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Carbon Nanotube Reinforced Heterostructure Electrochemical Sensor for the Simultaneous Determination of Morphine and Fentanyl in Biological Samples

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Abstract
In this study, an electrochemical sensor for simultaneous measurement of morphine and fentanyl based on a modified pencil graphite electrode with a semiconductor nanocrystalline structure was developed.The first layer of the sensor has a core of thioglycolic acid-bonded cadmium selenidequantum dot (TGA-CdSe), surrounded by a second layer, zinc sulfide quantum dot (ZnS). Functionalized carbon nanotubes (FCNT) have also been used to reinforce the sensor structure (TGA-CdSe/ZnS@FCNT). Measurements were performed by differential pulse voltammetry (DPV) and cyclic voltammetry (CV).The synthesis of nanostructures was confirmed by FTIR, EDX, SEM and XRD. In order to optimize the effective factors in the performance of this sensor, the Taguchi orthogonal array (OA16) design has been utilized. TheCV voltammograms showed irreversible oxidation peaks at potentials of 0.9 V and 0.38 V for fentanyl and morphine respectively. The transfer coefficients (α) of 0.96for morphine and 0.95 for fentanyl obtained. The diffusion coefficients gained on the electrode surface by chronoamperometrywere $3.84\times10^{-6}cm^2\ s^{-1}and1.615\times10^{-6}cm^2s^{-1}$ for morphine and fentanyl, respectively. Under optimal conditions, the linear concentration range and detection limit for morphine were 0.08-100 μM, and 0.024 μM. For fentanyl two linear ranges of 0.02-8μM, 8-100 μM and 0.006 μM were obtained. The fabricated sensor can be well used for the simultaneous measurement of morphine and fentanyl in biological samples with acceptable relative recoveries in the range of 98.3-102.

Keywords
Morphine; Fentanyl; Cadmium Selenide Quantum Dot; Zinc Sulfide Quantum Dot; Multi-Walled Carbon Nanotubes.

1. INTRODUCTION

Morphine with the chemical formula ($C_{17}H_{19}NO_3$) andIUPACname (4R,4aR,7S,7aR,12bS)-3-methyl-2,3,4,4a,7,7a-13-hexahydro-1H-4,12-methano-1H-4,12methanobenzofuro[3,2-e]isoquinoline-7,9-diol (Fig. A S1) and fentanyl with the chemical formula ($C_{22}H_{28}N_2O$) and IUPAC nameN-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]propanamide (Fig. B S1) are known as analgesic opioid drugs[1]. Morphine is a natural alkaloid extracted from opium, and fentanyl is a potent quasi-morphine derived industrially from the structural change of morphine.Morphine andfentanyl are drugs with analgesic, sedative, euphoric, hypnotic effects, along with physical and psychological dependence. In medicine, these substances are prescribed to relieve pain in patients after surgery according to the patient's clinical condition. The analgesic power and effectiveness of fentanyl is much higher than morphine[2,3]. Excessive use of this drug in drug forms or as illegal products can affect and damage the most important parts of the body, namely the brain, respiratory system, gastrointestinal tract and sex hormones, and ultimately cause death, so in order to prevent such complications, accurate measurement of fentanyl and morphine in the blood and urine has become very important inforensic medicine and pharmacological studie [4]. Today, there are many techniques for measuring fentanyl and morphine, such as, high-performance liquid chromatography, liquid[5] chromatography-Tandem mass spectrometry[6], gas chromatography[7], capillary electrophoresis[8,9], optical techniques such as spectrophotometry[10,11], chemiluminescence and electrochemiluminescence[12], atomic absorption and emission spectroscopy[13] and electrochemical techniques [14], Among these techniques, chromatography and optical techniques are not only time consuming due to the long preparation of the sample but also expensive due to the need to utilize materials with high purity. Moreover, even in some cases due to their lower sensitivity, these methods are less considered. Today, electrochemical techniques are being highly favored because of their selectivity, simplicity, high speed and sensitivity, reasonable price, low detection limit and the availability of a wide variety of electrodes[15].

Modification of the electrode surface with nanomaterials has been to better facilitate electron

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transfer and eliminate limitations such as interference with other species in real samples and increase sensitivity, selectivity, and low detection limits at the micro and nano- levels.

Among the materials used to modify the surface of the electrodes, semiconductor quantum dots such as CdSe, coated with another semiconductor such as ZnS are welcomed due to their positive features, such as: their small size, electrical conductivity and high catalytic activity, low toxicity, high stability, increased band gap, high surface area, increased electrical properties and high surface-to-volume ratio [16]

Multi-walled carbon nanotubes have a regular structure consisting of a number of concentric tubes. These graphene nanotubes with a high length to radius ratio have properties that include low weight, high strength, permeability and electrical conductivity and high stability, and a high specific surface area (from several tens of square meters per gram to one thousand square meters per gram) that accelerates electrochemical reactions and facilitates electron transfer. Therefore, their utilization in performing electrochemical reactions is more desirable[17]. Simultaneous use of the quantum dot core / CdSe / ZnS shell and multi-walled carbon nanotubes on the electrode surface as reinforcing agents leads to an integration of the characteristics of both groups and builds a strong sensor for the chosen analyses and other important organic species and minerals.

In view of the above, the present study focuses on the construction of a new sensor based on the emission of TGA-CdSe / ZnS core / shell quantum dots and multi-walled carbon nanotubes on the graphite pencil rod(PGE) and its use as a working electrode for simultaneous determination of morphine and fentanyl.

Due to the importance of investigating the effect of important parameters on the efficiency of the method and determining the optimal laboratory conditions for simultaneous measurement of two analytes, the Taguchi experimental design method was used to optimize the chemical process. Taguchi method, using orthogonal arrays (OA), greatly reduces the number of experiments and allows to obtain simultaneous optimal conditions for multiple responses and examine factors with different levels. In order to save cost, time and other factors, an attempt was made to design a simple and efficient method for extracting and measuring the analytes [18] .

Although there is much research into the measurement of fentanyl and morphine separately, library research shows that simultaneous measurement of these two drugs using a modified electrode has not been reported. Considering the advantages of employing electrochemical methods using modified electrodes, the main purpose of this study was to fabricate a sensitive electrode with high selectivity and low detection to simultaneously identify morphine and fentanyl in the matrix of biological samples, such as, blood and urine and prove the performance of the electrode.

2. EXPERIMENTAL

2.1. Chemicals

High purity morphine sulfate and fentanyl citrate (<%99) were obtained from Daru Pakhsh Company (Tehran, Iran). All chemicals (ethanol, acetone, methanol, hydrogen peroxide, acetic acid, n-n dimethyl formamide(DMF), acetonitrile, hydrochloric acid, phosphoric acid, boric acid, potassium hexacyanoferrate(III), potassium hexacyanoferrate(II) trihydrate, dichloromethane, zinc acetate, sodium sulfide) were procured from Merck (Darmstadt, Germany) with analytical purity degree and were used without any purification.

The multi-walled carbon nanotubes were prepared by chemical vapor precipitation. These nanotubes were purchased from US Research Company with a purity of more than 95%, density 2.1gr/cm^3, specific surface area of 110m^2/g, inner and outer diameter of 5-10nm and 20-30 nm respectively, a length of 10-30 microns and electrical conductivity above 100 S/cm.

The inert environment in the manufacture of TGA-CdSe / ZnS @ FCNT was created via Nitrogen gas with a purity of 99.99% that was obtained from Alpha Company (Tehran, Iran).

The electrochemical probe was made up of a solution of potassium chloride 0.1 M and [Fe (CN) 6] -3 / [Fe (CN) 6] -4mM. Double-distilled water (ddH_2O) was used in all experiments, and the electrical conductivity of the ultra-pure water was 5.5×10^{-6}MΩ.CM^{-1} .

Stock solutions of morphine and fentanyl in ethanol at a concentration of 1000 mg / L were prepared. Further, the Robinson buffer solution was prepared from 10 ml of 0.2M boric acid, 0.2M phosphoric acid, and 0.2M acetic acid which were added to a 100 mL flask and made up to volume with deionized water, plus 0.2 M sodium hydroxide and 0.2 M phosphoric acid were used to adjust the pH.

2.2 Devices

Electrochemical experiments were performed in this research using a potentiostat/galvanostat apparatus model BHP2066 made by the Behpajooh engineering company (Tehran, Iran).Three electrodes; Ag/AgCl(KCl 3 M) electrode as the reference electrode, platinum wire as the auxiliary electrode and TGA-CdSe / ZnS @ FCNT coated pencil graphite electrode as the working electrode The pencil graphite rods (Know brand, 2B, China

with internal thickness of 0.7 mm and length of 9 cm) used to make working electrodes.
The prepared sensors were characterized. To record the IR spectrum,the Bruker Optik GmBH. FT-IR spectrometer (Ettlingen, Germany) was used. The X-ray diffraction studies were performed using a Siemens AG, (Karlsruhe, Germany). While determining morphology was carried out using a Scanning Electron Microscopes (SEM) (model EV018, CarlZeiss AG, Germany), the EDX analysis was performed using a silicon detector. The synthesis of cadmium selenide was done using a Hielscher ultrasonic homogenizer model UTR200 (Teltow, Germany), and the pH of the solutions was adjusted using the pH meter of Metroham Laboratory model PHS-550 (Herisau Switzerland).

2.3. Synthesis of primary quantum dots, TGA-CdSe / ZnS @ FCNT

Water-soluble quantum dot core / shell TGA-CdSe / ZnS was used for sensitive detection of morphine and fentanyl in aqueous samples. The quantum dot core / shell / synthesis was performed by the co-precipitation method in two steps: first, quantum dots of cadmium selenide grafted with thioglycolic acid (TGA) were prepared and second,ZnS was coated on CdSe. In the first step, 0.4567 gr (2 m.mol) of cadmium chloride ($CdCl_2.2.5H_2O$) was dissolved in 100 ml of distilled water and poured into a 250 ml three-mouth flask 0.5 ml of thioglycolic acid was added. Then, the container containing the material was placed on a magnetic heating stirrer to mix the solution for a few minutes and make it uniform. The pH of the solution was then raised to 11 with 0.1 M sodium hydroxide, and nitrogen gas was passed through it to remove oxygen and other interfering agents. A few minutes after addition, 0.1 gr of sodium borohydride ($NaBH_4$) and 0.111 g of selenium dioxide were included and the resulting solution was refluxed into the nitrogen atmosphere, and the modified quantum dots of cadmium selenide was obtained for 4 hours at 100 ° C at different time intervals[19].
In the second step, two precursors of Zn (OAC) $2.2H_2O$ and sodium sulfide (Na_2S) were used as sources of zinc and sulfur 100 ml of TGA-CdSe solution (2mmol) was poured into a flask and then gently 0.44 g (2mmol) of zinc acetate and 0.156 g (2 mmol) of sodium sulfide was slowly added at room temperature while the material was placed on a magnetic stirrer plate that stirred the solution for 6 hours, thus preparing TGA-CdSe / ZnS [20].

2.4. Oxidation of carbon nanotubes

In order to purify carbon nanotubes to remove catalytic impurities and amorphous carbon, they were first heated in a furnace for 40 minutes at 400 ° C. In the next step, in order to oxidize the purified nanotubes first, 10 ml of nitric acid and 30 ml of concentrated sulfuric acid was mixed in a ratio of 3 to 1.0.5gr of raw nanotubes were added to the solution. The mixture was placed on a shaker at a temperature of 80 ° C with a stirrer speed of 200 rpm and reflux occurred for 8 hours.
By mixed acid oxidation hydroxyl, carbonyl and carboxyl groups immobilized into CNT surface and inside the nanotube layers. Oxidation leads to increasing specific surface area of the multi-walled carbon nanotubes.
To remove excess acid, the carbon nanotubes were washed in several steps with a sufficient amount of deionized water to bring the pH of the water to about 7. The active nanotubes were then dried in an oven at 70 ° C for 4 hours and stored in a desiccator.

2.5. Electrode preparation

2.5.1. Electrode preparation before surface correction

Before modifying the surface of the pencil graphite electrode and in order to prepare it, first the electrode was washed several times with double distilled water, then the pencil graphite rod was put in acetone for 10 minutes to remove organic compounds. Finally, it was washed several times with double distilled water. After the above operation, the graphite rods were dried at room temperature for 24 hours.

2.5.2. Modifying the electrode surface

In order to modify the electrode surface (according to the optimal values), 0.5 mg of activated multi-walled carbon nanotubes and 0.5 mg of quantum dot core/shell / TGA-CdSe / ZnS were placed in 10 ml of n-n dimethylformamide(DMF) and was then positioned under ultrasound for 15 minutes to homogenize its suspension. Then, under optimal conditions, the pencil graphite rod was placed in this suspension three times, each time for 4 hours, and each time, after immersion, it was dried at room temperature for 24 hours.

2.6. Preparation of biological samples

2.6.1 Preparation of blood and urine samples

0.6 ml of blood serum was added to 0.5 ml of acetonitrile [21], in order to precipitate protein was added to the blood sample and centrifuged at 3500 rpm for 10 minutes, to separate the precipitated serum proteins. The standard addition method was utilized to determine the concentration of morphine and fentanyl, in this way, different amounts of the standards were added to 1 ml of serum (the real sample), and with a Robinson buffer it was brought to a volume of 10 ml to pH7

in order to obtain different concentrations (0, 0.1, 0.2,0.3, 0.4, 0.6, 0.8, 0.9, 1, 1.2 µM).
Urine samples were taken from healthy volunteers and centrifuged at 3500 rpm for 10 minutes. The supernatant clear solution was then diluted 10 times with Robinson buffer pH7, and different amounts of analytes were added. Thus, spiked urine samples were obtained with different concentrations (0, 0.1, 0.2,0.3, 0.4, 0.6, 0.8, 0.9, 1, 1.2 µM)[22].

3. RESULT AND DISCUSSION

3.1. Characterization of TGA-CdSe/ ZnS core / shell quantum dots

To characterize the synthesized quantum dot core / shell cadmium selenide / zinc sulfide, scanning electron microscopy (SEM) was used. The TGA-CdSe / ZnS structure studied by scanning electron microscope (SEM) and result is shown in Fig. (1), which shows the homogeneous spherical particles.

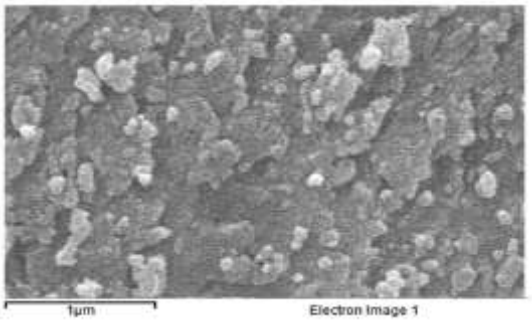


Fig. 1. SEM image from the surfaces ofTGA-CdSe / ZnS QDs powder.

In order to study the crystal structure and calculate the crystal size of the synthesized TGA-CdSe / ZnS, X-ray diffraction patterns were performed from crystalline powder samples at ambient temperature.
The reference model was compared with the Joint Diffraction Standards Committee (JCPDS). Fig. (2A) (quantum dot cadmium selenide (JCPDSNo. 19-0191)); and the quantum dots of the cadmium selenide / zinc sulfide (JCPDSNo. 77-2100) are shown in Fig. (2B).

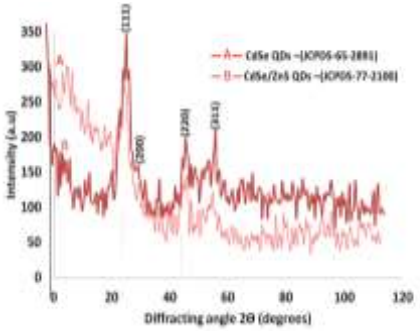


Fig. 2. (A): XRD pattern of TGA capped CdSeQDs, (B): TGA-CdSe / ZnS QDs.

The CdSe shows three index peaks at 28, 25.2 ~ 2 θ°, 43.2θ ~ and 51.062 2θ°, which can be assigned according to the crystal plane (111), (220) and (311), which is related to the structure of the cdse cube network. Similarly in CdSe / ZnS there are peaks at angles at 26.4θ ~ 2θ°, 43.6θ ~ and 51.6θ ~ 2θ° corresponding to the crystal plate (111), (200), (220) and (311) which have a cubic structure of zinc. The CdSe/ ZnS QD diffraction peaks were moved to higher angles compared to CdSe due to the growth of the Zns shell on the CdSe.
The mean particle size (D) was calculated based on the Debye-Scherrerequation

$$D = \frac{K\lambda}{\beta cos\theta}$$

Where D was the mean particle size,λ wavelength of X-ray radiation (K_α copper radiation equal to λ = 1.54056), β is the peak width at half height (FWHM) and θBragg angle (range 2θ from 5 to 99 degrees). The crystal size of CdSe andCdSe / ZnS QD nanoparticles from FWHM was determined with the highest intensity of the highest mentioned peaks (111) from the XRD pattern reflectanceusing theScherer equation. The average particle size according to Debye-Scherrer equation is 5.42 nm for CdSe QDs and 5.98 nm for CdSe / ZnS QDs. The calculated average size of CdSe / ZnS QD was higher than CdSe QD, so the increase in the size of the ZnS layer on CdSe QDs was confirmed.
FT-IR spectroscopy of the CdSe / ZnS core / shell quantum dots was studied, the results of which are shown in Fig. (3) by a peak of about 3400 cm^{-1}, due to OH vibrations appearing in the hydroxyl group.

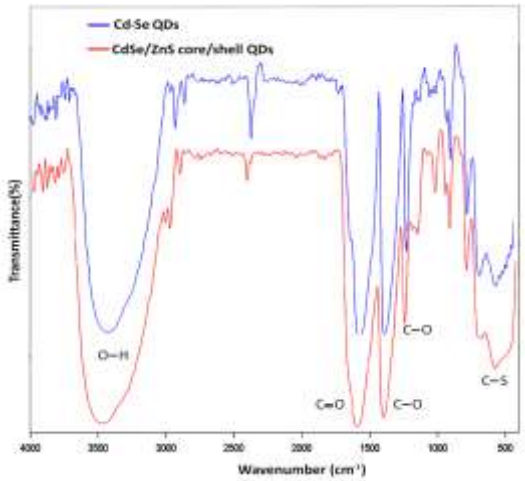


Fig. 3. FT-IR spectra from CdSe capped with TG and TGA-CdSe / ZnS QDs.

Two sharp peaks of about 1680 and 1400 cm^{-1} were observed due to the stretching vibrationsof the thiol, confirming that the thiols were coated on the surface of the quantum dot CdSe. The COO- group became the main link between the QDs and the main thiol group. The peak of CdSe samples

limited between 1600-1650 cm^{-1} in the absorption band (symmetric and asymmetric stretching vibrations) showed the TGA carboxylate anion.
The absence of S-H vibration at 2556–2654 cm indicated that the S – H bond was broken to form the –CdSe – S – CdSe– bond. In fact, TGA binds to the CdSe QD surface through its sulfur atoms.
The chemical composition of the TGA-CdSe and TGA-CdSe / ZnS quantum dots synthesized by EDX analysis was investigated (Fig.4).
The results illustrated in the chemical composition of TGA-CdS quantum dots in Fig. (4A) show the presence of Cd, Se, O and S elements. Also, the percentage of elements obtained in the EDX analysis is reported in Fig. 4B. The results show the stoichiometric ratio; Cd to Se was 1 to 3 and the presence of the elementsO and Sindicates that TGA was coupled to CdSe.
The EDX analysis TGA-CdSe / ZnS represented the presence of the elements(See Fig. 4C). The existence of the elements Cd, Se, Zn and S has been confirmed; also, the percentage of TGA-CdSe / ZnS elements obtained in the EDX analysis is reported in Fig.4D

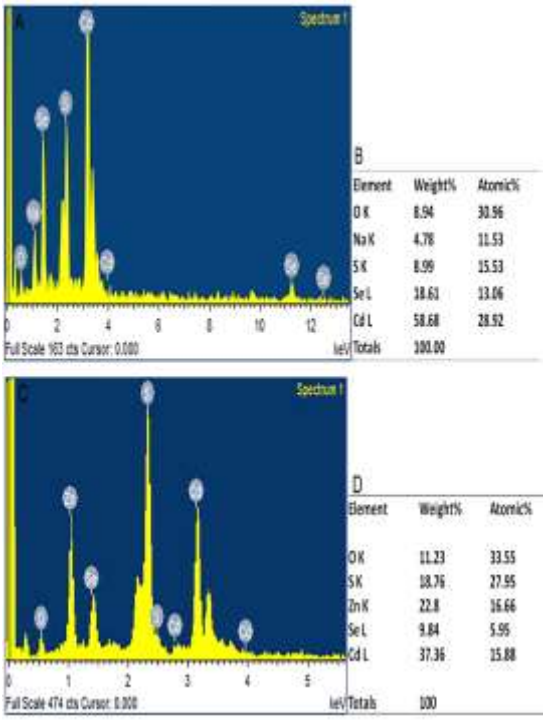


Fig. 4. (A): EDS spectra of CdSe capped with TGA. (B): TGA-CdSe / ZnS QDs. The elements obtained in the EDS analysis, (C): CdSe capped with TGA, (D): TGA-CdSe / ZnS QDs.

By coating the ZnS on CdSe, the stoichiometric ratio remained constant at a ratio of 1 to 3, but the Zn peak appeared at 1.1 eV and the S peak also at 2.33 eV. This indicated the formation of a ZnS shell on CdSe.

FT-IR spectroscopy of oxidized carbon nanotubes was shown in Fig.S2; symmetric and asymmetric stretching vibrations of C-H could be seen in the frequencies of 2825.12 cm and 2854.13 cm^{-1} and the C = C bond in 1636.56 cm^{-1} in the oxidized nanotubes. The wide peak was related to O-H stretching vibrations at the frequency of 3420. 22 cm^{-1} and the C = O peak at the frequency of 1712.62 cm^{-1}, belonging to the carbonyl group, confirmed the oxidation of the nanotubes.

3.2. Morphology of the electrode surface modified with TGA-CdSe / ZnS @ FCNT

The scanning electron microscopy was used for structural and morphological studies. Graphite electrode surface coated with TGA-CdSe / ZnS @ FCNT were evaluated by the SEM technique. Fig. S3 (A) shows the surface of an unmodified PGE electrode. By adding the TGA-CdSe / ZnS @ FCNT nanocomposite on the PGE, the surface area increased dramatically (Fig. S3 (B)). In fact, the addition of the multi-walled carbon nanotubes and hybrid quantum dots provide a more porous structure for PGE. This increases the active surface area of the electrode.

3.3. Determining the active surface area of the electrode

As the active surface of the electrode increases, the speed of electron transfer increases. To determine this parameter and understand the electrochemical properties to calculate the effective electrochemical active surface of the modified and unmodified electrodes using the cyclic voltammetry technique, Fig. (S4), the Randles-Swick equation is used.

$$I_{pa} = (2.69 \times 10^5) n^{3.2} A D^{1.2} C_{\circ} V^{1.2}$$

Where$I_{pa}$is the anodic current peak, n; number of electrons participating in the redox reaction, A; the electrode surface area (cm^2), D; diffusion coefficient (cm^2s^{-1}), V;the scan speed (vs^{-1}), and $C_{\circ}$;analyte concentration (M).The electrochemical properties of modified sensor has been studied using the 1.0mMredox couple $[Fe(CN)_6]^{3-/4-}$containing 0.1M KCl. The diffusion co-efficient for 1 mM $[Fe(CN)_6]^{-3}$ in 0.1 M KCl can be obtained by plotting I pa vs. $\nu_{1/2}$in the case of n=1, Do=6.5×10^{-6} cm2/s. On replacing the above mentioned values, by measuring the anodic current of the solution at different scan rates in the range of 25 to 200 mV /s, effective surface area of the electrode obtained from the slope of the curve. Accordingly, the effective surface area of the raw graphite electrode,the modified electrode with multi-walled nanotubes and the modified TGA-CdSe / ZnS @ FCNT electrode were 0.15, 0.45 cm^2 and 0.68 cm^2.

The results show that the modified electrode surface TGA-CdSe / ZnS @ FCNT accelerates the load transfer process and increases the active sites on the electrode surface.

3.4. Electrochemical behavior of fabricated electrodes

Electrochemical behavior of morphine and fentanyl on the raw, modified electrodes with FCNTs and modified electrodes with TGA-CdSe / ZnS @ FCNT core / shell quantum dots were evaluated using differential pulse voltammetry techniques (Fig. 5-A) and cyclic voltammetry (Fig. 5-B) under optimal conditions in Robinson buffer (pH 7) with scan rate0.1v.s^{-1} in the potential range of 0 to 1.25 volts. The results in a 10 µM solution of morphine and fentanyl show that two irreversible oxidation peaks appeared at potentials of 0.9V for fentanyl and 0.38V for morphine. Simultaneous use of TGA-CdSe / ZnSand FCNTs to modify the surface of electrode increases the effective surface area of the electrode as well as the charge transfer capacity due to the catalytic effect of multi-walled carbon nanotube and the unique properties of CdSe / ZnS core / shell quantum dots.

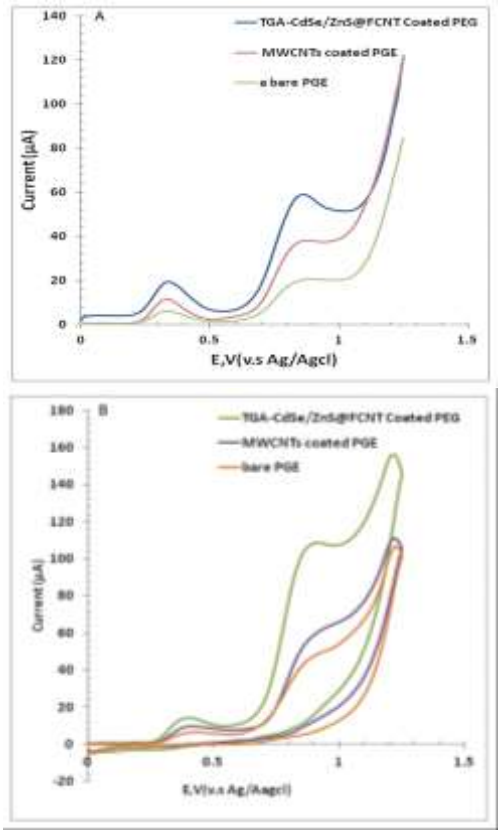


Fig. 5. (A): *Differential pulse* and (B): cyclic voltammograms of fentanyl, and morphine a bare PGE , carbon nano tubes coated PGE and TGA-CdSe/ZnS@FCNT coated PGE at 10 µM concentration of Fentanyl and 10 µM of morphine and10 µM of fentanyl at the presence of 0.1 molar Britton Robinson buffer (pH = 7) and scan rate of 0.1 mV.S^{-1}.

This surface coating significantly enhances the peak current and shifts the potential of the peaks to smaller values, indicating the positive effect of electrode surface in facilitating the simultaneous oxidation process of morphine and fentanyl. Therefore, the use of a sensor modified with TGA-CdSe / ZnS @ FCNT for measuring morphine and fentanyl in quantitative analysis was proposed.

3.5. Experimental design with Taguchi software

The Taguchi technique expresses an orthogonal standard representation for the effect of selected factors on the target value and defines the design of experiments. The Taguchi array design has been used to study the correlation between the effective variables, to optimize the studied parameters, to evaluate the performance, and to carry out the statistical analysis of the results. Taguchi is a powerful tool for performing minimal tests in the shortest time, reducing costs and obtaining data in a controlled manner to analyze the effect of parameters influencing the process. In order to investigate factors such as the concentration of carbon nanotubes and TGA-CdSe / ZnS core / shell nanoparticles, the time and number of immersions were optimized by Taguchi software with orthogonal (4×4) L_{16} presentation. For this purpose, each of these four factors was listed in four levels according to Table S1. Mini-Tab 16 software was used to design the experiment and statistical analysis. The sensor response and the corresponding S / N value for this orthogonal representation L_{16} are shown in Table S2. In this table, the sum of the currents resulting from the peak oxidation of morphine and fentanyl, in a solution of 10 µM of each of these materials, was measured based on DPV voltammetry techniques. In order to increase reproducibility, the analysis were repeated three times under the same conditions. Mean responses were also calculated for each factor at different levels.

3.5.1. Evaluation of variables

The average signal-to-noise ratio S / N of the electrode response for each level of the factors is shown in Fig. 6.

Indeed, the optimal amount of factors were; the concentration of TGA-CdSe / ZnS nanocomposite is equal to 0.0005 g and the concentration of carbon nanotubes is 0.0002, the immersion time 4 hours and the frequent immersion of the electrode in the coating suspension is 3 times.Therefore, the most influential factor is the concentration of nanocomposite. The time and number of immersions are insignificant on the response.

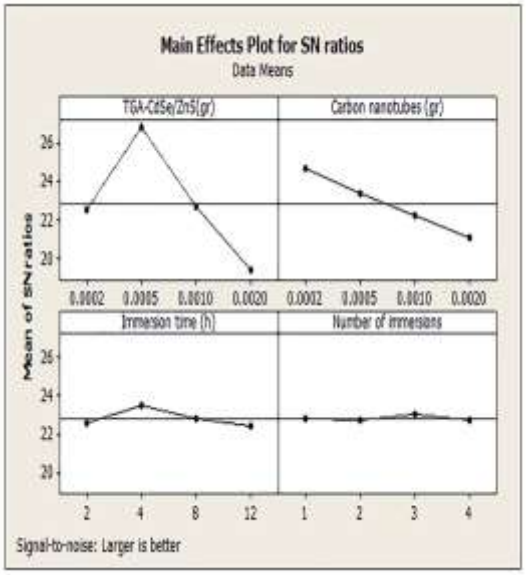


Fig. 6. Results of Taguchi analysis of morphine and fentanyl.

3.5.2. Statistical analysis of variance (ANOVA)

The ANOVA is used for the analysis of the results obtained by the Taguchi method. ANOVA Table (1)shows the results of a regression analysis. Based on these results, P values for the main effects (the amount of TGA-CdSe/ZnS nanoparticles and the concentration of carbon nanotubes had the lowest P value, which means that these factors are statistically significant on the electrochemical determination of fentanyl and morphine. Whereas, the immersion time (in hours) and number of immersions (n) have the highest P value, which means that it has less importance and effect on the process. The Fisher test[23] is a statistical tool for examining design parameters that has a significant effect on qualitative characteristics. In this analysis, F is to determine the importance of the coating concentration factor (91.13%) thus it is the most effective and the least effective factor is immersion time in terms of 1.68%.

3.6. The oxidation mechanism of morphine and fentanyl with TGA-CdSe / ZnS @ FCNT sensor

To obtain some information on the mechanism of electrochemical reactions of morphine and fentanyl at the surface of the TGA-CdSe / ZnS @ FCNT electrode, the cyclic voltammetry at different scan rates (2 to 300 mV) at a concentration of 10 mMof Morphine and fentanyl were used and the results are reported in Fig. S5.

According Fig. S5(B) and S6 (B), there is a linear relationship between the current intensity (I_p) and the square-root of the scan rate ($v^{½}$), according to which its equations for morphine y = 3.1553x + 2.5976 with a correlation coefficient R^2 = 0.9908, and for fentanyl, y = 29.769x - 0.0948 with a correlation coefficient R^2 = 0.9938were obtained. These relationships indicate that the oxidation process of the species is under the effect of the diffusion process.

In addition, plot of logarithm of peak current, log (I_p), versus the logarithm of scan rate, log V, was studied (Fig. 5C and 6C). If the slope of the equations is close to the theoretical value of 0.5, the process is controlled based on the diffusion; otherwise, when it is more than 0.5, and the electrode reaction is controlled based on surface adsorption phenomena[24,25]

Since the slope of fentanyl equation is 0.57, and that is near to the theoretical value of 0.5 for a diffusion controlled process. And the slope of morphine is 0.41. It can be concluded that the oxidation of this drug is mainly controlled by the diffusion process.

There is a good linear relationship between the peak oxidation potential (E_P) of morphine and fentanyl and the log v, (Fig. 5D and 6D).Therefore, according to the Laviron equation, the irreversible anodic reactions controlled by the diffusion process have a peak potential (E_P) of the following equation:

$$Ep = E_0 + 2.303\ RT/\alpha nF)\ \ln v$$

In this equation, E_0 is the standard formal potential, v is the potential scanning velocity, α is the electron transfer coefficient, n is the number of exchanged electrons, F is the Faraday number (96493 $Cmol^{-1}$), R is the universal gas constant (314/8 $J.mol^{-1}.K^{-1}$), T the ambient temperature in Kelvin (298^0K), and K_s is the electron transfer rate constant.

The slope values of the morphine and fentanyl equations are 0.0538 and 0.0561, respectively. If the value of the transfer coefficient (α) in irreversible systems is considered 0.5, the number of electrons exchanged in the reaction (n) for morphine and fentanyl is 2.09 (approximately equal to 2.1) and 2.01 (approximately equal to 2), respectively, which is consistent with the results obtained for the oxidation mechanism of morphine and fentanyl[26] .

3.7. pH effect

One of the most important factors influencing the performance of an electrochemical sensor is the pH.The electrochemical behavior of morphine and fentanyl with the designed sensor in 10 μM solution was investigated for both medications pH in the range of 2 to 10.

Table 1. ANOVA table for Taguchi approach

Source	d_f[a]	Ssd[b]	MS[C]	Adj MS	F-Value	P-Value
TGA-CdSe/ZnS(gr)	3	351.354	351.354	117.118	41.46	0.006
Carbon nanotubes (gr)	3	88.267	88.267	29.422	10.42	0.043
Immersion time (h)	3	18.935	18.935	6.312	2.23	0.263
Number of immersions	3	10.837	10.837	3.612	1.28	0.422
Residual error	3	8.475	8.475	2.825		
Total	15	477.868				

S = 1.68075 R-Sq = 98.23% R-Sq(adj) = 91.13%; a Degree of freedom ; b Sum of squares; C Mean S

According to the results (Fig. S7) the highest peak current for morphine has appeared at pH 6, and for fentanyl at pH 8 , therefore, pH7 was chosen as the optimal pH for the simultaneous measurement of the two drugs.

Based on Fig. (S (8A) and S (9A), the peak oxidation potentials for morphine and fentanyl shifted to more negative potentials with further increase in the pH. These results indicate that protons are also involved in the oxidation process of morphine and fentanyl. According to the below equation :

$$Ep = (-0.592m \ / \ n)\, pH + b$$

Where n and m are the number of electrons and protons involved in the electrochemical process, respectively. When we plot the EP versus pH, we get linear graphs (Fig. S(8B) and S (9B) whose equation for morphine is E_p = -0.0573$pH_{morphine}$+ 0.876; with a correlation coefficient of R^2 = 0.9899 and for fentanyl it is equal to E_p = -0.0566$pH_{fentaneyl}$ + 1.3096 with a correlation coefficient of R^2 = 0.9942.

The slope of these equations for morphine and fentanyl is 0.0573 and 0.0566, respectively. The value of this slope corresponds to the theoretical value of 0.0592, which indicates that the number of electrons and protons in the oxidation process of morphine and fentanyl are the same.

Due to the importance of the role of carrier electrolytes in electrochemical reactions, in addition to buffer Robinson, different carrier electrolytes such as phosphate buffer solution and acetate buffer and saline phosphate were selected for the study. This study was performed to determine the best carrier electrolyte to measure the oxidation current of two drugs. The results show that the electrochemical signal of morphine and fentanyl oxidation in Robinson buffer was higher and more repeatable than in acetate buffer and phosphate buffer on the surface of this electrode. Therefore, in subsequent studies, a 0.1 M Robinson buffer (pH 7) was used to measure the oxidation of morphine and fentanyl at the designed electrode surface.

3.8. Determination of electron transfer coefficient and emission coefficient

The TOEFL curve was used to determine the electron transfer coefficient of morphine and fentanyl on the surface of the modified electrode.

For this purpose, data related to linear scanning voltammograms were used in the areas where the kinetics control the process (ascending part of the diagram), (Fig. S (11A) and S (12A). The slope of the Tafel plot, Fig. S(11B) and S(12B) is equal to n (1-α) F / 2.303RT. According to the known values for R=8.314Jmol $^{-1}$.K^{-1} , and F= 96493C. mol^{-1} the value of α is calculated.

The Tafel plot,is shown for the20 μM morphine and fentanyl at a scan rate of 0.1 v/s using a cyclic voltammogram. The Tafel curve slopes indicating that the electrocatalytic process is the single electron charge transfer process. According to the observed slope and the Tafel plot equation, the transfer coefficients (α) are 0.96 and 0.95 for morphine and fentanyl, respectively. The chronoamperometric method was used to determine the diffusion coefficient of morphine and fentanyl. The modified electrodes were applied in the presence of different concentrations of morphine and fentanyl to record chronoamperograms with a potential of 0.5 V against Ag / AgCl (KCl3M) reference electrode. In order to record morphine and fentanyl oxidation chronoamperometric graph and to determine the diffusion coefficient, the Cottrell curve of these compounds was plotted at different concentrations. The resulting curves are linear with respect to the Cottrell relation. According to the following Cottrell equation:

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

Where, n is the exchanged electrons, A is the active surface of the electrode (cm^2), D is the diffusion coefficient ($cm^2.s^{-1}$), C is the concentration of the analyzed compound ($mmol.L^{-1}$), t is the time (s), F is the Faraday constant 96493 $Cmol^{-1}$.

From its slope, the diffusion coefficient of electroactive species can be obtained. The slope of the graph is equal to $nFACD^{1/2}/\pi^{1/2}\ t^{1/2}$

Morphine and fentanyl chronoamperometry plot with concentrations of 10, 20, 30 demonstrated in (Fig. 7A and 8A), respectively. Flow charts against I-$t^{-2.2}$ were plotted for each of the plots (Fig. 7B and 8B). Then I-$t^{-1.2}$ slope diagrams were drawn against the drugs (Fig. 7C and 8C). By substituting constant values of F, A, π and n, the diffusion coefficient for morphine and fentanyl was 3.84×10^{-6} and 1.615×10^{-6}cm^2 s^{-1}, respectively.

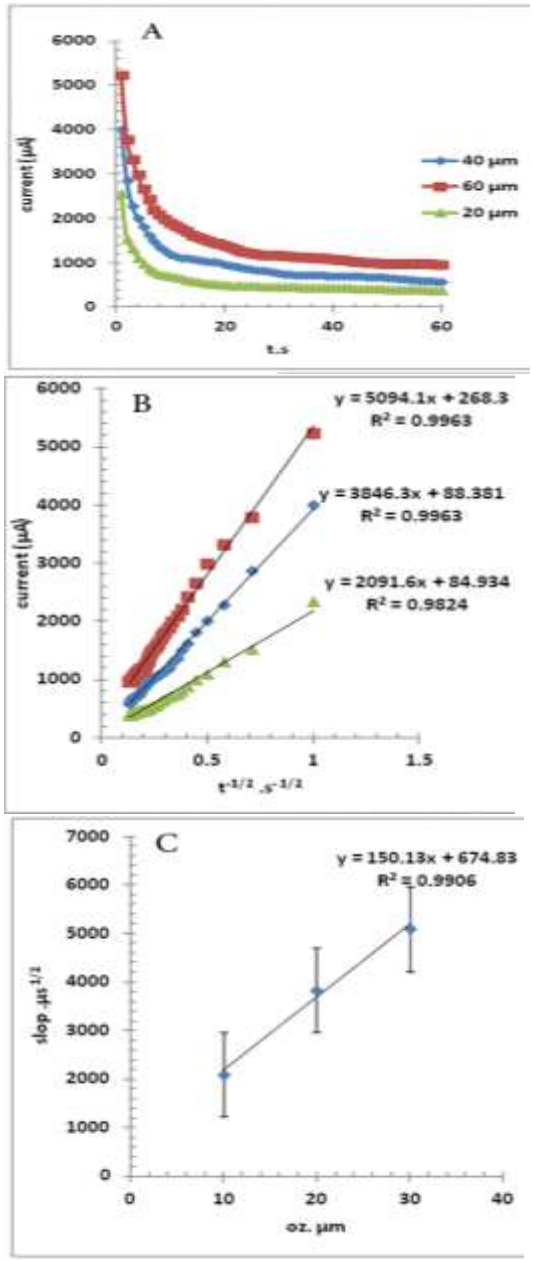


Fig. 7 (A). Chronoamperometric responses of TGA-CdSe/ZnS@FCNT coated PGE at a potential step of 800 mV in at the presence of 0.1 molar Britton Robinson buffer (pH 7).in the presence of 20, 40, and 60 mMmorphine.(B) Inset A shows the related plot of I vs. $t^{-1/2}$(C) shows the slopes of the resulting straight lines versus the morphine

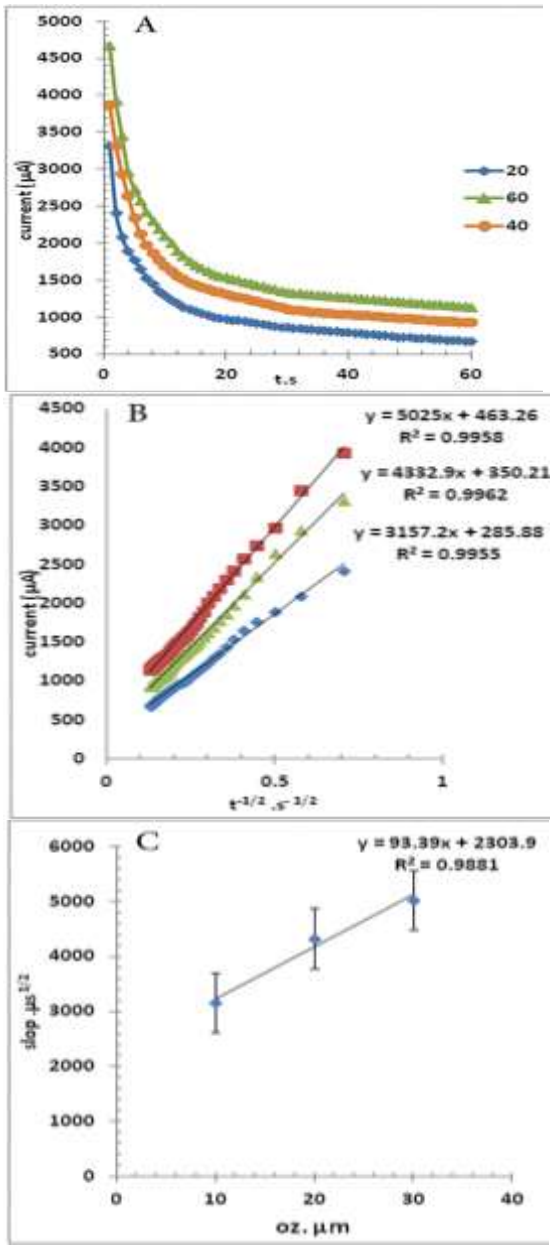


Fig. 8. (A). Chronoamperometric responses of TGA-CdSe/ZnS@FCNT coated PGE at a potential step of 500 mV in at the presence of 0.1 molar Britton Robinson buffer (pH = 7).in the presence of 20, 40, and 360 mMfentanyl.(B) Inset A shows the related plot of I vs. $t^{-1/2}$. (C) Shows the slopes of the resulting straight lines versus the fentanyl concentration.

3.9. Performance of the prepared sensor

Under optimal experimental conditions, the performance of the modified TGA-CdSe / ZnS @ FCNT sensor was determined by simultaneous measurement of standard morphine and fentanyl solutions by differential pulse voltammetry. To simultaneously determine the analytes under optimal conditions, DPV was performed at pH = 7, where only the concentration of one target molecule changed and the concentration of the other molecule was kept constant.

The peak oxidation current of morphine increased linearly with an increase in its concentration in the range of 0.008 to 100 μM and the peak oxidation current of fentanyl remained unchanged at a concentration of 2 μM (Fig. S13). Similarly, fentanyl was determined in the concentration range of 0.02 to 8 μM in the morphine-containing solutions at a constant concentration of 2 μM (Fig. S14). The peak current of morphine was unchanged and the peak current remained constant while the peak current of fentanyl increased with an increase in concentration. Simultaneous morphine and fentanyl determining was also investigated using the DPV method in the modified TGA-CdSe / ZnS @ FCNT sensor. Fig. (S16)shows DPV voltammograms of morphine and fentanyl at different concentrations and that the modified electrode can be used to simultaneously measure morphine and fentanyl over a wide range of concentrations of both drugs. As mentioned, there is a good linear relationship between the peak oxidation current of morphine and fentanyl and their concentration. In morphine the linear range is from 0.08 to 100 μM (Fig. S13B) and in fentanyl there are two linear ranges (1×10^{-4} to 8×10^{-6}) and (2×10^{-8} to 8×10^{-6}), (Fig. S(14B)).

The Calibration curve equations for morphine y = 0.0332x + 0.2236 with correlation coefficient R^2 = 0.9996 and for fentanyl two linear ranges y = 0.7677x + 0.4819 with correlation coefficient R^2 = 0.997 and y = 0.2011x + 3.9108 with correlation coefficient R^2 = 0.9982. The performance of the sensor for simultaneous measurement of two drugs was also evaluated. DPV voltammograms(Fig. S15A) and calibration curves (Fig. S15B) related to different concentrations of morphine and fentanyl show the equations related to the calibration curve for morphine equal to y = 0.0049 x + 2.069 with correlation coefficient R^2 = 0.9958, and for fentanyl y = 0.154x + 4.4383 with correlation coefficient R^2 = 0.9958. As can be seen, the modified electrode has a very good ability to measure two drugs simultaneously.

These results indicate that individual or simultaneous measurement of the drug with this sensor is satisfactorily possible without any interference with its performance.

To evaluate the practical application of the nano-sensor, competence cultivars such as the correlation coefficient (R^2), corresponding regression equation (slope of calibration curve indicating sensitivity), LOD detection limit and linear mechanical range (LDR) were investigated under optimal conditions (Table 2).

Table 2. Statistical results of morphine and methadone studies.

Statistical calculations	Morphine	Fentanyl
Calibration Equation (μM)	y=0.3723x+2.5303	y=0.7677x+ 0.4819 y=0.2011x+3.9108
LDR[a] (μM)	0.08-100	0.02-8 8-100
LOD[b]	0.024±0.0013	0. 006±0.0009
R^2 [c]	0.9988	0.9982 0.9984
RSD% (n=3)	2.23	4.07

a Linear dynamicl range; b Standard deviation of slope; c Correlation coefficient

The detection limit is an analytical method for measuring analytes, compared to other methods. A practical approximation to the detection limit accepted by analytical chemists is the analyte concentration, which has a response equal to the response of the control sample (y_b) plus 3 times the standard deviation of the control sample (S_b).

$$LOD = y_b + 3S_b \qquad \text{Equation 15-4}$$

In this equation, S_b shows the standard deviation of the white solution. The detection limits of the method for morphine and fentanyl were 0.0167 and 0.027 μM, respectively. The reproducibility of the method was assessed by performing 6 continuous measurements by a modified TGA-CdSe / ZnS @ FCNT sensor in a 10 μM solution of morphine and fentanyl. In addition, the standard deviation values (RSD) for measuring morphine and fentanyl were 1.84 and 3.62, respectively. The repeatability of the modified electrode was investigated by replicate recordings of voltammogram at the optimized condition. The coefficient of variance (%CV) for the peak currents based on seven replicates is 1.62%, indicating an acceptable repeatability of the response at the modified electrode.

To evaluate the stability of the prepared sensor, the electrode was kept at room temperature for 30 days, which showed no significant change (less than 7%) in the electrode response to both drugs, indicating proper sensor stability. The electrode analysis was compared with other electrodes made to date to measure morphine and fentanyl and is shown in Table 3.

The sensor modified with TGA-CdSe / ZnS @ FCNT has a wider linear amplitude range and lower detection limit than most previous reports.

3.10. Interference studies

To evaluate the selectivity and sensitivity of the fabricated electrode, the possible interference of some common molecules was studied.The selectivity of this electrode was measured at a

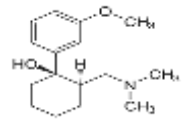
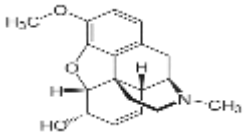
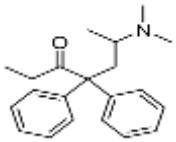
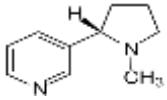
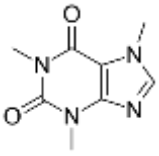
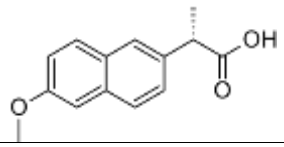
concentration of 1.0 µL of morphine and fentanyl (pH 7)in the presence of interferents at the relevant concentration, and several times higher than the analytes.The disturbance limit was considered as the concentration of interferents that caused a change in the analyte signal, with an error of more than 5%. The results are depicted in Table 4.

Table 3. An analytical comparison of the current method and some previous techniques on the determination of morphine and fentanyl

Working electrode	Method	LDR Morphine (µM)	LOD Morphine (µM)	Recovery% Morphine	Linear range fintanyel one (µM)	LOD (µM) Fintanyel	Recovery fentanyl	Ref.
TFMWCNT/IL/CPE[a]	SWV	0.025-600	0.015	98-102.4	-	-		[27]
MCNP/SPCE[b]	DPV	0.1-600	0.02	97.5 103.73	-	-		[28]
Au/MB/ds-DNA [c]	DPV	0.05-500	0.01	103.2-105.8	-	-		[29]
Zn (II)-MOF/ SPCE[d]	DPV	-	-		1–100	0.3	100-103	[30]
CNOs / GCE [e]	DPV				1 to 60	0.3	98-103	[31]
GO/CdSe-PGE	DPV	0.08-100	0.0027	98.75-101.4	0.02-8 8-100	0.018	98.3-102	This work

a TiO2/Fe3O4/Multiwalled Carbon Nanotubes Nanocomposite; b Magnetic core shellmanganese ferrite nanopartical modified screen printed electrode ; c Mercaptobenzaldehyde and ds-DNA modified Au electrode.; d screen-printed carbon electrode modified with the open-ended channels of Zn(ii)-MOF; e glassy carbon electrode (GCE) modified with the carbon nanoonions (CNOs).

Table 4. Investigating the selectivity effect

Drugs	Structure	Tolerance limit morphine (µM)	RSD% (morphine (n=3)	Tolerance limit fentanyl µM)(	RSD% (fentanyl (n=3)(
Tramadol		300	3.92	200	4.6
Codeine		10	4.46	100	4.87
Methadone		300	4.7	10	4.8
Nicotine		100	4.3	50	4.4
Coffeine		500	4.31	50	4.87
Naproxen		100	4.11	10	4.14

*: The tolerance limit is the maximum concentration of interferent which causes an error less than±5%.

3.11. Application of electrochemical sensors for analyses of real samples

In order to evaluate the efficiency of the proposed method for simultaneous measurement of morphine and fentanyl by the fabricated sensor in biological samples, urine and blood samples were prepared and then evaluated.

Urine samples were taken from a healthy volunteer. Urine samples were stored in the refrigerator. Then, 4 ml of urine solution was centrifuged for 5 minutes (1500 rpm) to separate the suspended particles. The prepared sample was passed a suitable filter. The solution was diluted 50%with the buffer solution. The prepared sample was stored in the refrigerator at 4 °C.

Blood samples were collected from a healthy volunteer. The blood was shaken and centrifuged at 6,000 rpm for 30 min to separate out plasma. 9 mL acetonitrile was added to 1 mL plasma and centrifuged at 6,000 rpm for 30 min to deproteinate it. The supernatant serum thus obtained was stored at 20°C and filtered for subsequent analysis.

Two samples of urine and blood, after initial preparation, were spiked with different concentrations of morphine (0.2, 0.4, 0.6, 0.8 and 1 μM) and fentanyl(0.1, 0.3, 0.6, 0.9 and 1.2 μM). Then analytical measurements were performed using the standard addition method, the results of which are listed in Table 5. The relative recovery percentage indicates the accuracy obtained and the applicability of the proposed sensor for measuring simultaneous low amounts of morphine and fentanyl in pharmaceutical and biological samples.

4. CONCLUSION

In the present study, a simple and efficient protocol was used for synthesizing cadmium selenide/zinc sulfide core /shell quantum dots and functionalized multi-walled carbon nanotubes, materials and applied in fabricating an electrochemically pretreated morphine and fentanyl sensor.

The designed electrode has several advantages such as simplicity, increased surface area, acceptable electron transfer rate, accuracy and reproducibility, low detection limit and good linear dynamic range. Simultaneous use of quantum dots; cadmium selenide / zinc sulfide core / shell and carbon nanotubes increase the electro-active surface area and improve electron transfer process, cause a significant increase in peak current density due to reduced overvoltage. The use of this nanocomposite leads toa significant increase in response, and sensitivity, eventually leading to the successful determination of the drugs at low levels in biological samples.To prove the practical applications of the optimized electrochemical procedure, it was successfully applied for the determination of morphine and fentanyl in real samples. The results indicated acceptable precision and accuracy towards the simultaneous determination of two drugs under the optimized conditions.

Table 5. An analytical results for simultaneous determination of fentanyl and morphine in serum and urinesamples

	Added (μM)		Found ± δ (μM)		RSD%[a] (μM)		RR%[b]	
sample	Fentanyl	Morphine	Fentanyl	Morphine	Fentanyl	Morphine	Fentanyl	Morphine
	0	0	0	0	0	0	0	0
Sample	0.2	0.2	0.197±0.0024	0.2028± 0.0076	1.22	3.7	98.5	101.4
# ١	0.4	0.4	0.4±0.013	0.401± 0.008	3.25	1.99	100	100.25
Blood	0.6	0.6	0.603±0.015	0.5932± 0.013	2.48	2.19	100.5	98.87
serum	0.8	0.8	0.7985±0.011	0.79± 0.0096	1.38	1.21	99.81	98.75
	1	1	1.02±0.026	0.9904± 0.018	2.5	1.82	102	99.04
	0	0	0	0	0	0	0	0
Sample	0.1	0.1	0.99660±.0025	0.0993±0.0020	1.94	2.1	99.9	99.3
# 2	0.3	0.3	0.2987±0.0068	0.302±0.0045	1.14	1.003	99.6	99.67
Blood	0.6	0.6	0.60± 0.007	0.598±0.006	1.17	1.49	100	100.66
serum	0.9	0.9	0.898±0.018	0.896±0.017	2.004	1.89	99.78	99.5
	1.2	1.2	1.18±0.02	1.19±0.011	1.69	0.92	98.33	99.16
	0	0	0	0	0	0	0	0
	0.2	0.2	0.2003±0.0049	0.1997± 0.0042	2.46	2.08	100.17	99.83
Sample	0.4	0.4	0.3997±0.003	0.4013± 0.003	0.8	0.76	99.91	100.33
# 1	0.6	0.6	0.606±0.009	0.6± 0.0137	1.51	2.29	101	100
Urine	0.8	0.8	0.8±0.0115	0.7973± 0.01	1.44	1.256	100	99.67
	1	1	0.985±0.030	1.012± 0.025	3.12	2.5	98.5	101.2
	0	0	0	0	0	0	0	0
Sample	0.1	0.1	0.102±0.003	0.1±0.0017	2.94	1.732	102	100
# 2	0.3	0.3	0.2987±0.007	0.3007±0.0055	2.35	1.83	99.55	100.22
Urine	0.6	0.6	0.607± 0.011	0.5997±0.0011	1.812	0.19	101.17	99.94
	0.9	0.9	0.9013±0.003	0.8987±0.0195	0.33	2.17	100.15	99.85
	1.2	1.2	1.2173±0.028	1.1927±0.0109	2.3	0.919	101.44	99.38

RR%: Relative Recovery%

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حسگر الکتروشیمیایی ناهمساختار تقویت‌شده با نانولوله کربنی برای تعیین همزمان مورفین و فنتانیل در نمونه‌های بیولوژیکی

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چکیده

در این مطالعه، یک حسگر الکتروشیمیایی برای اندازه‌گیری همزمان مورفین و فنتانیل بر اساس یک الکترود گرافیتی مغز مداد اصلاح‌شده با ساختار نانوکریستالی نیمه‌رسانا ساخته شد. لایه اول سنسور دارای هسته ای از نقطه کوانتومی سلنید کادمیوم متصل به تیوگلیکولیک اسید(TGA-CdSe) است که توسط لایه دوم، نقطه کوانتومی سولفید روی (ZnS) احاطه شده است. نانولوله های کربنی عامل دار نیز برای تقویت ساختار حسگر (TGA-CdSe/ZnS@FCNT) استفاده شده است. اندازه‌گیری‌ها با ولتامتری پالس تفاضلی و ولتامتری چرخه‌ای انجام شد. سنتز نانوساختارها توسط FTIR، EDX، SEM و XRD تأیید شد. به منظور بهینه سازی عوامل موثر در عملکرد این سنسور، از طرح آرایه متعامد تاگوچی (OA16) استفاده شده است. ولتاموگرام‌های ولتامتری چرخه‌ای پیک‌های اکسیداسیون برگشت‌ناپذیر را در پتانسیل‌های ۰/۹ ولت و ۰/۳۸ ولت برای فنتانیل و مورفین نشان دادند. ضرایب انتشار به دست آمده بر روی سطح الکترود توسط کرونوآمپرومتری $۳/۸۴ \times ۱۰^{-۶}$ و $۱/۶۱۵ \times ۱۰^{-۶}$ سانتی‌متر مربع بر ثانیه برای مورفین و فنتانیل به ترتیب بود. در شرایط بهینه، محدوده غلظت خطی و حد تشخیص مورفین ۱۰۰–۰/۰۸ میکرومولار و ۰/۰۲۴ میکرومولار بود. برای فنتانیل دو محدوده خطی ۸–۰/۰۲ میکرو مولار، ۱۰۰–۸ میکرومولار و ۰/۰۰۶ میکرومولار به دست آمد. سنسور ساخته شده می تواند به خوبی برای اندازه‌گیری همزمان مورفین و فنتانیل در نمونه‌های بیولوژیکی با بازیابی نسبی قابل قبول در محدوده ۱۰–۹۸/۳ استفاده شود.

واژه‌های کلیدی

مورفین؛ فنتانیل؛ نقطه کوانتومی کادمیوم سلنید؛ نقطه کوانتومی روی سولفید؛ نانولوله‌های کربنی چند جداره.