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Single-Walled Carbon Nanotube Network Electrodes for the Detection of Fentanyl Citrate

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ABSTRACT: We prepare disposable single-walled carbon nanotube network electrodes for the detection of the potent opioid fentanyl, currently a leading cause for opioid overdose deaths in the USA. We show repeatable dry transfer of single-walled carbon nanotube (SWCNT) networks to produce robust electrodes. This process directly produces highly conductive SWCNT electrodes without the need for any further modifications required for conventional carbon electrodes. The realized electrode showed low background currents combined with spontaneous enrichment of fentanyl, resulting in a high signal-to-noise ratio. With this electrode, a detection limit of 11 nM and a linear range of 0.01–1 μM were found for fentanyl. In addition, selectivity is demonstrated in the presence of several common interferents.

KEYWORDS: fentanyl citrate, SWCNT, DPV, voltammetry, electrochemical sensor

1. INTRODUCTION

Fentanyl (N-phenyl-N-[1-(2-phenylethyl)piperidinyl]-propanamide) is a highly potent synthetic μ-opioid receptor agonist, an analgesic widely used to treat acute and chronic severe pain. It has a fast onset and a short duration of action. As fentanyl has a very low bioavailability after oral administration,1 either transdermal or transmucosal administration is used. Unfortunately, also illicit drug use of fentanyl is rapidly increasing. According to a report by the Center for Disease Control and Prevention, fentanyl was involved in over half of the opioid-related overdose deaths.2 Thus, there is an acute and increasing need for methods for rapid and inexpensive screening of fentanyl.

Because of its high potency, 50–100 times higher than that of morphine,3 the therapeutic plasma concentrations of fentanyl are extremely low.4 Even in cases of fatal acute poisoning, concentrations remain in the range of 100 nM.5−6 Currently, determination of fentanyl requires specialized and highly sensitive equipment such as gas chromatography−mass spectrometry (GC-MS) or liquid chromatography−mass spectrometry (LC-MS). These methods are, however, usually laborious, time-consuming, expensive, and confined to specialized laboratories and are therefore poorly suited for point-of-care testing. In addition, routine immunoassay screens will not detect synthetic opioids, such as fentanyl, that have little structural similarity to morphine and other commonly tested opioids.7 In contrast, electrochemical detection is relatively rapid and simple and does not require complex sample processing.8,9 Cathodic adsorptive stripping voltammetry has previously been used to detect fentanyl.10 However, the limited water window, the workable potential window between which water is neither oxidized nor reduced, of most novel high surface area materials limits the applicability of this method. Ahmar et al.11 have also reported the detection of the synthetic fentanyl analogue sufentanil through anodic oxidation. They used liquid extraction into an organic phase to achieve high sensitivity, and therefore the method requires the use of organic solvents and more complex instrumentation. Recently, carbon-based electrodes have been used to detect fentanyl for screening illicit substances with modified screen-printed electrodes.12,13

In recent years, single-walled carbon nanotubes (SWCNT) have attracted attention in electrochemical sensing due to their favorable properties, including high electrical conductivity, possible electrocatalytic properties, and high surface area.14 SWCNTs have been widely used to detect drugs and biomolecules. Moreover, large areas of highly conductive SWCNT networks can be grown and patterned to produce electrodes without the need for modification of conventional electrodes.15–17 In this process shown in Scheme 1, SWCNTs are synthesized by means of aerosol chemical vapor deposition.
interactions can facilitate the adsorption of various molecules.

The glass was precleaved into 1 cm x 1 cm x 1 mm dimensions of 17 µm - 25.5 µm. The hydrophobicity of SWCNT networks is used to enhance the adsorption due to the large entropic driving force.

To the best of our knowledge, electrochemical detection of fentanyl at SWCNT electrodes has not been previously reported. Moreover, electrochemical detection of fentanyl at close to physiologically relevant concentrations has not been reported. In the current work, SWCNT network electrodes are fabricated with a simple and fast procedure. After thorough characterization, we demonstrate spontaneous enrichment and highly sensitive detection of fentanyl.

### 2. EXPERIMENTAL SECTION

#### 2.1. Single-Walled Carbon Nanotube Synthesis

SWCNTs were synthesized by high-temperature floating catalyst chemical vapor deposition (CVD). The process is described in greater detail in refs 15, 23, and 24. Briefly, in this process, iron nanoparticles are formed through thermal decomposition of an iron-containing precursor in a carbon monoxide atmosphere. The iron nanoparticles catalyze the decomposition of carbon monoxide, leading to nucleation and growth of SWCNT in the gas phase inside a quartz laminar flow reactor. The formed SWCNTs form bundles in the gas phase19 and are collected on a 44 µm nitrocelulose filters, resulting in a SWCNT network with dimensions of 17 × 25.5 cm².

#### 2.2. Electrode Fabrication

The SWCNT network was press-transferred onto glass (Thermo Scientific, ISO 8037-1) and densified with a drop of ethanol (99.5 wt %, Alfa, Finland). Prior to transfer, the glass was precleaned into 1 × 2 cm² pieces and cleaned by sonication in acetone (Analar NORMAPUR, Merck). The dry transfer process is described in detail in refs 15 and 26. Briefly, the SWCNT networks were cut and placed on the glass pieces with the SWCNT side down and pressed between two glass slides. After carefully peeling off the filter backing, the films adhered SWCNT network with a few drops of ethanol. After drying for 2 h, the electrode was covered with a PTFE film (Saint-Gobain Performance Plastics CHR 2255-2) with a prepunched 3 mm hole.

#### 2.3. Characterization

Visible Raman spectroscopy was performed with a LabRAM HR (Jobin Yvon Horiba) confocal Raman system. An argon laser with a wavelength of 514 nm (power 10 mW) and a BX41 (Olympus) microscope with a 100x objective (spot size of <1 µm) was used. For optical characterization, the SWCNT network was press-transferred directly onto transparent quartz slides (HQ5300, Heraeus). The optical absorption spectra and transmittance were measured with an Agilent Cary 5000 UV–vis–NIR spectrometer (Agilent Technologies, Inc.). Prior to UV–vis analysis, the samples were baked on a hot plate set to 250 °C for 2 h to desorb atmospheric dopants and reveal the intrinsic optical properties of the SWCNT network. For transmission electron microscopy (TEM) investigations SWCNT networks were press-transferred directly onto S147AH Au TEM grids with holey carbon films (Agar Scientific). The TEM study was performed with a JEOL 2200FS double-aberration-corrected electron microscope (JEOL, Japan), which is equipped with a X-ray EDS detector and a Gatan 4k UltraScan 4000 CCD camera. The microscope was operated at 200 kV with a field-emission electron gun.

The samples used in X-ray photoelectron spectroscopy (XPS) and X-ray absorption spectroscopy (XAS) were separately press transferred on highly boron-doped (<0.005 Ω cm) (100) Si pieces (Siegert Wafer, Germany). The XPS measurements were performed with an Axis Ultra electron spectrometer (Kratos Analytical, Manchester, UK) with monochromatic Al Kα irradiation at 100 W under neutralization. Pre-evacuation was performed overnight before the measurements. Survey and high-resolution spectra of C 1s, O 1s, and Fe 2p were recorded at 3–4 locations for each sample. Elemental quantitation was performed with CasaXPS software. Filter paper (Whatman) was used as an in situ reference for charge correction.

The X-ray absorption spectra were acquired at the Stanford Synchrotron Radiation Lightsource (SSRL), beamline 8-2, with a 55° beam incidence angle (magic angle) at which the absorption intensity does not depend on the molecule orientation. Beamline 8-2 uses a bending magnet and is further equipped with a spherical grating monochromator, operated using 40 × 40 µm slits corresponding to a resolution of around 200 eV. The spot size at the interaction point was around 1 x 1 mm² with a flux of 1E10 photons/s. With this flux, the beam damage is not noticeable even for extended exposure. The X-ray energy for the C 1s, O 1s, and Fe 2p edges was scanned from 260 to 350 eV, 520 to 580 eV, and 695 to 735 eV, respectively. The data were collected in total electron yield (TEY) mode by using the drain current amplified by a Keithley picoammeter. The incoming flux was recorded using a nickel grid with Au sputtered film.

#### 2.4. Electrochemistry

The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed with a CH Instruments (CHI630E) potentiostat. A three-electrode cell with a Ag/AgCl reference electrode (+0.199 V vs SHE; Radiometer Analytical) and a Pt wire as counter electrode was used. Fentanyl citrate ampules (50 µg/mL, Hameln) were purchased from the University Pharmacy, Helsinki, Finland. Each 2 mL ampule contains the equivalent of 100 µg of fentanyl as fentanyl citrate. All other chemicals were obtained from Sigma-Aldrich. Fresh stock solutions were prepared every measurement day by diluting in pH 7.4 phosphate buffered saline (PBS) solution to achieve a concentration of 30 µM fentanyl. Henceforth, the concentrations will be given in molar concentrations of fentanyl. Different concentrations of fentanyl citrate solution were obtained by injecting the stock solution into the cell with 20 mL of PBS. Similarly, solutions with different interferents were prepared in pH 7.4 PBS. For the interference studies fentanyl citrate solution was injected into the cell. All DPV measurements were conducted with a pulse amplitude of 50 mV and a scan rate of 20 mV/s, and all accumulation of analyte was performed under open circuit conditions. Prior to all measurements, the solutions were purged with
nitrogen for at least 15 min to remove any dissolved oxygen. The cell was blanketed throughout the measurements.

3. RESULTS AND DISCUSSION

3.1. Characterization. The Raman spectrum of the SWCNT network is shown in Figure 1A. The SWCNT sample shows the characteristic features for SWCNT. These modes include the first-order single-resonance radial breathing modes (RBM) and the multiplex feature G mode around 1580 cm$^{-1}$. In addition, several harmonics and combination modes, including iTOLA, M, and G' bands as well as a weak disorder-induced D band, are also observed around 1315 cm$^{-1}$. Peak fitting revealed an $I_D/I_G$ ratio of 0.102 ± 0.003. The small intensity of the D band indicates low presence of amorphous carbon and a small number of defects. Previous work has shown that the SWCNT network is a mixture of metallic and semiconducting SWCNT. The inset in Figure 1A shows a magnification of the RBM region, with peaks corresponding to SWCNT diameters in the range of ~1.2–2.1 nm. Under ambient conditions SWCNT can adsorb oxygen and moisture, causing p-doping. Doping of the SWCNT films can improve the conductivity and suppress optical transition in van Hove singularities by CNT Fermi level shifts. It has been shown that baking at relatively low temperatures can desorb these atmospheric dopants and reveal the intrinsic optical properties of the SWCNT network. After some time under ambient conditions the SWCNT is redoped, and the electrical and optical properties return to their original values. Figure 1B shows the optical absorption spectra for the pristine (ambient stored) and baked SWCNT networks. After 2 h baking at 250 °C, the intensity of the absorption peak S11 is significantly increased, indicating dedoping of atmospheric dopants. The baking also caused an increase in the sheet resistance from 88 to 380 Ω/sq of the SWCNT film with a transmittance of 86.6%. A mean diameter of the SWCNT of 2.1 nm was calculated based on the absorption spectra and Kataura plot. These results are in good agreement with the Raman spectra.

Figure 1C shows an overview bright-field TEM micrograph of the SWCNT network where the catalyst particles appear in black. This assignment was confirmed by TEM EDS analysis, where iron and carbon were detected in spots on the catalyst particles (Supporting Information Figure S1). In contrast, on the SWCNT bundles only carbon was detected. Figure 1D shows a high-resolution TEM micrograph where both individual SWCNTs and carbon nanotube bundles are clearly displayed. Both smaller 5 nm iron catalyst particles and larger particles are observed. More detailed HR TEM micrographs of the catalyst nanoparticles are shown in Figures S2 and S3. These TEM micrographs show that the catalyst particles are encapsulated by a few layers of carbon. The fast Fourier transform of the micrographs shown in the Supporting Information reveals $d$-spacings corresponding to those of iron carbide (cementite, Fe$_3$C). It has been previously shown...
that Fe₃C particles can be formed during the growth process after the decomposition of ferrous catalyst and that CNTs can grow from these structurally fluctuating Fe carbide particles. 31 The larger ∼10 nm particles show similar d-spacings corresponding to iron carbide. The crystal structures of the catalyst particles were further studied by selected area electron diffraction (SAED) analysis in three randomly selected spots (see Figures S4 and S5). The electron diffraction images confirm the presence of Fe₃C. In addition, diffraction spots that could be explained by the presence of metallic Fe were also observed.

XPS survey scans were conducted to further study the elemental composition of the SWCNT networks. The XPS wide spectrum shown in Figure 2A indicates the presence of 71.7 ± 0.2 at. % carbon, 8.7 ± 0.2 at. % oxygen, and 0.1 ± 0.01 at. % iron. In addition, 19.5 ± 0.3 at. % Si was found in the XPS analysis. The Si and most of the oxygen are likely due to the partially exposed Si wafer with native oxide, as previous work has shown that SWCNT networks with similar thickness do not fully cover the Si wafer surface. 32 This is further supported by the low amount of oxygen detected in the EDS scans (see Figure S1). The large oxygen content found in the XPS analysis could, however, also be partially explained by a higher surface loading of oxygen on the SWCNT network.

More detailed studies of the surface chemistry were performed with high-resolution X-ray photoelectron spectroscopy and X-ray absorption spectroscopy. The XAS spectra are shown in Figure 2, and the peaks were assigned based on extensive literature. 33,34,43–42 The XPS C 1s, O 1s, and Fe 2p spectra are shown in Figure S6. A sharp peak in the XPS C 1s spectrum at 284.4 eV can be observed due to the sp² carbon.

The peak shape is indicative of a highly ordered low defect density SWCNT film. The XAS C 1s shows two prominent bands attributed to unoccupied π* states (284.9 eV) and sp² carbon long-range order and is consistent with previously reported XAS spectra for SWCNT. 33,34 These peaks indicate sp²-rich material with a clear long-range order. Moreover, the C 1s XAS spectrum clearly shows the presence of both ketone/aldehyde (287.5 eV) and carboxyl (288.8 eV) groups, despite a low oxygen content, as determined by TEM EDS. Because of the high surface loading of silicon oxide, the C–O bonding is convoluted in the XPS O 1s spectrum (see Figure S6). Peaks previously attributed to oxygen-containing functionalities can, however, be resolved in the O 1s XAS spectrum. Similarly, the contribution of C–O bonds and iron oxides in the O 1s XAS spectrum are obscured by the silicon wafer native oxide (535–570 eV).

Because of the low surface loading of iron, the XPS is barely able to resolve the Fe (see the Supporting Information). Nevertheless, the Fe 2p high-resolution spectrum indicates the presence of iron and oxidized species. The peak at 707 eV could also be explained by presence of iron carbide. 44,45 This is further supported by the lack of oxygen in the EDS spectra acquired on the catalyst particles (see Figure S1). Moreover, the Fe 2p XAS spectrum very closely matches those previously observed for iron carbides. 45 These results are further supported by the TEM analysis, showing the presence of iron carbides. Nevertheless, it should be noted that the spectrum could also arise from a mixture of metallic and oxidized iron species. 35,36 The low surface loading of iron and the convolution of the O 1s spectra make unequivocal identification of the oxidation state of Fe challenging. While
all catalyst particles imaged by TEM appear to be iron carbide, the network likely consists of a mixture of iron and iron carbide. Based on these results, however, the local presence of noncrystalline surface iron oxides can also not be completely ruled out.

3.2. Electrochemical Measurements.

3.2.1. Cyclic Voltammetry. SWCNT network electrodes on insulating substrates have previously been shown to have small background currents and high signal-to-noise ratios in electrochemical analysis. For this reason, the apparent double-layer capacitance was determined by measuring the charging current with CV (see Figure S7 and Table S1). An apparent double-layer capacitance ($C_{dl}$) of 10.3 ± 1.0 μF/cm² was obtained. While this background current is considerably larger than that observed by Bertoncello et al. for a similar SWCNT network electrode, double-layer capacitances of 33 ± 6 μF/cm² have been reported by McDermott et al. for glassy carbon. Moreover, the obtained double-layer capacitance is comparable to electrodes known for low $C_{dl}$ values, such as boron-doped diamond and ultrasmooth amorphous carbon thin film electrodes.

Figure 3A shows the cyclic voltammogram of 15 μM fentanyl recorded with different scan rates, and Figure 3B shows the oxidation currents as a function of the scan rate. From the figures it is evident that the oxidation of fentanyl citrate gives two oxidation peaks at 866 and 967 mV, respectively (100 mV/s). The separation between the two oxidation peaks remains relatively constant at 88 ± 7 mV at all scan rates above 100 mV/s. At scan rates below 100 mV/s, however, the second oxidation peak can no longer be clearly observed as it appears as a shoulder of the main peak. The oxidation peak potentials can be seen to increase with increasing scan rate (see Figure S8), finally reaching values of 1.033 and 1.139 V, respectively, at the scan rate of 10 V/s.

Figure 3. Cyclic voltammetry of 15 μM fentanyl in PBS (pH 7.4). (A) Effect of scan rate and (B) the oxidation peak currents as a function of the scan rate. The inset in (A) shows a cyclic voltammogram of the supporting electrolyte (dashed line) and 15 μM fentanyl (solid line) with a scan rate of 100 mV/s. The error bars in (B) show the standard deviation of three electrodes.

Figure 4. (A) Differential pulse voltammetry of 1 μM FEN in phosphate buffer with pH 11 to 3. (B) Oxidation currents and (C) oxidation potential as a function of pH. Accumulation time: 300 s.
This is consistent with early work by Masui et al.\textsuperscript{50} postulating the abstraction of an electron from the lone pair of electrons from the amino nitrogen as the first and the rate-limiting step of the anodic oxidation. The proposed oxidation mechanism for aliphatic tertiary amines provides a secondary amine and an aldehyde. The second peak is likely due to the further oxidation of a secondary amine.\textsuperscript{50} The overall reaction was found to be irreversible even at the scan rate of 10 V/s, consistent with previous studies.\textsuperscript{12,50} A separate experiment also confirmed that citrate alone gives no oxidation peaks even at concentrations as high as 10 mM (not shown). After the first scan, a new redox couple with a peak potential separation of 28.3 mV and a formal potential of 69.9 mV emerges (100 mV/s). The peak potential separation increases only to 41 mV with a scan rate of 10 V/s, implying a quite reversible two-electron process. In DPV experiments with 15 μM fentanyl the oxidation peak related to this redox couple intensifies with increasing scan number and is likely due to an aldehyde that forms as a reaction product. At the same time a 26% loss of signal was observed over 10 DPV scans (see Figure S9). In addition, two further oxidation peaks related to reaction products could be observed in DPV scans around 165 and 338 mV.

As seen from Figure 3B, the oxidation current scales linearly with the scan rate, indicating an adsorption controlled process. Similarly, Figure S10 indicated adsorption controlled oxidation also at scan rates ranging from 5 to 100 mV/s. The hydrophobic nature of the relatively defect free SWCNT sidewall and π–π electron interactions likely strengthens this effect. It should be noted, however, that similar effects can also be caused by thin layer diffusion at porous electrodes.\textsuperscript{51} Therefore, further research is required to determine if the observed behavior is caused by thin-layer diffusion or an adsorption-controlled oxidation process.

3.2.2. Effect of pH and Accumulation Time. Figure 4 shows the DPV scans of 1 μM fentanyl in phosphate buffer with varying pH. The peak potential shifts as a function of the pH, indicating a proton coupled oxidation process. Figure 4B shows a slope of −0.085 V/pH, indicating transfer of an uneven amount of protons and electrons. At pH 9 and above the oxidation potential shows only a very weak dependence on the pH, and thus the oxidation is no longer proton coupled. This suggests deprotonation of the tertiary amine and a pK_a slightly above 8. These results are in good agreement with previously reported pK_a values for fentanyl at room temperature.\textsuperscript{52} Furthermore, they support the previously suggested oxidation mechanism for aliphatic tertiary amines, where the transfer of two electrons occurs in two separate steps, separated by proton transfer and followed by rapid reaction with water.\textsuperscript{50} At pH 4 and below only a very weak peak was observed for the oxidation of fentanyl. A DPV scan in a wider window at pH 3 reveals a collection of new peaks around −150, 15, and 390 mV, likely due to decomposition products of fentanyl. Fentanyl has previously been found to rapidly degrade into several reaction products in acids.\textsuperscript{53,54} The chemistry of fentanyl is complex, and several degradation products have been identified after chemical oxidation. More studies are required to fully understand the oxidation mechanism of fentanyl. The oxidation current shown in Figure 4C was also found to be dependent on the pH, and the highest sensitivity was found at pH 8. Similar oxidation currents were obtained in the PBS solution with pH 7.4, and therefore all further measurements were performed in PBS solution.

To further study the adsorption behavior, an accumulation study was carried out. Figure 5 shows the effect of accumulation time with 1 μM fentanyl in PBS solution under open circuit conditions. A linear increase in the oxidation current was found in the time interval of 30–240 s. The maximum oxidation current was obtained after 300 s of accumulation, after which the current saturated and no further enrichment was observed. Relatively large variation in the oxidation currents was observed with accumulation times shorter than 300 s due to different rates of accumulation of analytes for each of the measured three electrodes. After 300 s accumulation, however, a relative standard deviation of 5.7% was observed, indicating that despite the slightly different rates of accumulation, the saturated signal gives adequate repeatability. This spontaneous enrichment of fentanyl further indicates strong adhesion of fentanyl on the SWCNT surface, likely due to hydrophobic and π–π electron interactions. Because of the positive charge of fentanyl at neutral pH, electrostatic interactions may also influence the adsorption process. Further research is required to fully understand the adsorption mechanism and the enrichment of fentanyl at the SWCNT electrode.

3.2.3. Determination of Fentanyl Citrate. DPV measurements of concentrations of fentanyl are shown in Figure 6A, and Figure 6B show the linearization of the currents...
for measurements with four different electrodes. Under the optimized DPV conditions, the peak current had a linear relationship ($R^2 = 0.9931$, total sum of squares = 0.0566) with the concentration of fentanyl citrate in the range from 10 nM to 1 μM. At higher concentrations the current deviated from the linear calibration curve. The current at the 1 μM concentration is close to the highest peak current observed in the enrichment study in Figure 5, suggesting that the deviation from the linear relationship may be due to saturation of the electrode surface. The standard deviation of the blank signal together with the sensitivity was used to calculate the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated as LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the blank signal and S the sensitivity, giving LOD and LOQ of 11 and 36 nM, respectively. Based on this a linear dynamic range of 36 nM–1 μM was found.

Table 1 gives a list of reported methods and materials for electrochemical detection of fentanyl. As can be seen, the detection limits of this work are substantially lower than those obtained with various other methods with comparable instrumentation. The more complicated cathodic stripping voltammetry method using a dropping mercury electrode also showed a slightly higher detection limit and comparable linear range. Furthermore, no interference studies were performed, and a longer preconcentration time of 10 min with negative biasing of the working electrode was required to achieve optimal sensitivity. In contrast, the SWCNT electrode of this

Table 1. Direct Electrochemical Detection of Fentanyl and Analytical Performance (Physiologically Relevant Concentration Range 1–100 nM)

| method                | electrode material                                      | detection limit (μM) | linear range (μM) | ref |
|-----------------------|----------------------------------------------------------|----------------------|-------------------|-----|
| potentiometry         | PVC fentanyl phosphotungstate membrane electrode         | 5.43                 | 10–10000          | 55  |
| cathodic stripping voltammetry | dropping mercury electrode                        | 0.05                 | 0.1–1             | 10  |
| cyclic square-wave voltammetry | IL-modified screen-printed carbon electrode       | 5                    | 5–105             | 12  |
| square-wave voltammetry          | IL-MWCNT-modified SPCE                       | 10                   | 10–100            | 13  |
| differential pulse voltammetry     | SWCNT                                   | 0.011                | 0.01–1            | this work |

Figure 6. Differential pulse voltammetry of increasing concentrations of fentanyl in pH 7.4 PBS: (A) increasing concentrations of fentanyl and (B) calibration curve. The error bars show the standard deviation of four electrodes.

Figure 7. Interference study. (A) DPVs of interferents without addition of fentanyl (dashed line) and mixture of interferents and fentanyl (solid line). (B) Sensitivities of fentanyl in the range 0.1–1 μM. (C) Background subtracted oxidation currents of 500 nM fentanyl with and without interferent. The vertical lines in (B) and (C) show the standard deviation of (B) the sensitivity and (C) the oxidation current of fentanyl for four electrodes.
work showed faster spontaneous accumulation under OCP conditions.

3.2.4. Repeatability and Stability. At high concentrations of 15 μM, relatively rapid passivation of the electrode was found (not shown). Similarly, very rapid passivation of an ionic liquid modified screen-printed carbon electrode was observed by Goodchild et al.12 in 50 μM fentanyl. It has previously been shown that the radical cations formed during the electrochemical oxidation of aliphatic amines can covalently attach to carbon electrode surfaces.6 For these reasons, a passivation study was performed with a concentration within the linear range of fentanyl. In this study, DPVs were recorded in PBS with 500 nM fentanyl with 5 min accumulation between scans. After 12 scans no loss of signal was observed, and the relative standard variation (RSD) of the measurements was 1.5%, confirming that no rapid passivation occurs at concentrations closer to the physiologically relevant concentration range. The repeatability was assessed according to the ICH guidelines57 with three electrodes at three different concentrations. At concentrations of 100, 250, and 500 nM relative standard deviations of 4.5, 6.5, and 7.4%, respectively, were obtained. Despite this adequate repeatability, the larger RSD compared to consecutive single electrode determinations shows that there is electrode to electrode variation. The stability of the electrode was also studied by measuring an electrode stored for 30 days prior to measurement in 1 μM fentanyl. The same signal was achieved with this electrode as for a fresh electrode, indicating good stability during storage.

3.2.5. Interference Studies. To study the selectivity, fentanyl citrate was measured in the presence of several common interferents, including 5 mM glucose, 1 mM citrate, 250 μM ibuprofen, 250 μM ascorbic acid, 250 μM uric acid, and 100 μM paracetamol (acetaminophen). Fentanyl citrate was injected into the cell to achieve concentrations within the dynamic linear range starting from 100 nM. Figure 7A shows DPV measurements in PBS solution with 500 nM fentanyl and with fentanyl together with each interferent. Each measurement was performed with a separate electrode. The dashed lines denote the signal in PBS with only the interferent for each electrode. For fentanyl alone the dashed line denotes the PBS background and solid line 500 nM fentanyl in PBS. The sensitivity toward fentanyl with and without each interferent is shown in Figure 7B while the background subtracted oxidation currents are shown in Figure 7C. It can be seen that all measurements are within the standard deviation as measured with four electrodes in 500 nM fentanyl in PBS. Thus, none of the measured interferents cause interference at the measured concentrations.

4. CONCLUSIONS

The paper shows highly sensitive electrochemical detection of fentanyl with differential pulse voltammetry. We show that SWCNT network electrodes with high purity and low defect density spontaneously enrich fentanyl under open circuit conditions, enabling high sensitivity. These results present a simple electroanalytical method for quantitative detection of fentanyl citrate with detection limits and linear range in physiologically relevant concentrations. Moreover, selectivity is achieved in the presence of various common interferents. Despite these interesting results, further validation of the proposed method is required to show the applicability in real applications.

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**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsanm.9b01951.

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