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Original Research

Development of a New Method Based on Copper Sulfide Nanoparticles for the Determination of Fentanyl in Biological Samples

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Abstract

Background: Fentanyl is an opioid analgesic drug extensively used to alleviate pain with no consciousness loss. To address the fentanyl crisis from the criminal and medical perspectives,the development of a free drug determination methodology for this drug is crucial.

Methods: A fluorescent nanoprobe based on copper sulfide nanoparticles (CuS NPs) is developed for the determination of fentanyl in exhaled breath condensate (EBC). NPs are synthesized according to a hydrothermal method and their size and morphology are characterized via X-ray diffraction (XRD), Fourier-transformed infrared (FTIR), and TEM. The fluorescence intensity of the nanoprobe is enhanced in the presence of fentanyl. The affinity of CuS NPs to complex formation with fentanyl results in blocking non-radiative e–/h+recombination defect sites on the surface of NPs and consequently enhancing the signal intensity. One at a time optimization method was used for the optimization of reaction conditions.

Results: Under the optimized conditions, a low limit of detection (LOD) of 0.008 µg mL-1 was obtained for fentanyl determination. Furthermore, a linear relationship is found between the analytical response and the concentration of fentanyl in the range of 0.01-2.0 µg mL-1 with a relative standard deviation of<2.5%.

Conclusion: The validated method is applied for the determination of fentanyl in the EBC of patients receiving fentanyl treatment.

Introduction

Fentanyl is an opioid analgesic drug extensively used to alleviate pain with no consciousness loss. This lethal drug offers 100-times more intensive than morphine due to its strong acting on µ-opioid receptors. Compared to morphine, fentanyl is more desirable for routine/anesthetic surgery due to its remarkable anesthesia and analgesia. Fentanyl could simply enter the plasma and central nervous sites and metabolized rapidly in the liver owing to its high lipophilicity.[1] Despite the admirable pain relief of fentanyl, fentanyl abuse could cause several adverse effects including death, respiratory failure, nausea, drowsiness, and dizziness.[2] The unbound fentanyl is responsible for pharmacological effect; however, total concentrations are measured for therapeutic drug monitoring purposes.[3] Measurement of the free concentration of fentanyl can lead to reducing the prescribed dose, reducing these side effects, and increasing its efficiency. Therefore, the development of a free drug determination methodology for this drug is crucial from the criminal and medical perspectives to address the fentanyl crisis. Exhaled breath condensate (EBC) is one of the biological fluids sustaining low interfering compounds compared to plasma, urine, and blood matrices for drug monitoring. Proof that fentanyl is exhaled was confirmed by Wang et al[4] efforts. Using gas chromatography-mass technique, they showed that fentanyl was exhaled as part of the aerosol droplets in fentanyl-treated children. In another work conducted by Berchtold et al,[5] the detectability of fentanyl in EBC samples was performed even in lower concentrations (with about 5.0 pg mL^{-1}). Apart from the type of biological sample, introducing sensors with fentanyl detection capability would be vital to capitalize on the effectiveness of law enforcement officers and healthcare providers. Based on the literature, some methods such as chromatography-based methods,[6] surface-enhanced Raman spectroscopy,[7] and optical fiber[8] have been used for the quantification of fentanyl in biological fluids like blood and urine. These methods take low throughput and are time-consuming in nature which is unable to meet the increasing requirements for rapid quantification of fentanyl despite the good accuracy and sensitivity of these methods. Therefore, the development of fast, reliable, stable, and reproducible sensors for the analytical detection of fentanyl has garnered rapid advancement in recent years due to their importance in EBC samples.[9]

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With the prompt progress of nanotechnology in recent years, nanomaterials have been extensively employed in sensory fields.[10,11] Among numerous categories of nanomaterials, fluorescent nanomaterials such as copper sulfide nanoparticles (CuS NPs) catch the spotlight regarding their exceptional applications and features in sensors. CuS represents two prominent forms of stable stoichiometries including sulfur-rich covellite (CuS) phases and copper-rich chalcocite (Cu_2S). Covellite as a p-type semiconductor metal chalcogenide is intensively considered as many stable and metastable phases when exposed to oxidizing conditions.[12] Moreover, covellite NPs act as a p-type semiconductor by having Cu vacancies in their lattice and showing variable localized surface plasmon resonances in the near infrared range. Additionally, CuS is a class of nanomaterials displaying unique properties such as metal-like electrical conductivity,[13] recyclability, low toxicity, easy availability, and stoichiometry.[14]

In this study, a CuS NPs-based fluorometric probe was developed for the determination of fentanyl in EBC samples. A spectrofluorometric process based on CuS NPs was considered and used for the fentanyl analysis. The observed enhancement in fluorescence intensity of the nanoprobe might be associated with the construction of a stable non–luminescent complex between CuS NPs and fentanyl owing to the chelating ability of fentanyl and copper in the structure of CuS NPs. Generally, the affinity of CuS NPs to form a complex with fentanyl cause blockage of non-radiative e^-/h^+ recombination defect sites on NPs surface and consequently enhance the intensity of the signal. According to this mechanism, a "turn–on" probe was confirmed for fentanyl determination in the EBC samples.

Materials and Methods

Reagents and solutions

Ethylene glycol monomethyl ether ($C_3H_8O_2$), thiourea (CH_4N_2S), and sodium hydroxide (NaOH) received from Merck and copper (ll) nitrate trihydrate ($Cu(NO_3)_2 \bullet 3H_2O$) was obtained from Sigma-Aldrich in analytical grade for preparation of CuS NPs. The solutions were prepared and used freshly by diluting them with de-ionized water. Sodium hydroxide (NaOH) as well as sodium dihydrogen phosphate (NaH_2PO_4) was purchased from Merck (www.merck.com) to adjust different pHs. A stock solution of fentanyl with 50 μg mL^{-1} was daily used for the preparation of water-diluted fentanyl.

Apparatus and software

For recording the fluorescent spectrum, A FP-750 spectrofluorometer (Jasco Corp., Japan) was applied with 5 nm and 10 nm slit width in excitation and emission slit width paths. The instrument was equipped with a Peltier thermostated single cell holder model ETC-272 (JASCO Corp., Japan) for temperature control. A digital pH-meter model 744 (Metrohm Ltd., Switzerland) was employed for the adjustment of pH. A CM30 transmission electron microscopy (TEM, Philips, The Netherlands, www.philips.com) was used for evaluating the size and shape of NPs. To verify the phase crystallinity and purity, powder X-ray diffraction (XRD) patterns were accomplished in the range of $2\theta = 20\text{-}\ 70^{o}$ with filtered Cu-Kα radiation. Furthermore, Bruker Tensor 270-Fourier transforms infrared (FT-IR) spectroscopy was performed for the successful synthesis of CuS NPs.

Synthesis of CuS NPs

A surfactant-free procedure was used for the synthesis of CuS NPs in this study.[15] In brief, 20 mL of ethylene glycol and 0.6 g thiourea was mixed with 0.456 g of Cu $(NO_3)_2 \bullet 3H_2O$ in a 50 mL three-neck flask. To minimize air contact, nitrogen gas was introduced to the obtained mixture. Afterward, the solution was heated to 110°C. after 10 minutes, a solution containing 5 mL ethylene glycol and 5mL of NaOH (1 M) was inserted into the flask and kept for a further 5 minutes. The produced CuS NPs were isolated by centrifuging for 5 minutes at 10 000 rpm after cooling to room temperature. To remove the excessive precursors and ions, CuS NPs were washed several times with water. Finally, the as-prepared CuS NPs were dried in a vacuum desiccator to prevent additional air oxidation.

Samples preparation

Using a lab-made setup, EBC samples were collected from healthy sample donors for optimization and validation of the method.[16,17] EBC is a low-protein and diluted aqueous matrix so that samples taken are directly used without any pretreatment procedure or sample preparation.

General procedure

A batch analytical method was used in a 2 mL microtube. For that, 10 μL of phosphate buffer with a concentration of 0.1 mol L^{-1} (pH 10.0) was added to the microtube with 200 μL of EBC spiked with various concentrations of fentanyl solution (0.01–2.0 μg mL^{-1}). Then, 200 μL of CuS NPs were added to the mixture. After setting the final volume of the solution up to 0.5 mL with water, the samples were incubated for 5 minutes. Finally, with an excitation wavelength of 315 nm, the analytical response was recorded at 410 nm.

Results and Discussion

Characterization of synthesized copper sulfide NPs

In this study, a fluorometric sensing nanoprobe was established using CuS NPs for the detection of fentanyl in EBC samples. To determine the size and shape of the prepared NPs, TEM was employed. As illustrated in Figure 1a, CuS NPs showed almost uniform and spherical shapes with individual particle sizes mostly below 20 nm. The size distribution histogram of CuS NPs given in the inset of Figure 1a was obtained from TEM images by measuring almost 20 particles. The results represent a well-dispersion and spherical shape of NPs in an aqueous

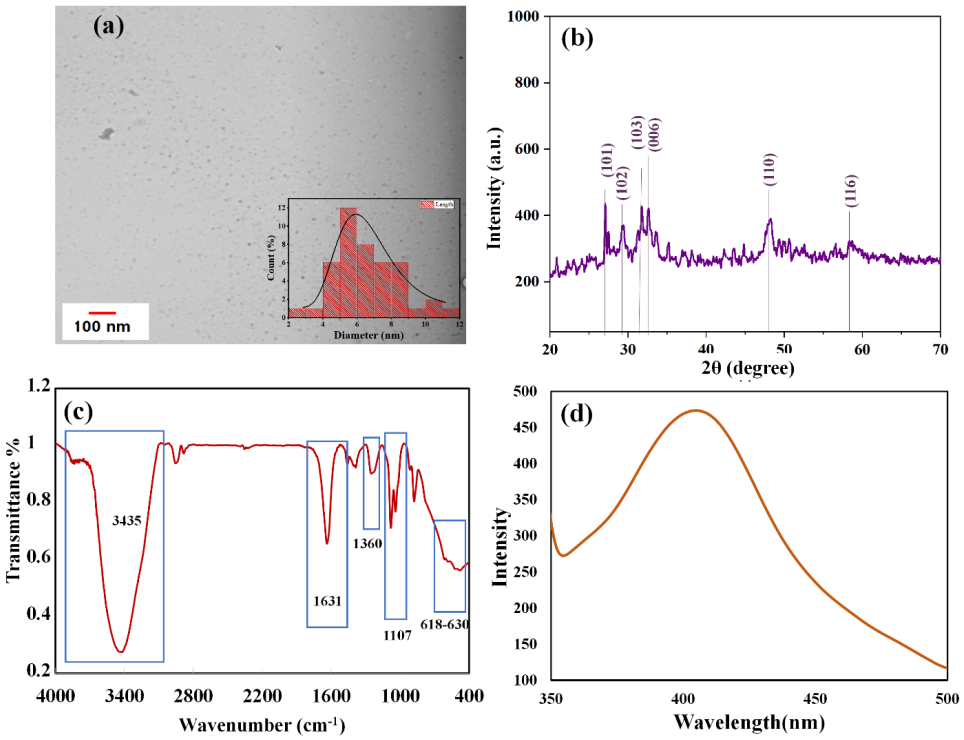

Figure 1. (a) TEM image (inset: Size distribution), (b) XRD patterns, (c) FTIR spectra, and (d) emission spectra (under excitation wavelength at 315 nm) of as-synthesized CuS NPs.

solution with an average size of 7.69 ± 1.5 nm.

The successful synthesis of the CuS NPs and their crystalline structure were investigated by XRD analysis in the 2θ range of 4-70°. As shown in Figure 1b, the presence of diffraction peaks at 2θ of 29.53, 31.96, 32.71, 48.19, and 58.93° relating to (102) (103), (006), (106), (110), and (116) planes represent the hexagonal crystallinity of the fabricated CuS NPs sample. These results are in well-agreement with the covellite phase standard data.[18] Furthermore, obtaining peaks of (103) and (006) weaker than (110) signifies polysulfide in CuS NPs.

To get an additional indication of the successful synthesis of the CuS NPs, the sample was subjected to FTIR spectroscopy. As can be seen in Figure 1c, the characteristic band at 3435 cm^{-1} was ascribed from the vibration -OH group of adsorbed water on the sample. Moreover, the absorption band located at 1631 cm^{-1} corresponds to the -OH bending of water. The unique absorption bands at 1098 cm^{-1} could be attributed to the distribution of the sulfate groups. The presence of a characteristic peak at 1360 cm^{-1} could be indicative of the polysulfate on its surface. The infrared vibration peaks detected at 1107 cm^{-1} corresponded to asymmetric stretching of the carbonyl group (C = O). Furthermore, Cu-S stretching mode was shown at 630/618 cm^{-1} indicating the formation of CuS crystals.[19]

To explore the optical properties of the synthesized CuS NPs, their fluorescence spectra were also studied at room temperature. According to Figure 1d, an intense emission peak at 410 nm with an excitation at 315 nm represented the successful synthesis of CuS NPs.

Proposed detection mechanism

The CuS NPs show a high emission intensity at 410 nm, which was increasingly enhanced with fentanyl addition in the range of 0.01-2.0 μg mL^{-1} (Figure 2). This practical enhancement may be associated with the formation of a stable non–luminescent complex between CuS NPs and fentanyl due to the chelating ability of fentanyl and copper.[20] Generally, the affinity of CuS NPs to form a complex with fentanyl cause blockage of non-radiative e^-/h^+recombination defect sites on the NPs surface and consequently enhance the intensity of signal. So, consistent with this mechanism, a "turn–on" probe was authenticated for fentanyl determination in the EBC samples.

Optimization of reaction parameters

The influence of important parameters such as pH, CuS NPs concentration, and the temperature was investigated to obtain the optimal response in the determination of fentanyl. In this case, the analytical response was $\Delta F(F\text{-}F_0)$ in which F and F_0 are the fluorescence intensity of the mixture with and without fentanyl. For optimization training, fentanyl at a concentration of 1.0 μg mL^{-1} was applied. The impact of pH on fentanyl quantification was primary estimated in the pH range of 6.0-11.0. The overall applied condition was: CuS NPs, 200 μL; EBC, 200 μL; fentanyl concentration, 1.0 μg mL^{-1} and final volume of the solution, 0.5 mL. The results in Figure 3a reveal that

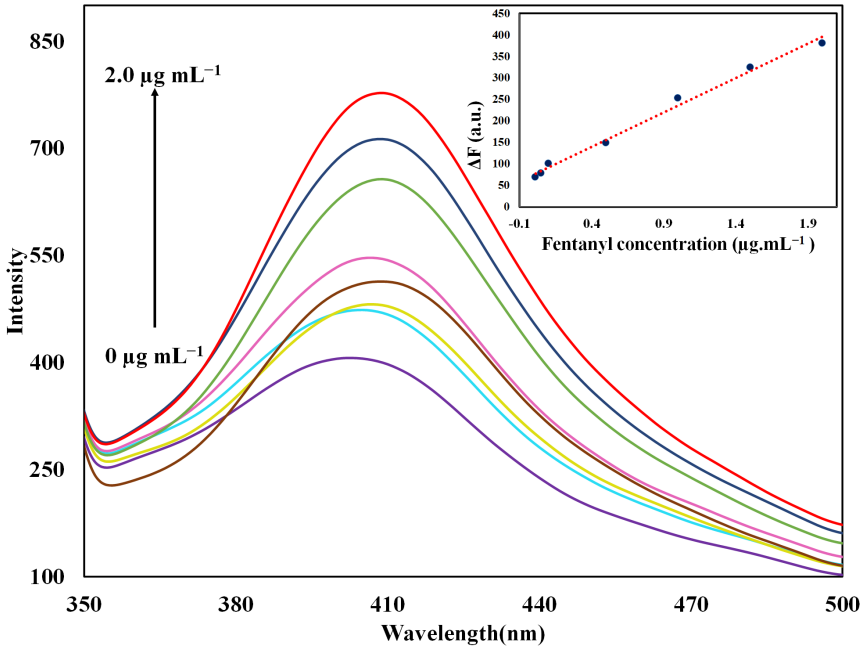

Figure 2. Fluorescence spectra of CuS NPs in EBC (n = 3) in the absence and presence of fentanyl concentrations (0.01–2 µg mL^{-1}); Inset: Calibration curve for CuS NPs response toward different concentrations of fentanyl. Experimental condition: 200 µL of EBC, 10 µL of phosphate buffer with pH 10.0 (0.1 mol. L^{-1}), 200 µL of CuS NPs, final volume:0.5 mL with water.

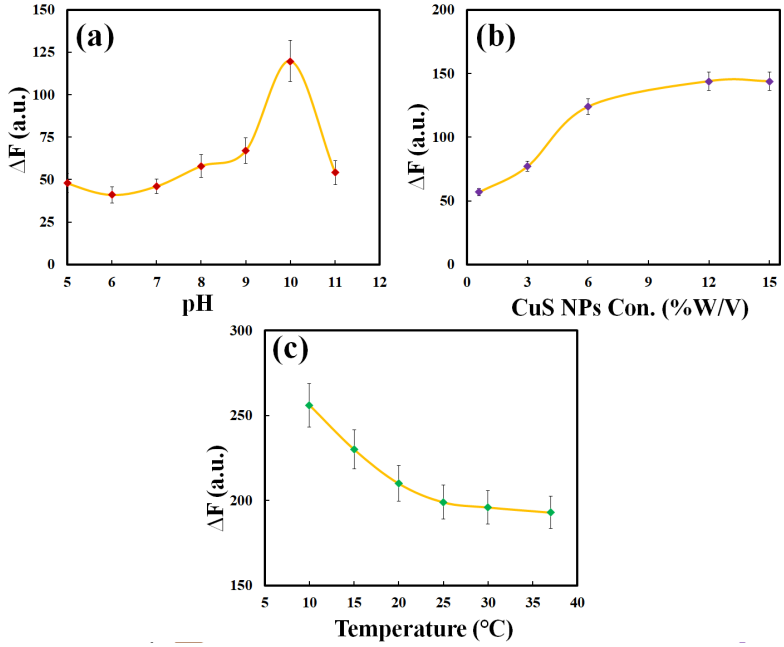

Figure 3. Effect of (a) pH changing, (b) CuS NPs concentration, and (c) temperature on the response of nanoprobe

the system's highest response was achieved at pH = 10.0. The weak basic pK_a of fentanyl is reported to be 8.3, which means that fentanyl would be a negative charge at pHs more than 8.3, and could be successfully absorbed on CuS NPs with a positive charge surface. Furthermore, the effect of CuS NPs concentration on analytical response was also investigated in the range of 0.6-15.0 × 10^{-3} %W/V. Figure 3b reveals that the analytical response rises with increasing the amount of CuS NPs, getting a maximum of 15.0 × 10^{-3} %W/V. Finally, the effect of temperature in the reaction system was studied in the range of 10-37°C. The findings in Figure 3c showed that response of the probe was significantly decreased by increasing the temperature. In temperature above 10°C due to non-radiated relaxation resulting from increasing incidences between molecules in the high temperatures.

Investigation of interferences

The study was examined in the incidence of over-the-counter or other co-administrated drugs in the same

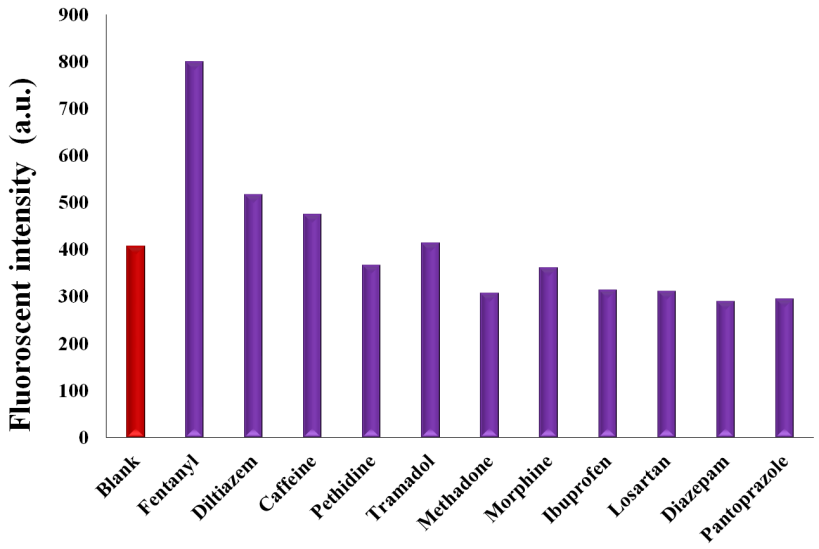

Figure 4. The study of interferences on the developed probe using some possible over-the-counter or co-administrated drugs with concentrations of 2.0 μg mL^{-1}.

optimal condition. Each pharmaceutical was used at equal concentrations to fentanyl, and subsequently the system's fluorescence signal was compared. The intensity of the fluorescence was measured and summarized in Figure 4. According to the results, caffeine, pethidine, tramadol, and morphine had little effect on the fluorescence response of the investigated probe compared to fentanyl. However, among the commonly used drugs investigated for selectivity studies, diltiazem, methadone, ibuprofen, losartan, diazepam, and pantoprazole represent significant interference with fentanyl determination. It is proposed that this method could be performed for fentanyl tracing in the EBC of the patients not receiving these drugs or an extraction method could be used before direct determination.

studied compounds including diltiazem, methadone, ibuprofen, losartan, diazepam, and pantoprazole represent little effect on the fluorescence response of the investigated probe compared to fentanyl. The obtained results specify that the proposed approach has respectable selectivity toward fentanyl detection.

Analytical figures of merit

At optimum conditions, the analytical evaluation for the determination of fentanyl in the EBC samples as an investigated biological matrix was performed by assessing the calibration plot, the limit of quantification (LOQ), the limit of detection (LOD), accuracy, and the precision. For plotting the calibration curve, ΔF was drawn against the known concentrations of fentanyl. With a regression coefficient of 0.9909, a good linear relationship was obtained in the fentanyl concentration range of 0.01-2.0 μg mL^{-1} (inset of Figure 2). The LOD and LOQ reached 0.008 μg mL^{-1} and 0.02 μg mL^{-1} for the determination of fentanyl in EBC samples by employing 3 S_b/m and 10S_b/m (m: calibration slope; S_b: blank's the standard deviation), respectively. In this study, S_b was reached by measuring five blank samples. Additionally, the precision of the proposed approach was assessed by relative standard deviations (RSDs %) of replicated analysis of 1.0 μg mL^{-1} fentanyl on different days and also on the same day. The inter days and intra-day RSDs % were calculated as 2.5 % and 1.1 %, respectively representing respectable repeatability and reproducibility of the validated system for the determination of fentanyl. A comparison of the recent process with other methods reported in the literature is summarized in Table 1. Based on the results, the validated approach is comparable and respectable with other approaches for fentanyl detection.

Analytical application

For investigation of the method's accuracy, recovery

Table 1. Comparison of analytical characteristics of the presented method with other reported literature-based methods for fentanyl detection

Method	Sample	LOD (μg mL^{-1})	Linear range (μg mL^{-1})	Reference
Potentiometry	Ampoule	1.82	3.36-336	[21]
EC	-	0.0028	0.003-33.6	[22]
CSWV	-	3.3	3.3-67.3	[23]
DPV	Blood serum, urine and ampoule	0.10	0.33-20.1	[24]
Polarography	Ampoule	0.05	0.03-0.33	[25]
SWV	-	3.36	3.36-33.6	[26]
HPLC-UV	Plasma	0.8×10^{-3}	0.005-0.1	[27]
GC-MS	Breath sample	0.1×10^{-4}	$(0.5\text{-}8.0)\times10^{-4}$	[4]
CuS NPs-based Fluorometry	EBC	0.008	0.01-2.0	This work

EC, electrogenerated chemiluminescence; CSWV, cyclic square wave voltammetry; DPV, differential pulse voltammetry; HPLC, High performance liquid chromatography; GC-MS, gas chromatography–mass spectrometry; CuS NPs. copper sulfide nanoparticles; EBC, exhaled breath condensate; LOD, limit of detection.

Table 2. Determination of fentanyl in the spiked EBC samples

Sample	Added (μg mL^{-1})	Found ± SD (μg mL^{-1})[a]	Recovery (%)[b]
EBC			
	0.50	0.46 ± 0.11	92.28
	1.00	0.96 ± 0.02	94.27
	2.00	2.12 ± 0.03	106.45

[a] Mean of three determinations ± standard deviation
[b] Recovery (%) = [(Found–Base)/Added] × 100. "Found" and "Base" refer to the amount of the analyte in samples after and before spiking, respectively.

tests are performed by spiking the appropriate amount of fentanyl in EBC samples. As can be seen in Table 2, the recovery percentages were in the range of 92.28% to 106.45% signifying that the offered approach has good reliability for fentanyl detection in EBC samples.

Conclusion

In this investigation, CuS NPs have been designated for fentanyl analysis in the EBC samples. A spectrofluorometric method based on CuS NPs was considered and applied for fentanyl analysis in EBC samples. The system employed a selective interaction between CuS and fentanyl to deliver suitable specificity to fentanyl. Additionally, quick response time and the high sensitivity of the validated method made it an appropriate process for the determination of fentanyl in biological samples.

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Author Contributions

SM: Investigation; ZK: Data curation, Writing-review & editing. All authors have read and approval of the manuscript.

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Ethics Issues

Not applicable.

Conflict of Interest

There is no conflict of interests.

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