Economical Voltammetric Sensor for Sensitive Rapid Determination of Ondansetron in the Presence of Opioid Antagonist Naltrexone

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Abstract
The electrochemical actions of ondansetron (OND) were investigated on the modified electrode with Polyvinyl Pyrrolidone (PVP) and multi-walled carbon nanotube (MWCNTs). The oxidation peak current was enhanced to be doubled when compared to the bare electrode as a result of the synergistic effect of nanomaterial and cation-exchange polymer on the electron transfer rate. Following optimization of the experimental conditions, the Square Wave Voltammograms (SWV) method was employed to determine ondansetron in pharmaceutical formulations. Moreover, the modified electrode MWCNTs/PVP/CPE displayed high selectivity in the voltammetric measurements of ondansetron and co-administrated opioid antagonist drug naltrexone (NAL) with strong potential separation (422 mV). The response peak currents had a linear relationship with drug concentration in the range of (2.00–200.0 nmolL⁻¹) with detection limits 430 pmolL⁻¹ and 456 pmolL⁻¹ for ondansetron and naltrexone, respectively. The electrode was successfully used to detect both medications electrochemically in human plasma samples.

Keywords Electrochemical sensor · Polyvinyl pyrrolidone · Multi-walled carbon nanotube · Ondansetron · Naltrexone · Plasma

Introduction
Cancer is the second leading cause of mortality in the world after cardiovascular disease; ondansetron (OND) is a selective 5-HT3 receptor antagonist commonly used in the treatment of emetogenic side effects associated with cancer chemotherapy or radiotherapy. It was also the first-line therapy for preventing nausea and vomiting after surgery [1]. Moreover, OND is used to alleviate irritable bowel syndrome, pruritus, alcohol dependence, anxiety, sleep disturbances, opiate withdrawal syndrome, and vertigo [2]. Ondansetron also has a beneficial impact when co-administered with another drugs, such as naltrexone (NAL), since the combination of OND and NAL is more effective in reducing ethanol intake than either medication alone [3].

Naltrexone is a type of opiate drug selected for the short-term management of post-surgical, acute pain, and the relief of mild to extreme chronic pain in a cancer patient. Naltrexone is chemically similar to naloxone but has greater oral bioavailability and a longer time of action. NAL is one of the first drugs used in the treatment of opioid overdoses, dependency, and later in the treatment of alcoholism [4].

Different analytical techniques have been described for the determination of OND which include, reported by the British Pharmacopoeia, non-aqueous titrimetric and HPLC methods [5] spectrophotometry [6–9], spectrophuorimetry [10], potentiometry [11], voltammetry [12], radioimmunoassay [13], flow injection [14, 15], HPTLC [16, 17], HPLC [18–21], and (LC–MS/MS) method [22, 23].
In comparison to the other methods, voltammetry offers numerous benefits in the pharmaceutical analysis, being fast, inexpensive, low energy consumption, fast and no needing sample preparation, adaptable to tiny sample volumes, high performance, and large quantitation range [24–26]. In our suggested method, we modified a carbon paste electrode (CPE) to overcome the disadvantages of the bare ones such as low sensitivity, slow electron transfer, and lack of reproducibility, using an ionic liquid [27], multi-walled carbon nanotube, nanomaterial [28], transition metal compound [29], and polymer film [30].

Multi-walled carbon nanotubes (MWCNTs) took a great attention in recent years [31, 32], as it promotes effective electronic transfer. These distinguishing characteristics are due to their large surface area, small over-voltage, high electrical conductivity, and enhanced electrode kinetics which in turn lead to low detection limits and quick reactions. Polyvinyl Pyrollidone (PVP) may act as a surface stabilizer, a nanoparticle dispersant and a binder in many pharmaceutical tablets [33]. PVP is a bulky, non-toxic [34], non-ionic [35] C = O polymer besides containing CH₂ and C–N functional classes [36] which are commonly used in nanoparticle (NP) synthesis.

To date, no voltammetric technique for determining OND and NAL in human plasma samples has been published in the literature; the method is the first one to use PVP in sensor fabrication, so the modified electrode MWCNTs/PVP/CPE was effectively utilized as an electrochemical sensor for determining OND in the presence of NAL at trace levels in biological samples. Thus, the goal of our work is to advance, examine, and enhance the experimental conditions for the determination of OND in different matrices via new sensor MWCNTs/PVP/CPE as a fast and sensitive method.

**Experimental**

**Materials and Reagents**

Ondansetron was purchased from Sigma-Aldrich (St. Louis, MO, USA); its purity was certified to be 99.88%. NAL was obtained from Te mad Pharmaceutical (Tehran, Iran); its purity was certified to be 99.75%. A “Zofran” tablet (batch no: B74319C) claimed to contain 8.0 mg OND was purchased from the local market. Samples of plasma were gifted by Vacsera Co. (Giza, Egypt).

As supporting electrolyte, Britton-Robinson (BR) buffer was prepared by mixing a solution of 0.04 molL⁻¹ phosphoric acid (Sigma-Aldrich), 0.04 molL⁻¹ acetic acid (Loba Chime Co., India), and 0.04 molL⁻¹ boric acid (El-Nasr pharmaceutical Company, Cairo, Egypt). Buffer solutions have been adapted to the acceptable quantity. Buffer solutions have been adjusted by using acceptable quantity of 0.2 molL⁻¹ sodium hydroxide (Win lab, Leicestershire, U.K.) to reach the preferred pH. Phosphate buffer was prepared by mixing monosodium and disodium phosphate salts in proper ratios forming buffer concentrations ranging from 8.0 to 100.0 mM and pH values ranging from 5.7 to 8.0. Graphite powder, paraffin oil, MWCNTs, and PVP were purchased from Sigma-Aldrich (Steinem, Germany). A Milli-Q ultrapure water (Millipore, Bradford, USA) was used to prepare normal solutions and buffers.

**Apparatus**

The electrochemical workstation Biologic SP150 was used for all voltammetric measurements. As an auxiliary electrode, a platinum wire (BAS, USA) was used. All cell potentials were assessed with an Ag/AgCl reference electrode (3.0 mol L⁻¹KCl) (BAS, USA). A glass cell (5.0 mL) was used for pH measurements, and a JENWAY 3510 pH meter (Staffordshire, England) with a single-rod measuring chain constructed of glass was used. All electrochemical tests were carried out at room temperature of 25 °C. A JEM 1400 electron microscope was used to perform transmission electron microscopy (TEM) observations (Japan Electro Company).

**Stock and Working Standard Solutions of Drugs**

**Standard Solutions of OND**

The stock solution of OND (1.0 × 10⁻³ molL⁻¹) was prepared in volumetric flask 50.0 mL by dissolving 14.7 mg OND in distilled water and the volume was brought up to the mark using the same solvent. Working solutions of OND (1.00–100.0 µM) and (2.00–700 nM) were made by dilution of the stock solution with distilled water to the required concentration.

**Standard Solutions of NAL**

Working solutions (1.0 × 10⁻⁴–1.0 × 10⁻⁶ molL⁻¹) were made by diluting the stock solution with distilled water.

**Working Electrode Preparation**

MWCNTs/PVP/CPE modified electrode was formed by hand-blending of 1.0% of MWCNTs and 0.5% PVP with 98.5% graphite powder. Then the cocktail was blended with an appropriate quantity of paraffin oil in a glassy mortar till a homogeneously whetted paste was formed. A portion of the paste was pressed into the hollow of the electrode frame and
flattened on a clear-out paper until having a glossy appearance without contacting the surface.

**Construction of Calibration Curve**

To analyze OND, the Square Wave Voltammetry (SWV) method was utilized. Aliquots equal to 1.00–100.0 µM and 2.00–700 nM OND were transferred with a micro-pipette into a 5.0-mL volumetric flask and complete to the mark with phosphate buffer pH = 8.0. Voltammetric analyses were conducted and voltammograms of square waves were reported at MWCNTs/PVP/CPE. Calibration graphs were created by plotting the anodic peak current against a final molar concentration of the drug.

**Determination of OND in Pharmaceutical Preparation**

Ten tablets from Zofran were weighted, finely powdered, and thoroughly combined well. A quantity of the finely ground powder equivalent to two tablets was accurately weighed and dispersed in water in a 100.0-mL volumetric flask. The solution was sonicated for 15 min to ensure that the active component was completely extracted. After sonication, the sample was filtered using 0.45 µm Acrodisc GHP filters (Gellman, Ann Arbor, USA). An aliquot of the filtrate was transferred into a calibrated flask and diluted with water to produce a final claimed concentration of (1.0 × 10⁻⁴ molL⁻¹) OND. Dilutions were prepared with deionized water to cover the working concentration range. The voltammetric measurement was performed as described above, and the amount of OND in the pharmaceutical preparations using the standard addition technique. Aliquots of the OND standard solutions equivalent to (10, 20, and 40 µM) were added to fixed concentration of OND (20 µM) for recovery studies.

**Analysis of OND in Biological Sample (Plasma)**

Half a milliliter of untreated human plasma samples (collected from the Holding Organization for Biological Products and Vaccines (VACSERA, Egypt)) were mixed with 1.0 mL of OND (1.0 mmolL⁻¹), and 3.5 mL of acetonitrile was added into 10.0 mL centrifugal tube to precipitate plasma protein. A total of 1.0 mL of the supernatant was transferred into a volumetric flask of 5 mL and complete the volume with deionized water. Different concentrations equivalent to 2.00–700.0 nM were used to construct a calibration curve.

**Determination of OND in Presence of NAL in Biological Sample (Plasma)**

Aliquots of 0.5 mL of untreated human plasma samples were mixed with 0.5 mL of OND (1.0 × 10⁻³), and 0.5 mL of NAL (1.0 × 10⁻³) then 3.5 mL of acetonitrile were applied to the 10.0-mL centrifugal tube to precipitate plasma protein. A total of 1.0 mL of the supernatant was mixed with deionized water to achieve a concentration (0.1 mmolL⁻¹). Different concentrations equivalent to 2.0–200.0 nM were used to make a calibration curve. The calibration curve was developed by plotting the peak current (Iₚ) against drug concentrations (molL⁻¹).

**Results and Discussion**

**Characterization of the MWCNTs/PVP/CPE**

The characterization and morphological structure of PVP/CPE and MWCNTs/ PVP/CPE was investigated. The TEM images of PVP/CPE (Fig. 1a) showed the spherical...
nano-particle distribution yielding faceted nanoparticles in the hundreds of nanometers and the selective area electron diffraction (SAED) was detected. Figure 1b shows dark sheets of distributed amorphous-like tubes and the homogeneous porous network structure of the nanocomposite.

The electroactive area of the electrodes was measured using Cyclic Voltammetry (CV) via a solution of 5.0 mmolL⁻¹ K₃Fe(CN)₆ in 0.1 molL⁻¹ KCl. By recording the current at different scan rates, the electroactive area was calculated using the Randel’s–Sevcı́ equation for a reversible process [37].

\[ I_p = 2.65 \times 10^5 \frac{n^{2/3}}{v^{1/2}} \frac{A^{1/2}}{D^{1/2}} \frac{C^{1/2}}{v^{1/2}} \]  

Where \( I_p \) is the peak current, \( n \) is the number of electrons transferred in the electrochemical process, \( A \) is the electrode area, \( D \) is the diffusion coefficient, \( C \) is the redox probe concentration, and \( v \) is the applied voltammetric scan rate. The used diffusion coefficient was \( 7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1} \). The electroactive area was calculated from the slope upon plotting \( I_p \) versus \( v^{1/2} \). The calculated areas were found to be 0.0493 and 0.136 cm² for PVP/CPE and MWCNTs/PVP/CPE, respectively. This validates the MWCNTs/PVP/CPE having a larger electrode surface area, as illustrated in Fig. 1.

**Electrochemistry of OND**

At a scan rate of 100 mV s⁻¹, SWV demonstrated an anodic oxidation peak for \( 1.0 \times 10^{-4} \text{ molL}^{-1} \) OND in phosphate buffer pH = 8.0 at bare CPE, MWCNTs, PVP, and MWCNTs/PVP/CPE as seen in Fig. 2.

The oxidation peak current was established to be 12.1 A for OND at bare CPE. Upon using 1% of MWCNTs and 0.5% of PVP, the current was increased to be 17.15 A and 20.02 A, respectively, while the current was increased to double to be 23.5 A when a mixture of MWCNTs and of PVP for OND at MWCNTs/PVP/CPE was used, showing that the composite has high electron transfer kinetics compared to the other bare one and accelerates electron transport at the electrode surface. The influence of PVP concentrations on the method’s sensitivity was investigated. PVP/CPE immobilized in electrode matrix at concentrations ranging from 0.25 to 1.0%. The anodic peak current was increased up to a concentration 0.5% PVP. Upon using high concentration, the peak was deformed and the background current was increased as seen in Fig. 3.

**Effect of Different Supporting Electrolyte**

At pH 8.0, the power of various buffers such as BR buffers and phosphate buffers was studied. The oxidation peak has the largest current and lowest potential when phosphate buffer is used; moreover, the morphology of the peak was more promising, as demonstrated in Fig. 4.

**Influence of pH**

The pH of the buffer used has a significant impact on electrochemical sensitivity and voltammetric peak differentiation. Figure 5a illustrates the electro-catalytic oxidation of OND at varied pH values of the phosphate buffer (6.0 to 8.0). Figure 5b demonstrates that the oxidation peak potential
of OND rises dramatically as pH increased, reaching their maximum values at pH 8.0.

The peak current at pH 8.0 has a higher response as it operates similar to the pKa value (7.4) of OND [38]. As a result, the pH of the buffer was adjusted to 8.0 in all subsequent tests. The electrochemical oxidation peak potential of OND is pH-dependent, as illustrated by the Nernst Eq. (2):

\[ E_p (V) = 1.75550 - 0.0580 \times \text{pH} \quad (R^2 = 0.9988) \]

(2)

The response of OND is comparable to the predicted Nernst value of 0.0592 V for the electrochemical phase for the identical electron and proton. As shown in Fig. 5c, the peak current response as a function of pH reveals that buffer pH 8.0 provides the greatest analytical current for OND.

**Effect of Scan Rate**

In addition, the influence of voltammetric scan rate on the electrochemical response of a 0.1 mmolL\(^{-1}\) solution of OND was measured in phosphate buffer pH = 8.0 at varied scan rates (v) (20–150 mV s\(^{-1}\)) as shown in Fig. 6a.

According to the regression equation [39], up to a scan rate of 100 mVs\(^{-1}\), the peak current increases linearly with the square root of the scan rate as seen in Fig. 6b.
Fig. 5  A SWV of 0.1 mmolL$^{-1}$ OND at different pH values using MWCNTs/PVP/CPE sensing platform, scan rate 100 mVs$^{-1}$. B shows a plot of anodic peak potential of OND versus pH at modified electrode MWCNTs/PVP/CPE, and C a plot of anodic peak current of OND versus pH at modified electrode MWCNTs/PVP/CPE.
Where \( I_p \) is the peak current (µA), \( n \) is the number of electrons occurring in half-reaction for the redox couple, \( \nu \) is the scan rate at which the potential is swept (Vs\(^{-1}\)), \( C \) is the analyte concentration, \( A \) is the electrode area (0.052 cm\(^2\) for MWCNTs/PVP/CPE), and \( D_o \) is the diffusion coefficient (cm\(^2\) s\(^{-1}\)). The apparent diffusion coefficient, \( D_{app} \), of OND in phosphate buffer (pH 8.0) was estimated using square wave voltamogram (SWV) studies, and it increased from 8.2 × 10\(^{-7}\) cm\(^2\) s\(^{-1}\) at bare CPE to 6.1 × 10\(^{-5}\) cm\(^2\) s\(^{-1}\) when the functionalization of bare CPE surface with MWCNTs/PVP nanoparticles. This suggested a quick mass transfer of analyte molecules from bulk solutions to the MWCNTs/PVP/CPE surface, as well as a fast electron transfer mechanism of electrochemical oxidation of the analyte molecule at the electrode–solution interface [40].

In the range 20–150 mVs\(^{-1}\), a direct connection was obtained between the logarithm of oxidation peak currents and the logarithm of scan rate (20–150 mVs\(^{-1}\)) (Fig. 6c), yielding the following equation:

\[
I_p = (2.69 \times 10^5) n^{3/2} A C_0 D_o^{1/2} \nu^{1/2}
\]  

(3)

Fig. 6 A SWV of 0.1 mmolL\(^{-1}\) OND in phosphate buffer (pH 8.0) at different scan rates (20.0–150.0 mVs\(^{-1}\)) using MWCNTs/PVP/CPE. B shows the relation between anodic peak current and square root of scan rate at modified electrode. C depicts the relation between log anodic peak current and log scan rate at MWCNTs/PVP/CPE. D corresponds to the relation between peak potential and log scan rate at MWCNTs/PVP/CPE.

Schematic 1 Electrochemical oxidation reaction of OND

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**Electrochemical oxidation reaction of OND**

-CE

\[\text{OND} \rightarrow \text{OND}^+ \]

\[\text{OND}^+ + \text{H}^{+} \rightarrow \text{OND} + \text{H}^{2+}\]
Fig. 7 A SWV for different concentrations of OND (2.00 nM–100.0 µM) in phosphate buffer (pH 8.0) at a scan rate of 100 mVs⁻¹ using MWCNTs/PVP/CPE. B the calibration curve of OND in nM (2.00–700.0 nM molL⁻¹) in phosphate buffer (pH 8.0) at MWCNTs/PVP/CPE, and C the calibration curve of OND in µmol (1.00–100.0 µmolL⁻¹) in phosphate buffer (pH 8.0) at MWCNTs/PVP/CPE.
The slope of the established linear relations at MWCNTs/PVP/CPE is less than 0.5, showing that the electroactive species are transported via diffusion. Among the various investigated scan rates, 100 mVs\(^{-1}\) produced the best voltammograms and had the highest sensitivity. As demonstrated in Fig. 6d, increasing the scan rate resulted in a shift to more positive potentials.

\[
E_p(V) = 1.1192 + 0.0849 \log v \quad (R^2 = 0.9996) \quad (5)
\]

Lavern’s theory [41] for irreversible processes was used to calculate the number of electrons transported in order to establish the kinetic parameters of the electron-transfer process for the OND oxidation on the MWCNTs/PVP/CPE.

\[
E = \varepsilon + 2.303 \frac{RT}{anF} \log \frac{RTK^o/anF}{2.303T/anF} \log v \quad (6)
\]

Where, \( R \) is the gas constant (8.314 J K mol \(^{-1}\)), \( T \) denotes the temperature (298 K), \( F \) refers to the Faraday constant (96.485 C/molL), \( \alpha \) is the electron transfer coefficient, and \( n \) is the number of the electrons. \( an \) is determined by plotting the slope of potential versus log scan rate; in this system, the slope is 0.0849; \( an \) was calculated to be 0.697. Since \( \alpha \) was believed to be 0.5 for a fully irreversible electron transfer, then \( n \) was measured at 1.393, certifying that one electron was active in OND oxidation.

**Oxidation Mechanism of OND**

The voltammetric reaction of OND at the modified electrode was explained by a one-electron transfer to create the cation radical at the nitrogen atom in the imidazole ring. This finding suggested that the OND electrochemical reaction at MWCNTs/PVP/CPE was a surface-controlled mechanism [42]. A diagram illustrating the oxidation of OND at the MWCNTs/PVP is presented in Schematic 1.

**Method Validation**

According to the International Conference on Harmonization (ICH) Guideline [43], validation of the suggested technique was determined and linearity, LOD, LOQ, precision, specificity, robustness, and accuracy were calculated.

**Linearity**

Under the optimal condition mentioned above, the linearity of OND was done via SWV (Fig. 7a). Linear relationship was achieved between peak current and OND concentration in range of 2.00–700.0 nM molL\(^{-1}\) and 1.00–100.0 µM molL\(^{-1}\) as shown in Fig. 7b, c. The parameters are listed in Table 1.

The regression equation was computed and found to be:

\[
I_p(\mu A) = 0.0066 C (M) + 1.3368 \quad (r = 0.9998) \quad 1^{st} \text{ Linear segment}
\]

\[
I_p(\mu A) = 0.0495 C (M) + 7.0391 \quad (r = 0.9999) \quad 2^{nd} \text{ Linear segment}
\]

Where \( I_p \) is the peak current in µA, \( C \) is the concentration in M and \( r \) is the correlation coefficient.

**Table 2** Determination of OND in a pharmaceutical formulation using MWCNTs/PVP/CPE

| Sample          | Claimed conc (µM) | Amount added standard OND (µM) | Apparent recovery% OND |
|-----------------|-------------------|-------------------------------|------------------------|
| Zofran tablets  | 20.0×10\(^{-6}\)  | 2.00                          | 97.64                  |
| (contain 8.0 mg OND) | 20.00             | 100.09                        |                        |
| Recovery% ± RSD| 98.75 ± 1.254     |                               |                        |

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\[
\log I_p = 0.3651 + 0.4908 \log v \quad (R^2 = 0.9990) \quad (4)
\]
Limit of Detection and Quantification

Using the following formulae, the limits of detection and quantification (LOD and LOQ) were calculated to be 109.50 pmolL$^{-1}$, 33.184 pmolL$^{-1}$.

$$\text{LOD} = 3.3 \frac{\sigma}{S}, \quad \text{LOQ} = 10 \frac{\sigma}{S},$$

where “$\sigma$” is the standard deviation of the intercept and “$S$” is the slope of a calibration curve. The sensitivity of MWCNTs/PVP/CPE was confirmed by LOD and suggesting good sensitivity for the proposed sensor (Table 1).

Accuracy and Precision

Accuracy was checked by applying the proposed method on the determination of three different concentrations for OND in triplicates. The mean recovery percentages and standard deviation were calculated, and a suitable result was achieved (Table 1). Precision of proposed method was investigated intra-daily (repeatability) and on 3 successive days (intermediate precision) using three concentrations of OND; relative standard deviations were calculated and found to be less than 2% (Table 1).

Robustness

The procedure’s reliability was demonstrated by the reproducibility of the peak current with small variations in the experimental conditions.

The investigated variables were pH (8.0 ± 0.2) and the time taken before each measurement (20 s ± 4 s). These minor variations had no influence on the peak current intensity for the examined drug, reflecting the efficiency during routine use of the proposed system.

Table 3 Comparison of the square wave voltammetric method to the reported one for OND

| Technique used       | Linearity range                  | Detection limit (M) | Reference |
|----------------------|----------------------------------|---------------------|-----------|
| Electro-chemistry (M)| 1.0 × 10$^{-7}$–7.5.0 × 10$^{-6}$ M | 3.1 × 10$^{-8}$ M    | [12]      |
| Potentiometry (M)   | 5.0 × 10$^{-6}$–1.0 × 10$^{-2}$ M | 4.5 × 10$^{-6}$ M    | [11]      |
| HPLC methods        | 0.2–20 ng/mL                     | —                   | [18]      |
|                     | 30–70 µg/mL                      | —                   | [19]      |
|                     | 4–20 µg/mL                       | 0.28 µg/mL          | [20]      |
|                     | 46–7.2 µg/mL                     | —                   | [21]      |
| (LC–MS/MS) method   | 8–80 ng/mL                       | 1 ng/mL             | [22]      |
|                     | 5–1000 ng/mL                     | 5 ng/mL             | [23]      |
| HPTLC method        | 200–1200 ng/spot                 | 14.83 ng/spot       | [16]      |
|                     | 0.1–0.5 µg/spot                  | 90.3 ng/spot        | [17]      |
| Spectrophotometry (µg/mL) | 8–16 µg/mL                     | 0.1356 µg/mL        | [6]       |
|                     | 5–15 mg/mL                       | —                   | [7]       |
|                     | 2–10 µg/mL                       | —                   | [8]       |
|                     | 5–15 µg/mL                       | —                   | [9]       |
| Spectrofluorimetry  | 0.025–0.150 µg/mL                | 0.005 µg/mL         | [10]      |
| Radioimmunoassay    | 0.05–2.40 µg/mL                  | 1 ng/mL             | [13]      |
| Flow injection method| 20 µg/mL                        | —                   | [14]      |
| MWCNTs/PVP/CPE      | 2.00–200.0 n molL$^{-1}$         | 430 pmolL$^{-1}$    | This work |

Table 4 Determination of OND in plasma samples using the new proposed sensing protocol

| Parameters             | Proposed voltammetric method for OND | Official methoda |
|------------------------|--------------------------------------|------------------|
| MWCNTs/PVP/CPE        | 98.01                                | 100.05           |
| Mean                   | 2.206                                | 1.241            |
| SD                     | 6                                    | 6                |
| n                      | 4.866                                | 1.54             |
| Variance               | 0.368                                | —                |
| t (2.228) b            | 3.159                                | —                |

*HPLC method using ZorbaxC18 column and mixture of acetonitrile: phosphate buffer as mobile phase. The UV detection was performed at 216.0 nm using DAD detection.

bThe values in parentheses are the corresponding tabulated values at $p=0.05$.

Table 5 Statistical comparison between the results of determination of pure OND by the proposed voltammetric method and official method

| OND in human plasma sample | Taken conc. (nM) | Found conc. (nM) | Recovery% |
|----------------------------|------------------|------------------|-----------|
| 3.0                        | 2.90             | 96.74            |
| 20.0                       | 19.95            | 99.75            |
| 70.0                       | 68.65            | 98.08            |
| 300.0                      | 296.95           | 98.98            |
| 700.0                      | 704.26           | 100.60           |
| Mean                      | 98.83            |                  |
| SD                        | 1.49             |                  |
| %RSD                      | 1.51             |                  |

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Fig. 8  A SWV of OND at the MWCNTs/PVP/CPE recorded in phosphate buffer (pH8.0) at different concentrations of OND in the presence of constant concentration of NAL (200 nM) and inset demonstrates a calibration graph for the quantification of OND; B SWV of NAL at MWCNTs/PVP/CPE recorded in phosphate buffer (pH 8.0) at different concentrations of NAL in the presence of constant concentration of OND (200 nM) and inset demonstrates a calibration graph for the quantification of NAL; and C SWV for the solutions containing various concentrations of NAL and OND (2–200 nM) recorded at the MWCNTs/PVP/CPE in phosphate buffer at (pH 8.0) and inset demonstrates a calibration graph for the quantification determination of NAL and OND.
Specificity and Selectivity

The specificity of the described method was proven by its ability to determine OND in drug product without interference from excipients, organic- and inorganic-related substances that are commonly present.

Determination of OND in Pharmaceutical Preparation Using SWV Method

To estimate OND in the drug product, the proposed SWV method was successfully applied (Table 2); by applying the standard addition technique, the feasibility of the proposed SWV approach was further tested (Table 2).

The proposed SWV technique was successfully used to estimate OND with lower limit of detection and high sensitivity in comparison with the reported method, as shown in Table 3.

The results obtained for the analysis of OND were statistically compared with those obtained by applying the official method [5]. The values of calculated $t$-test and $F$ value at 95% confidence interval were less than the tabulated ones, revealing no significant difference between the suggested and reported method with respect to accuracy and precision (Table 4).

Determination of OND in Biological Fluid (Plasma)

SWV technique was used in spiked human plasma sample to evaluate OND. According to the regression equation, peak current was linearly related to OND concentrations over the range 3.0–700 nM molL$^{-1}$. The results in Table 5 shows a good recovery for studied drug in the pH 8.0 phosphate buffer.

\[ I_p (\mu A) = 0.0041 + 1.337 C \]  

(7)

Simultaneous Determination of OND and Co-Administered Drug NAL

The combination of OND and NAL is thought to have synergistic effects for reducing alcohol consumption. Preclinical and clinical investigations revealed a larger reduction in alcohol consumption when both drugs were administered concurrently compared either treatment alone [3].

The voltammetric response of NAL was also investigated at the MWCNTs/PVP/CPE interface to create a novel electroanalytical technique for simultaneous measurement of OND and NAL. SWV peak of different concentration OND in the presence of constant concentration of NAL (200.0 nM) was checked using MWCNTs/PVP/CPE as seen in Fig. 8a. Also, the electrode’s sensitivity to a constant concentration of OND (200.0 nM) in the presence of various concentrations of NAL was tested as shown in Fig. 8b.

These studies illustrated that the results are extremely close to the value obtained in the absence of NAL, showing that OND and NAL oxidation at the MWCNTs/PVP/CPE is independent. Figure 8c shows the SWV for solutions containing different concentrations of NAL and OND (2.0–200.0 nM). It can be observed that raising the concentration of NAL causes a rise in the associated peak currents, but there are no significant changes in the peak current and potential of OND.

The sensor of proposed method revealed strong electron mediating activities followed by well-separated oxidation peaks towards OND and NAL at 1.196 and 0.774 V, respectively.

Conclusion

For the first time, the study discusses the oxidative action and electrochemical detection of OND in the presence of NAL. The MWCNTs/PVP/CPE demonstrated a significant amplification impact on the voltammetric response of OND. This study presents a novel and easy electro analytical technique for directly quantifying OND. In comparison to the HPLC technique, the suggested sensor has several advantages, including high accuracy, quick response, reduced cost, and convenience. Furthermore, the MWCNTs/PVP/CPE demonstrated excellent selectivity in voltammetric measurements of OND in pure form, in pharmaceutical preparation, human plasma, and in the presence of co-administered opioid antagonist drug NAL, with a potential difference of 422 mV.

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Data Availability  Datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest  The authors declare no competing interests.
