. These make graphene oxide nanoribbons very good candidates for developed electrochemical and electronic tools in several areas, including lithium ion battery, super-capacitor, organic photovoltaic, massive tools production, great dielectric constant gate dielectrics, optoelectronics, and sensors [85-88].

Todays, screen printing technologies have been confirmed to produce thick film electrochemical transducers. They enjoy advantages for field analysis, such as little specimen dimension, high efficiency, quickness, and portability. In addition, screen printed electrodes are produced by cheap processes, that causes their disposablity. Such a property is clearly important when we test biological species, and therefore, prevents surface fouling side effects [89-96].

This research used graphene nanoribbons benefits for modifying a screen-printed electrode in order to explore electrochemical behavior of morphine and diclofenac.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

An Auto-lab potentiostat/galvanostat (PGSTAT 302N; Eco Chemie:the Netherlands) was employed for electrochemical experimentations; The system was monitored with a general-purpose electrochemical system. SPE (DropSens; DRP-110: Spain) comprised of 3 conventional electrodes: graphite counter electrode, unmodified graphite working electrode, and a silver pseudo-reference electrode. A Metrohm 710 pH meter instrument was utilized to measure pH. The reagents used in this work were analytical grade, which were obtained from Merck (Darmstadt:Germany). The pH value was adjusted with ortho-phosphoric acid and its salts.

2.2. Preparing Electrode

The bare electrode made by screen-printed method was coated with G composite. 1 mg of GONRs was distributed via ultra-sonication for 30 mins to prepare the $MoWS_2$ composite stock

solution in a 1 mL of aqueous solution. The 5 µl of G suspension aliquots was casted on the carbon working electrodes, followed by drying at room temperature.

2.3. Preparation of Real Samples

The morphine injection was 10 times diluted with water. Afterward, the diluted solutions were transferred into a 25 mL of volumetric flask, followed by diluting with PBS into the mark. The acetaminophen concentrations were measured with a standard addition method.

Five 100 mg of diclofenac pills (Tehran Chemie Pharmaceutical Co., Iran) were grinded for the preparation of a solution through dissolving 500 mg of powder in 25 ml of water via ultra-sonication. The prepared solutions were poured in 25 ml of flasks, followed by titrating with PBS to the mark point.

Refrigerated urine samples (10 ml) were centrifuged at 2,000 rpm for 15 mins, followed by filtering the supernatant using a filter with a 0.45 µm of pore size. The solutions were distributed into a 25 ml of volumetric flask and were diluted with PBS to the mark. Various doses of morphine and diclofenac were utilized for the analysis. The concentrations of morphine and diclofenac were measured with standard addition method.

3. RESULT AND DISCUSSION

3.1. Electrochemical Profile of the Morphine on the G/SPE

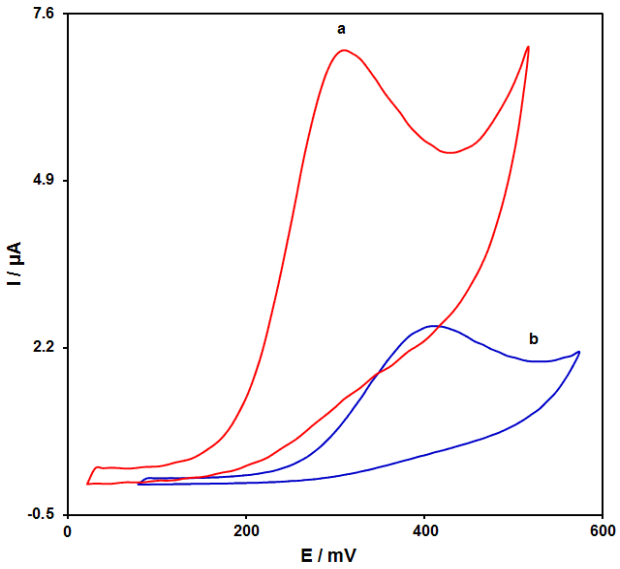

Figure 1. The CVs of a) G/SPE, (b) unmodified SPE in 0.1 M PBS at pH of 7.0 in the presence of 100.0 µM morphine at 50 mVs^{-1} scan rate.

Obtaining an optimum pH value is of high importance to obtain good outputs in studying electrochemical behaviours of pH depended morphine. We, thus, employed the modified electrode for

the characterizations at different pH values of from 2.0 to 9.0. As a result, reasonable outputs are attained at a value pH of 7.0.

Cyclic voltammograms (CVs, Figure 1) was conducted in 100.0 µM of morphine using the bare SPGE (Curve b) and G/SPE (Curve a) electrodes. The oxidation peak of morphine for the G/SPE occurs at around 300 mV which is around 200 mV more negative than that of the bare SPGE.

3.2. Impacts of Scan Rates on the Results

Augmented oxidation peak currents are observed after enhancing the scan rates as exhibited in the outputs from the morphine oxidation current (Figure2). Furthermore, a linear relationship between Ip and square root of the potential scan rate ($\nu^{1/2}$) is found, suggesting a diffusion-controlled acetaminophen oxidation process.

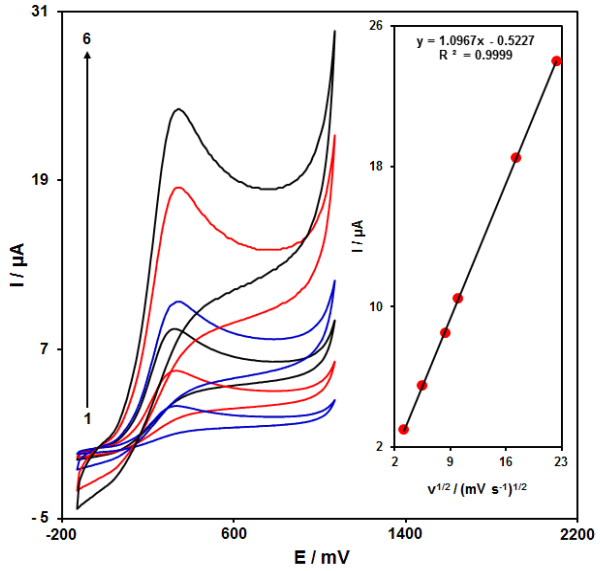

Figure 2. CVs of G/SPEin 0.1 M PBS at pH of 7.0 consisting of 100.0 µMmorphine at distinct scan rates; 1-6 corresponded to 10, 30, 70, 100, 300 and 500 mV s^{-1}. Inset; variations in the anodic peak currents vs. $\nu^{1/2}$.

3.3. Chronoamperometric Analyses

The chronoamperometry analysis for morphine was conducted with G/SPE at 0.35 V. Chronoamperometric outputs of the morphine with diverse concentrations at pH equal to 7.0 is exhibited in Figure 3. Cottrell equation is adopted for further analyses of the electroactive moiety:

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

where D is the diffusion coefficient (cm^2 s^{-1}). C_b is the exerted bulk concentration (mol cm^{-3}). Experimental results regarding I vs. $t^{-1/2}$ (Figure 3A) suggest a good fit toward distinct morphine concentrations. Final slopes of the straight lines (Figure 3A) are vs. morphine concentrations (Figure 3B). The D mean-value is equal to 3.0×10^{-6} cm^2/s according to Cottrell equation and resultant slopes.

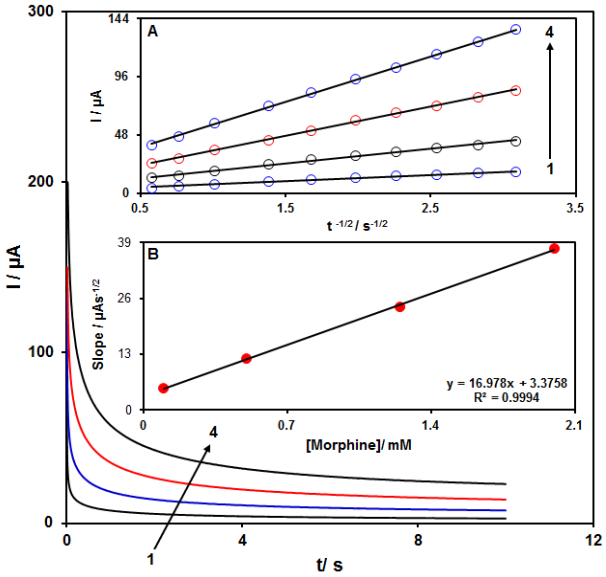

Figure 3. The chronoamperograms obtained at G/SPEin 0.1 M PBS at pH of 7.0 for different concentrations of morphine. It is notable that 1–4 correspond to 0.1, 0.5, 1.25, and 2.0 mM of acetaminophen. Inset A. the I plot versus $t^{-1/2}$ observed by chrono-amperograms 1 to 4. B. Slope plot of the straight line vs. concentration of morphine.

3.4. Calibration Curve

Morphine was quantitatively analysed in the water solution on the basis of the final peak currents of morphine using the G/SPE (Figure 4).

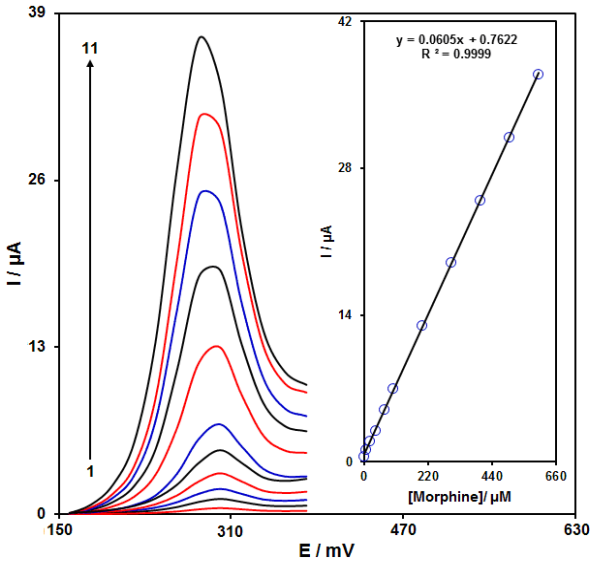

Figure 4. DPVs of G/SPEin 0.1 M PBS (pH 7.0) containing different concentrations of morphine. Numbers 1–11 correspond to 0.07, 5.0, 20.0, 40.0, 70.0, 100.0, 200.0, 300.0, 400.0, 500.0, and 600.0 µM of acetaminophen. The inset shows the plot of the peak current as a function of the morphine concentration in the range of 0.07-600.0 µM.

We employed the modified electrode (G/SPE) as a working electrode; the morphine concentration is 0.1 M PBS in DPV. As a result, a linear relationship between the peak currents and morphine concentrations is observed using a correlation coefficient that is equal to 0.9999. A comparison of analysis results for morphine using the prepared electrode and other previous reports is given in Table 1.

Table 1. Comparison the determination of morphine between G/SPE and modified electrodes reported in the literature.

Sensor	Analytical methods	Linear range (µM)	LOD	Ref
MWCNTs/SnO_2–Zn_2SnO_4/CPE	DPV	0.1-31.0 µM	0.009 µM	97
(CTAB)/GO/GCE	DPV	50.0-60.0 µM	0.36 µM	98
ZnO/MWCNTs/IL/CPE	LSV	0.1-700.0 µM	0.06 µM	99
M-CNFs/CPE	DPV	0.0033-245 µM	1.9 nM	100
AuNPs/ MIP/f-MWCNT/PGE	SWV	0.008-5.0 µM	2.9 nM	101
G/SPE	DPV	0.6-600.0 µM	20.0 nM	This Work

3.5. Concurrent Detection of Morphine and Diclofenac

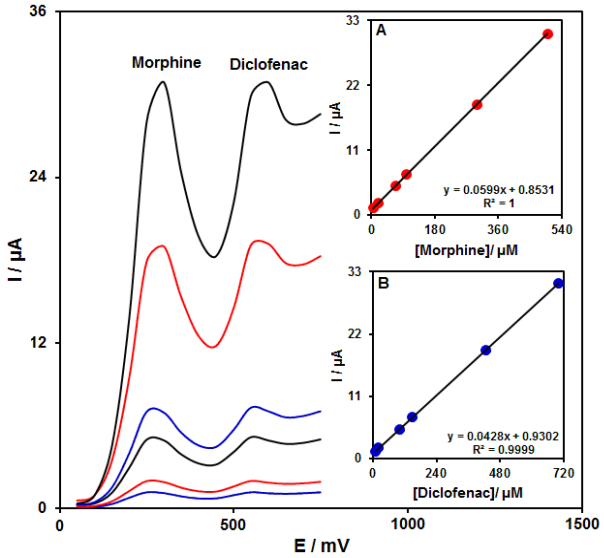

Figure 5. The DPV of the G/SPEin 0.1 M PBS at pH of 7.0 with various concentrations of morphine + diclofenac. Notably, 1–6 corresponded to 5.0+10.0, 20.0+20.0, 70.0+100.0, 100.0+150.0, 300.0+425.0 and 500.0+700.0 µM of morphine and diclofenac. Inset A. The Ip plot vs. morphine concentration. B. The Ip plot vs. diclofenac concentration.

The graphene nanoribbon modified SPE for detection of morphine and diclofenac is considered as the pioneering effort. There is drawback in the electrochemical detection of morphine in the presence of diclofenac using common electrodes. It is due to the concurrent changes in the analyte concentration of DPV (Figure 5).

Anodic peaks are found at 250 and 550 mV in oxidizing morphine and diclofenac which confirms the existence of G/SPE.

3.6. Analyzing the Real Samples

The prepared electrode was further evaluated in real samples, which enables the detection of morphine and diclofenac in diverse samples. A standard procedure is employed; the corresponding results are presented in Table 2.

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

Sample	**Spiked**		**Found**		**Recovery (%)**		**R.S.D. (%)**	
	Morphine	Diclofenac	Morphine	Diclofenac	Morphine	Diclofenac	Morphine	Diclofenac
Morphine Ampoule	0	0	9.0	-	-	-	3.2	--
	2.5	10.0	11.7	9.9	101.8	99.0	3.1	2.4
	5.0	15.0	13.8	15.5	98.6	103.3	2.1	1.6
	7.5	20.0	17.1	19.5	103.6	97.5	2.8	2.3
	12.5	25.0	21.0	25.3	97.7	101.2	1.7	3.3
Diclofenac Tablet	0	0	-	13.0	-	-	-	2.9
	5.0	2.0	4.9	15.2	98.0	101.3	1.7	3.2
	10.0	4.0	10.2	16.5	102.0	97.0	3.5	2.1
	15.0	6.0	14.9	19.5	99.3	102.6	2.4	2.8
	20.0	8.0	20.6	20.6	103.0	98.1	2.5	2.2
Urine	0	0	-	-	-	-	-	-
	5.0	7.5	5.1	7.4	102.0	98.7	3.5	1.8
	10.0	12.5	9.9	12.8	99.0	102.4	2.5	2.6
	15.0	17.5	14.7	17.9	98.0	102.3	1.9	2.3
	20.0	22.5	20.5	22.3	102.5	99.1	2.7	3.1

4. CONCLUSION

This research investigated graphene nanoribbon on the bare SPE surface and its use for electrochemical detection of morphine and diclofenac. It was found that the G/SPE showed influential

electro-catalytic activity toward morphine and diclofenac oxidation and eliminated overlapped peaks of morphine and diclofenac in two certain peaks through differential pulse voltammetric method. Due to its' electro-catalytic selectivity, ability, reproducibility, and sensitivity, morphine and diclofenac modified SPE has been so helpful for developing sensors for concurrent detection of morphine and diclofenac in electroanalytical chemistry.

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