ORIGINAL RESEARCH

Analytical Eco-Scale for Evaluating the Uniqueness of Voltammetric Method used for Determination of Antiemetic Binary Mixture Containing Doxylamine Succinate in Presence of its Toxic Metabolite

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Abstract

Green analytical procedures are gaining popularity in the pharmaceutical research area as a way to reduce environmental impact and improve analyst health safety. The current work presents a green and sensitive electrochemical carbon paste electrode that has been chemically modified with zirconium dioxide and multi-walled carbon nanotubes for estimation of pyridoxine HCl (PYR) and doxylamine succinate (DOX) using the square wave voltammetric technique. Under optimum conditions, the linearity ranges were 20.00–2000.00 ng mL^{-1} and 2.00–20.00 µg mL^{-1} for both drugs in the 1st linear segment and 2nd linear segment, respectively. Stability testing assesses how the quality of a drug substance changes over time, depending on environmental and laboratory factors. DOX was found to undergo oxidative degradation when refluxed for 7 h using 30% H_2O_2 and the degraded product (DOX DEG) (toxic metabolite) was successfully characterized utilizing LC–MS. The developed electrode showed selectivity for the determination of binary mixture in pure form, pharmaceutical form, and in the presence of DOX DEG and common interfering molecules with good recovery. The proposed method was found to be eco-friendlier than the reported method in terms of the use of hazardous chemicals and solvents, energy consumption, and waste generation.

Keywords Zirconium dioxide carbon paste · Multi-walled carbon nanotubes carbon paste · Green square-wave voltammetry · Anti-emetic drug · Stability indicating

Introduction

In recent years, there has been a significant increase in the use of green solvents in the development of green analytical methodologies. Green analytical chemistry (GAC) is defined as the reduction or elimination of harmful chemicals used in the analytical process, as well as the reduction of energy consumption and waste production, all while maintaining system performance requirements [1–3]. GAC aims to provide environmentally friendly techniques for routine pharmaceutical testing, which is a major concern among quality control analysts. The National Environmental Approaches Index (NEMI), Assessment of Green Profile (AGP), Green Analytical Procedure Index (GAPI), and eco-scale were a few methods for measuring the greenness of a suggested analytical methodology, which were largely based on 12 principles stated by Gauszka et al. [4]

The square wave voltammetric technique (SWV) is one of the fastest and most sensitive pulse voltammetry techniques. SWV has many advantages over other analytical methods in pharmaceutical research. These merits can be summarized in cost-effectiveness, low energy consumption, minor sample pre-treatment, versatility to small volume samples, and high efficiency with a wide range of applications [5–8].

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Seventy to eighty percent of pregnant women experience nausea and vomiting during their pregnancy [9]. Doxylamine succinate (DOX), a first-generation antihistamine medication, and pyridoxine hydrochloride (PYR), a water-soluble vitamin, are two well-known active compounds that have been used to treat a variety of pathological disorders [10, 11]. Their combination is currently generating a lot of attention as it is one of the few choices on the pharmaceutical market that has recently been approved by the FDA as a secure and successful treatment of morning sickness during pregnancy [12]. The chemical name of PYR, known as vitamin B6, is 4,5-bis (hydroxyl methyl)-2-methylpyridin-3-ol [13]. DOX is chemically named as dimethyl ({2-[1-phenyl-1-(pyridin-2-yl) ethoxy] ethyl}) amine, it has antimuscarinic and antihistaminic actions with a sedative effect [13].

Vomibreak® tablets are available in Egypt (each tablet containing 10.00 mg of PYR and DOX). It is prescribed to pregnant women experiencing morning sickness [14].

The isolation, detection, characterization, and quantification of the most likely and potential degradation products are of increasing importance to improve the consistency of the active pharmaceutical ingredient and its preparation. Stress tests are required by the ICH forced degradation guidelines to figure out the active drug's inherent stability characteristics [15].

Reviewing the literature for the assessment of a mixture of PYR and DOX in pharmaceutical preparations revealed many spectrophotometric approaches including the simultaneous equation method [16–18], first-order derivative [19], second derivative [20], absorbance correction method [21], PLS, and MCR-ALS chemometric methods [22]. Also, dissolution profiling was done for this combination in the pharmaceutical dosage form in both acid and buffer stages [23]. Also, chromatographic techniques were published for the estimation of this mixture namely, HPLC [24–28], TLC [29], and UPLC [30]. There was only one chromatographic method for the determination of the presented antiemetic drug mixture in presence of DOX DEG [31]. The literature reported that PYR was determined alone in pharmaceutical preparations by voltammetry [32–37]. But no voltammetric methods had been reported for the simultaneous estimation of PYR and DOX alone or in presence of one of their degradation products till now.

Carbon nanotubes paste electrode (CPE) is molecular-scale wires with high electrical conductivity and extremely high mechanical power. It has been used in a variety of applications, including sensing and catalysis [38]. The modified carbon paste electrode (MCPE) was found to be superior to the unmodified one because the unmodified electrode has some drawbacks such as slow electron transfer, low sensitivity, and reproducibility [39]. Nanomaterials, transition metal compounds, and multi-walled carbon nanotubes (MWCNT) are used to resolve these drawbacks. In contrast to single-walled MWCNT, can be manufactured in large quantities and are easy to be purified. As a result, MWCNT are used in a variety of scientific research fields with low production costs.

Zirconium dioxide (ZrO_2) is an inorganic oxide and an ideal material for the immobilization of biomolecules with oxygen-containing groups due to their thermal stability, lack of toxicity, and affinity for oxygen-containing groups of ZrO_2. Due to its unique properties, it has been used in various fields like sensing and catalysis [40]. Recently, ZrO_2-modified carbon electrodes have been used in chemical and biosensor applications [5].

The goal of the present work is to apply the first green voltammetric method for simultaneous determination of PYR and DOX in the presence of DOX DEG by using GAC, where a simple, sensitive, and low-cost modified nanoparticle electrode based on ZrO_2/MWCNT/MCPE was employed in comparison with bare CPE. In terms of eco-scaling for green assessment, the developed voltammetric method was compared to the published HPLC method [26]. Eco-scaling depending on penalty points (based on reagents and instruments). The eco-scaling of the established method was measured and subtracted from a base of 100 (the ideal green analytical method score) [41]. Statistical analysis of the developed technique was performed using the reported HPLC method, where no significant difference was found [26].

Experimental

Materials and Reagent

PYR and DOX were gently provided by Eva Pharma for Pharmaceutical Industry (Cairo, Egypt) and Mash Premiere for Pharmaceutical Industry (Cairo, Egypt), respectively, and their purities were found to be 99.73% for DOX and 99.25% for PYR, according to the official method [13]. Vomibreak® tablets, manufactured by Marcyrl Company were bought from the local market. Each tablet claimed to contain 10.0 mg for each PYR and DOX as active ingredients.

All the used chemicals and solvents were of analytical grade and were used without further purification. Acetate buffer solutions (pH 3.0–7.0) were used as supporting electrolytes. The electrochemical tests were carried out using a pH of 7.0. Acetate buffer (pH 7.0; 0.1 M) was prepared by dissolving 12.30 g sodium acetate in a 1000-mL volumetric flask and completing the volume with double distilled water then pH was adjusted using acetic acid or sodium hydroxide [42]. Bi-distilled water was used. Graphite powder and paraffin oil were supplied from Sigma-Aldrich (Cairo, Egypt). Acetone, chloroform, methanol, and ammonia 25% (El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt) were used.

Instrumentation

The BioLogic SP 150 electrochemical workstation was used for all voltammetric measurements in this study. A platinum wire from BAS was used as an auxiliary electrode (USA). All cell potentials were determined using a BAS Ag/Ag Cl (3.0 M NaCl) reference electrode (USA). For electrochemical measurements, a glass cell (5.0 mL) was used at room temperature. A JEM-1400 electron microscope was used to perform the transmission electron microscope (TEM) measurements (Japan Electro Company) for electrochemical characterization of the modified electrode. A XEVO TQD triple quadruple instrument, Waters Corporation, Milford, MA01757 USA, the mass spectrometer was used to carry out ESI–MS negative ion acquisition mode for determining the molecular weight of DOX DEG.

Preparation of Standard Stock and Working Solutions

PYR and DOX stock solutions (1.00 mg mL^{-1}) were produced by transferring 100.0 mg of each drug powder into a volumetric flask of 100.0 mL, dissolving with a minimum amount of bi-distilled water, then filling to the mark with the same solvent. These solutions were then diluted further to yield working solutions containing 0.01 mg mL^{-1} of each drug.

Preparation of Degradation Product

After dissolving 100 mg of DOX in 10 mL of methanol, 20 mL of 30% H_2O_2 was added and refluxed for 7 h. The solution was dried at room temperature, then re-dissolved in methanol, transferred to a 100-mL volumetric flask, and the volume was finished with methanol. DOX solution was newly made and kept away from light.

Preparation of Working Electrodes

For bare CPE, in a glassy mortar, graphite powder (0.50 g) and paraffin oil (0.30 mL) were ground together to produce carbon paste. The prepared carbon paste was poured into the electrode body's hole and smoothed out with filter paper until it was shiny.

For modified CPE (MCPE), various concentrations of zirconium dioxide modified carbon paste were prepared by hand-mixing of 3.0% (*w/w*), 5.0% (*w/w*), and 10.0% (*w/w*) of graphite powder and ZrO_2 in a glassy mortar, combined with a small amount of paraffin oil until a wetted paste became homogeneous. A shiny look of the electrode, without touching the surface, was reached by packing an optimum amount of the paste into its body's hole and then smoothed on a filter paper. ZrO_2 5.0%/MWCNT 0.5%/MCPE was prepared in the same way above by adding given quantities of zirconium dioxide nanoparticles and MWCNT 0.5% (*w/w*) to 94.50 times of their weights.

Optimization of Experimental Conditions

Effect of Different Electrodes Composition

Oxidation peaks for 0.1 mg PYR and DOX at bare CPE, ZrO_2 3%/MCPE, ZrO_2 5%/MCPE, ZrO_2 10%/MCPE, MWCNT 0.5%, MWCNT 1%, and ZrO_2 5%/MWCNT 0.5%/MCPE were measured and compared to each other.

Effect of pH

Since pH of the used buffer has a profound effect on the electrochemical sensitivity and voltammetric peak separation. Optimization of the electro catalytic oxidation of PYR and DOX at different pH of acetate buffer in the range (3.0 to 7.0) using bare CPE and ZrO_2/MWCNT/MCPE was performed.

Effect of Scan Rate

The influence of scan rate on the current and potential of 0.10 mg solution of PYR and DOX was investigated. The current was recorded at different scan rates (ν) 10.0–60.0 mV s^{-1} in the chosen optimum pH of acetate buffer.

Method Validation

Linearity

Different aliquots equal to (20.00–2000.00 ng mL^{-1}) and (2.00–20.00 μg mL^{-1}) for both drugs in the first linear segment and second linear segment, respectively, have been correctly transferred from the standard solutions to a series of 5-mL volumetric flasks. The volumes were filled to the mark with acetate buffer solution, pH = 7.0. Solutions were then transferred to an electrolytic cell for further processing. SWV with an amplitude of 25.0 mV, a phase potential of 5 mV, and a frequency of 100 Hz were used to measure the anodic peak present (Ip). The calibration curve was developed by plotting the peak current (Ip) against PYR and DOX concentrations and calculating the regression equation.

Accuracy

Different concentrations of PYR and DOX were tested for accuracy by using the suggested voltammetric technique, each within its linearity range, and the concentrations were determined using the corresponding regression equation.

Precision

Repeatability

Three concentrations of PYR and DOX (70.00, 1000.00, and 1200.00 ng mL^{-1}) in the first linear segment and (8.00, 10.00, 18.00 µg mL^{-1}) in the second linear segment were analysed in triplicates intra-daily using the previously mentioned procedure under linearity. SD% was then calculated for each sample.

Intermediate Precision

The above-mentioned PYR and DOX samples under repeatability were analysed in triplicates on three successive days using the procedures stated under linearity. SD% was then calculated for each sample.

Interference Study

Many substances that could interfere with the electro-analytical determination of PYR and DOX were investigated using recommended conditions. The interfering compounds were chosen from the substances which probably may be present in their pharmaceutical preparation. The main goal of electro-analysis research was to assess these species selectively. Therefore, the estimation of a mixture solution containing PYR and DOX in the presence of uric acid (UA) and ascorbic acid (AA) was done. The chosen interfering materials were glucose, sucrose, starch, cellulose, NaCl, Mg, K, and Na ions. By the addition of these materials, PYR and DOX response signals had been checked.

The Stability and Reproducibility of ZrO_2/MWCNT/MCPE

The assessment electrode was done by preparing nine electrodes as described before under experimental, for checking the reproducibility of the proposed electrode.

Application of the Proposed Electrochemical Method in Pharmaceutical Preparation

Ten Vomibreak® tablets were weighed, crushed, and thoroughly combined in a dry and clean mortar. A precisely measured fraction of the crushed powder needed to generate a solution of 1000.0 µg mL^{-1} was put into a 25-mL

volumetric flask, followed by 15 mL of bi-distilled water. After 30 min of sonication, the volume was completed with the same solvent. A working solution was made by putting 1.00 mL of the preceding solution into a 10 mL volumetric flask and filling the capacity with the same solvent. In the electrolytic cell, aliquots of the drug solution were deposited, and the same process as for pure pharmaceuticals was followed.

Results and Discussion

A review of the literature showed that no voltammetric approach has been used to assess PYR and DOX in drug substances or pharmaceutical formulations [16–30]. As a result, the goal of this work was to investigate and improve the experimental conditions to develop a green and smart stability-indicating voltammetric method for the determination of PYR and DOX in the presence of DOX DEG using a new modified carbon paste electrode with ZrO_2 5.0%/MWCNT 0.5%/MCPE as a rapid and sensitive method.

Electrochemical Characterization of the Modified Electrode

The nanoparticles have a variety of forms, the majority of which are almost cubic, and have a larger electrode surface area than other ZrO_2 phases, resulting in enhanced electro sensitivity, as shown in the TEM images (Fig. 1A). Figure 1B represents TEM investigation of MWCNT that has tabular-like structure and highly ordered walls. The outer region of the tube wall was constructed with many layers of carbon with graphite-like platelets and multi-wall carbon nanotubes associated with the inner shells. These results are attributed to a strong interaction between ZrO_2 nanoparticles and MWCNT due to the chemical affinity between them [42], as shown in Fig. 1C. The nanoparticles are almost uniformly distributed throughout the nanotube layer.

Electrochemistry of PYR and DOX

In all cases of square wave responses, two oxidation peaks were seen for PYR and DOX (Fig. 2A). The greatest voltammetric response occurred with small improvements in the voltammetric peak potentials by employing ZrO_2/MWCNT/MCPE. The electroactive area of the electrodes was determined using the Randles–Sevc'k equation for a reversible process and a solution of 5.0 mmol L^{-1} K3Fe (CN)$_6$ in 0.1 $molL^{-1}$ KCl [43].
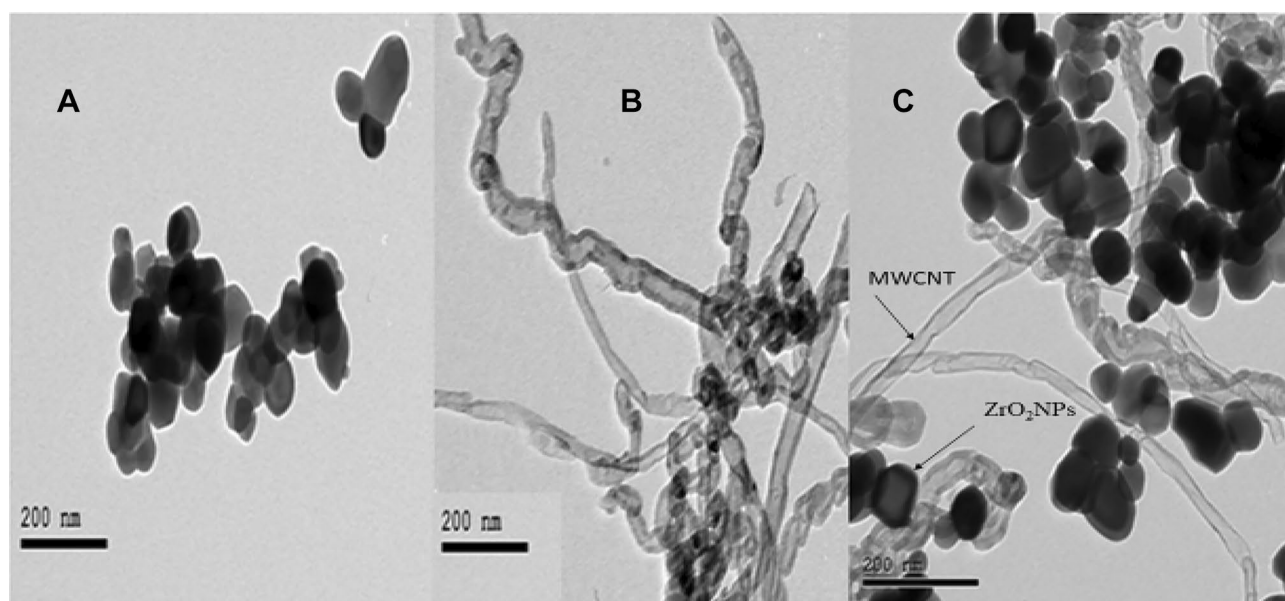
Fig. 1 Transmission electron microscope images (TEM) of **A** ZrO_2, **B** MWCNT, and **C** ZrO_2/MWCNT

$$I_p = 2.65 \times 10^5 \, n^{3/2} \, AD^{1/2} \, Cv^{1/2}$$

where Ip stands for peak current, n stands for the number of electrons transferred, A for electrode field, D for the diffusion coefficient, C is redox probe concentration, and v for voltammetric scan rate. The D value that was used was 7.6×10^{-6} cm^2 s^{-1}. The electroactive area was computed using the slope of the plot of I_p versus $v^{1/2}$ and the calculated areas were discovered to correspond to 0.058, 0.092, 0.098, and 0.109 cm^2 for bare CPE, ZrO_2/CPE, MWCNT 0.5%/CPE, and ZrO_2 5.0%/MWCNT0.5%/CPE, respectively. This revealed that the biggest electrode surface area is found in ZrO_2/MWCNT.

Optimization of Experimental Conditions

Effect of Different Electrodes Composition

Optimal anodic peak current appeared when modification of CPE with a quantity equivalent to 5.0% *w/w* of ZrO_2 and 0.5% *w/w* of MWCNT was done. More amounts of ZrO_2 or MWCNT nanoparticles increased the background current, decreased the response, and weakened the peak current. The effect of different modifiers on response is presented in Fig. 2A.

At bare CPE, the current of the oxidation peak was seen to be 3.09 and 5.5 µA, for PYR and DOX, respectively. Upon using ZrO_2/CPE, it increased to 8.7 and 15.02 µA for PYR and DOX, respectively. A further increase to 11.67 µA for PYR and 20.48 µA for DOX was seen upon adding MWCNT.

Modification of electrode by ZrO_2 5.0% and MWCNT 0.5% showed the highest oxidation peak current, and the potential was shifted negatively, which had a value of 14.68 and 27.43 µA, for PYR and DOX, respectively (Fig. 2B). In comparison to the other electrodes, the synergetic effect of ZrO_2 and MWCNT demonstrated that the composite had high electron transport at the electrode surface vastness. When the potentials of different electrodes were tested, it was discovered that ZrO_2/MWCNT/MCPE had the least negative potential, showing that the modified electrodes increase both the electrode kinetics and the facility of the electron transfer process.

Effect of pH

It is generally known that pH has a significant impact on the reaction of organic and inorganic drug compounds [44]. As pH of the used buffer and choice of scan rate number had a pronounced effect on the electrochemical sensitivity and voltammetric peak separation [45].

Currents of the oxidation peaks of PYR and DOX increased significantly as the pH increased and reached their maxima values at pH 7.0 (Fig. 3A). The pH of the used buffer was adjusted to 7.0 in all further studies. Figure 3B illustrates that the electrochemical oxidation peak potential for PYR and DOX is pH-dependent as shown by the following Nernst equations:

PYR: Ep $(v) = 1.0634 - 0.0549$ pH $(R^2 = 0.997)$

at $(ZrO_2/MWCNT/MCPE)$;

Fig. 2 **A** Square wave voltammograms of (0.10 mg) of PYR and DOX in acetate buffer (pH 7.0) at scan rate 20.0 mV s.$^{-1}$ at bare CPE, ZrO$_2$/MCPE, MWCNT/MCPE, ZrO$_2$/MWCNT/MCPE electrodes. **B** Effect of using different concentrations of ZrO$_2$/MCPE and different modified electrodes on current
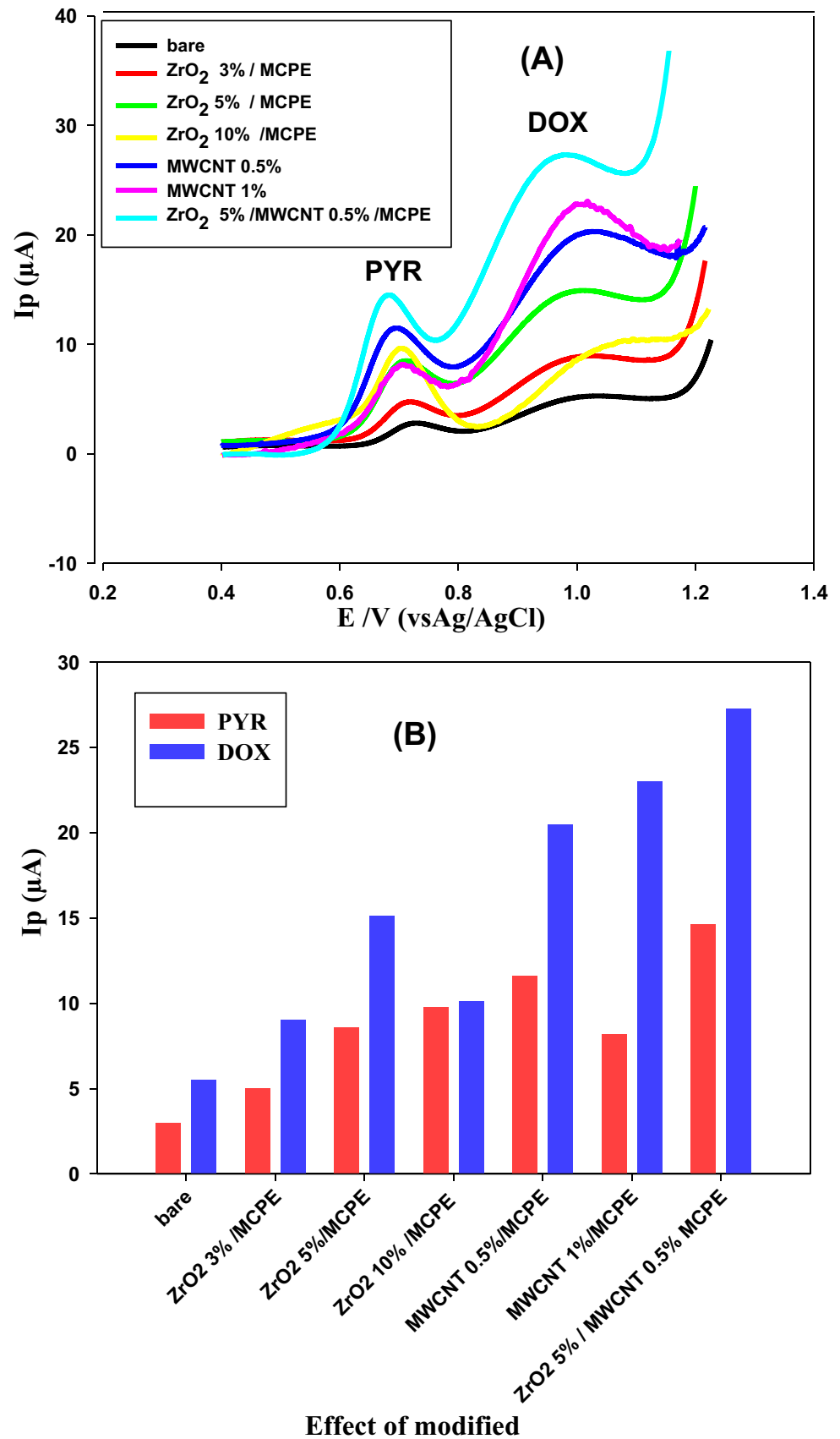
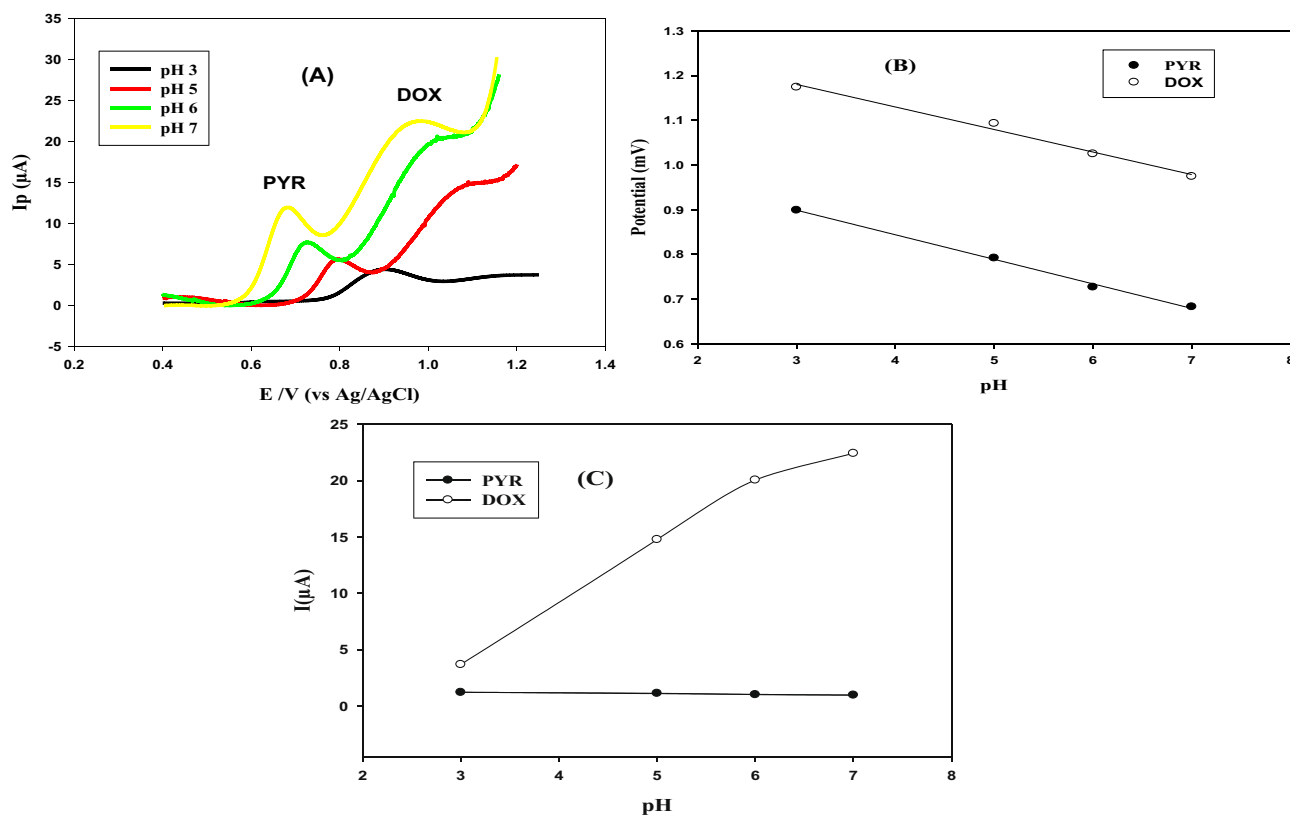**Fig. 3** **A** Square wave voltammetric responses of (0.10 mg) of PYR and DOX at different pH values using ZrO$_2$ 5%/MWCNT 0.5%/ MCPE sensing platform, Scan rate 20.0 mV s^{-1}. **B** A plot of oxida- tion peak potential of PYR and DOX versus pH at the bare and modi- fied electrode. **C** A plot of the anodic peak current of PYR and DOX versus pH at the modified electrode

DOX: Ep $(v) = 1.3311 - 0.0504$ pH ($R^2 = 0.988$)

at (ZrO$_2$/MWCNT/MCPE).

Both answers were consistent with the theoretical Nernst value of 0.0592 V for an electrochemical process involving equal amounts of electrons and protons. The responses of the peak current as a function of pH are shown in Fig. 3C, with acetate buffer at pH 7.0 supplying the highest anodic current for both PYR and DOX.

Scan Rate Optimization

A scan rate of 20.0 mV s^{-1} was the optimum chosen one according to the results shown in Fig. 4A which were then used to measure the relation between scan rate and potential or current. The interfacial reaction of both drugs at each electrode was determined and the findings are displayed in Fig. 4A. Up to a scan rate of 20.0 mV s^{-1}, the peak current increased with the square root of the scan rate, and a linear straight line was produced as shown in Fig. 4B, according to the regression equation:

$$I_p = 2.69 \times 10^5 \, n^{3/2} \, A \, Co \times D^{1/2} \, v^{1/2}$$

where Ip is the peak current (Acm^2), v is the potential sweep rate (V s^{-1}), n stands for electron's numbers in half-reaction for the redox pair, C is the concentration of analyte, A is the electrode area (to 0.058 and 0.109 cm^2 for bare CPE and ZrO$_2$/MWCNT, respectively), and the diffusion coefficient (cm^2 s^1) is denoted by Do. (cm^2 s^{-1}). SWV tests showed that the apparent diffusion coefficient, D app, of PYR and DOX in acetate buffer (pH 7.0) increased from 9.1×10^{-7} cm^2 s^{-1} (using bare CPE) to 5.84×10^{-5} cm^2 s^{-1} (after bare CPE surface functionalization with ZrO$_2$ and MWCNT). This suggested a rapid mass transfer of analyte molecules from bulk solutions to the ZrO$_2$/MWCNT surface, as well as a quick electron transfer process of electrochemical oxidation of analyte molecules at the electrode–solution interface.

In the range of 10.0–60.0 mV s^{-1}, there were direct relationships between log scan rate and log current. Figure 4C as the following equations:

Log $I = 0.3405 + 0.590$ log v ($R^2 = 0.989$) for PYR
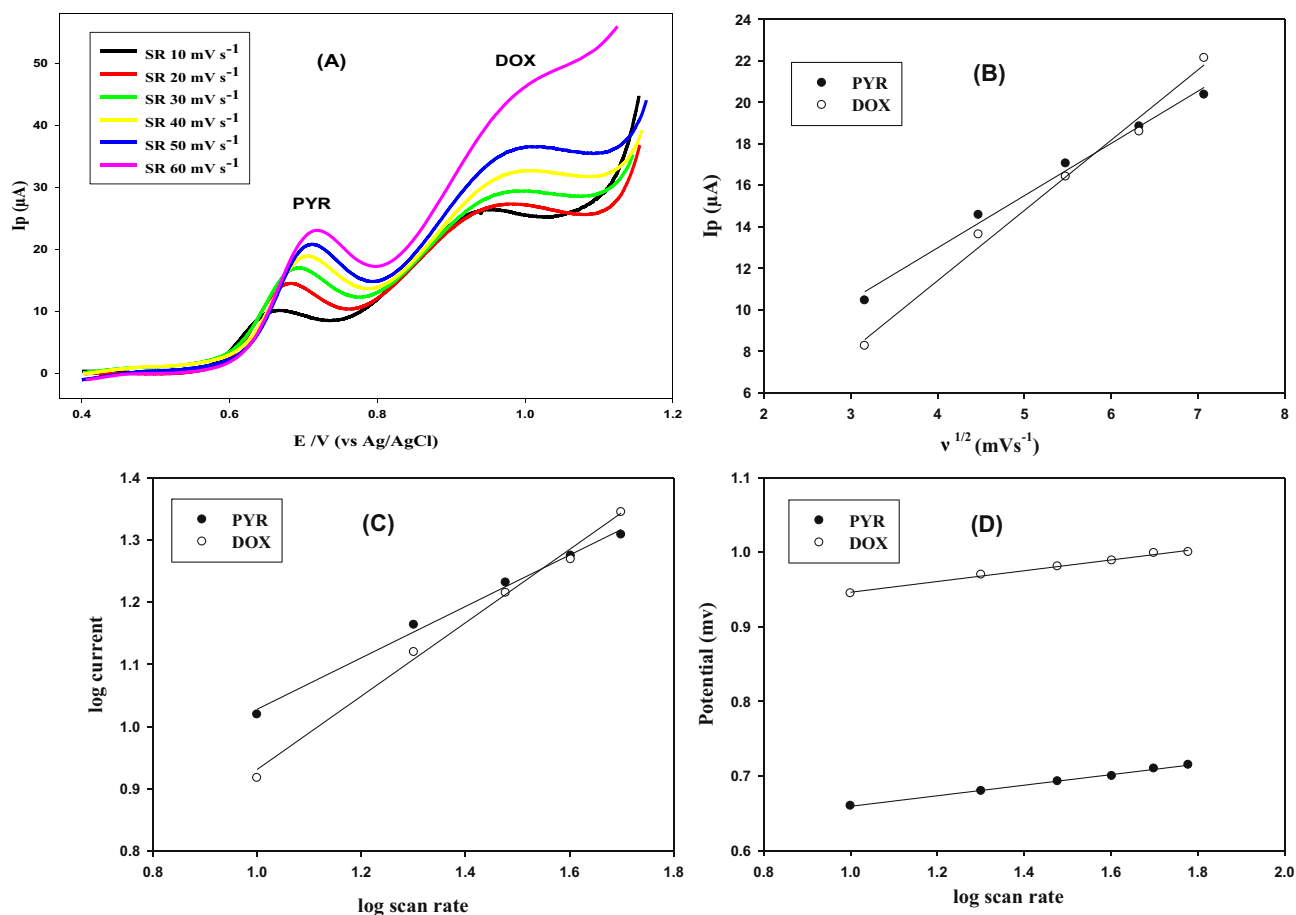
at (ZrO$_2$/MWCNT/MCPE);

Fig. 4 **A** Square wave responses of 0.10 mg of PYR and DOX in acetate buffer (pH 7.0) at different scan rates (10.0–60.0 mVs.$^{-1}$) using ZrO$_2$ 5%/MWCNT 0.5%/MCPE. **B** The relation between peak current and the square root of scan rate at the modified electrode. **C** Depicted the relation between log anodic peak current and log scan rate at the modified electrode. **D** The relation between peak potential and log scan rate at the modified electrodes

$$E_{p\,(V)} = 0.8741 + 0.0722 \; \log \; \nu \; (R^2 = 0.9926) \; \text{for DOX}$$
$$\text{at (ZrO}_2/\text{MWCNT/MCPE).}$$

At the modified electrode, the obtained PYR slope value was around 0.5, implying that the electroactive species are transferred by a diffusion and absorption mechanism. The DOX slope computed value was less than 0.5, showing that diffusion control is the major mechanism [46]. After checking various scan rates, it was discovered that 20.0 mV s^{-1} provided the best voltammograms and had the highest selectivity.

The relationship between log scan rate and potential is shown in Fig. 4D.

$$E_{p\,(V)} = 0.5885 + 0.0708 \; \log \nu \; (R^2 = 0.9969) \; \text{for PYR}$$
$$\text{at (ZrO}_2/\text{MWCNT/MCPE);}$$

$$E_{p\,(V)} = 0.8741 + 0.0722 \; \log \; \nu \; (R^2 = 0.9926) \; \text{for DOX}$$
$$\text{at (ZrO}_2/\text{MWCNT/MCPE).}$$

Moreover, kinetic parameters were calculated for the electrochemical process of PYR and DOX oxidation on the ZrO$_2$/MWCNT/MCPE, according to Lavern's theory [47].

$$E = E° + 2.303 \; RT/\alpha n \; F \; [\log \; \text{Rat}^{ko}/\alpha n \; F]$$
$$+ 2.303 RT/\alpha n \; F \; (\log \; \nu)$$

where R is the gas constant (8.314 J K mol^{-1}), F is the faraday constant (96.485 C KJ), T is the temperature (298 K), α symbolizes the electron transfer coefficient, and n shows the number of electrons. The slope of potential versus log scan rate can be used to determine n. In this system, the slope was 0.0708 and 0.0722 for PYR and DOX, respectively, αn was calculated to be 0.836 and 0.819 for PYR and DOX. Because for a completely irreversible electron transfer, α assumed as 0.5, then n was calculated to be 1.67 and 1.6 for PYR and DOX, respectively, which showed that two electrons were involved in the oxidation of PYR and DOX.

Oxidation Mechanism of PYR and DOX

Electro-oxidation was associated with the exchange of protons in acetate buffer solution (PH 7) as two electrons were released during the oxidation process due to the oxidation of the hydroxyl group in PYR [48]. Due to the oxidation of ternary amines in DOX, two electrons were liberated in the oxidation process [49]. Scheme 1 showed the oxidation of PYR and DOX at the modified electrode.

Validation of the Proposed Voltammetric Method

The voltammetric method was validated in compliance with the ICH guidelines [50].

Linearity

The electroanalytical response of ZrO$_2$/MWCNT was explored toward the sensing of two drugs in presence of

each other as a combination. This was carried out by altering the drug concentrations simultaneously using the ZrO$_2$/MWCNT/MCPE and recording the SWV, then comparing the results to bare CPE. The results showed clearly defined oxidation peaks at potentials of +0.679 and +0.977 V (vs. Ag/Ag Cl) for PYR and DOX, respectively. Figure 5A and B shows the plot of the peak current (I_p) as a function of PYR and DOX concentrations at the ZrO$_2$/MWCNT/ MCPE sensor. A good correlation between peak current and concentration was found for PYR and DOX over the concentration range of (20.00–2000.00 ng mL^{-1}) and (2.00–20.00 μg mL^{-1}) for both drugs in 1st linear segment and 2nd linear segment, respectively, as shown in Fig. 5C and D. The regression equations were: I_p (μA) = 1.653 + 0.0039 c, r = 0.9998 for PYR, and I_p (μA) = 3.068 + 0.0059 c, r = 0.9994 for DOX at 1st linear segment, and I_p (μA) = 8.9137 + 0.2835c, r = 0.9996 for PYR, and I_p (μA) = 16.886 + 0.5143 c, r = 0.9997 for DOX at 2nd linear segment. The calibration parameters are shown in Table 1.
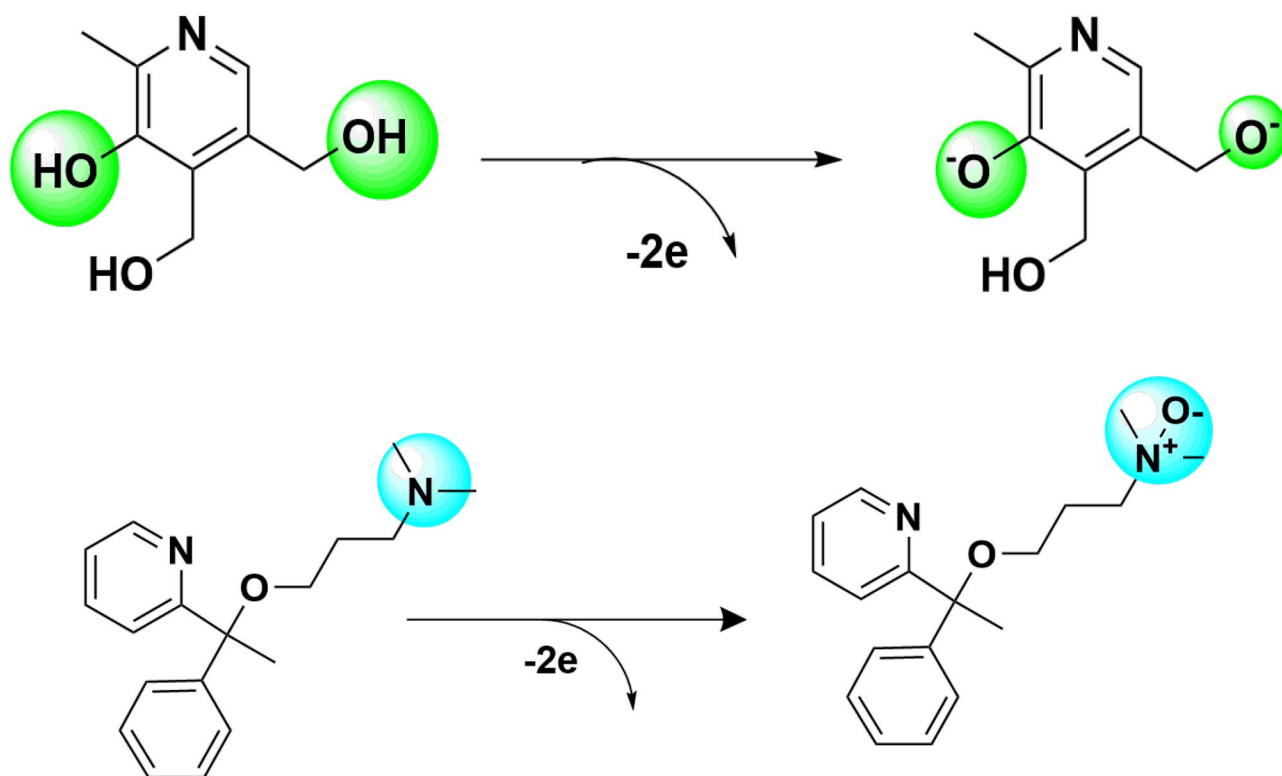
Scheme 1 Electrochemical oxidation reaction of **A** pyridoxine (PYR) and **B** doxylamine (DOX)
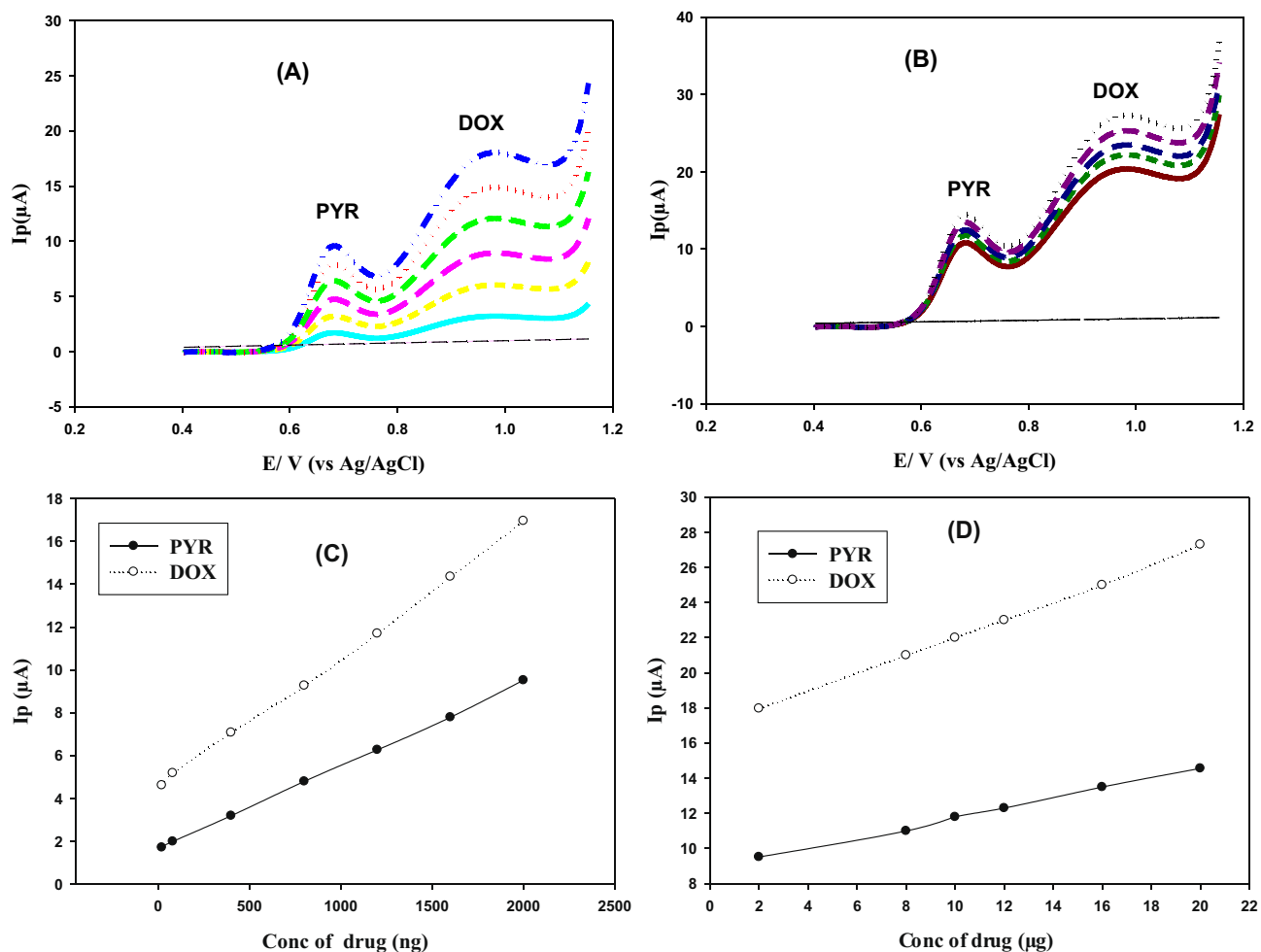
Fig. 5 Square wave voltammograms for different concentrations of PYR and DOX in acetate buffer (pH 7.0) at scan rate 20.0 mV s^{-1} using ZrO$_2$ 5%/MWCNT 0.5%/MCPE were showed in (**A**) at range (20.00–2000.00 ng mL^{-1}); (**B**) at range (2.00–20.00 μg mL^{-1}). Cali-bration curves of PYR and DOX were showed in (**C**) at range (20.00–2000.00 ng mL^{-1}) and (**D**) at range (2.00–20.00 μg mL^{-1}) DOX in acetate buffer (pH 7.0) at scan rate 20.0 mV s.$^{-1}$ using ZrO$_2$ 5%/MWCNT 0.5%/MCPE

Accuracy

The accuracy was determined by calculating the recovery percentage for the three replicates of three different concentrations covering the linearity range of PYR and DOX as shown in Table 1.

Precision

Repeatability and intermediate precision were calculated as RSD percentage and found to be less than 2%, which assured that the adopted technique was sufficiently precise to be applied during routine work as shown in Table 1.

Interference Study

The obtained results showed that the applied electrode had good selectivity and the electro-analytical determination of PYR and DOX was not affected by any interference. This was shown in Table 2.

The Stability and Reproducibility of ZrO$_2$/MWCNT/MCPE

The SD% for the I_p between electrodes was 2.41% for 0.1 mg of the two medications. The results proved that the modified electrode had satisfactory reproducibility. After 2 weeks of

Table 1 Validation parameters of the proposed voltammetric method for the determination of PYR and DOX in acetate buffer (pH 7.0) at ZrO$_2$/MWCNT/MCPE

Parameter	PRY		DOX	
	First linear segment	**Second linear segment**	**First linear segment**	**Second linear segment**
Linearity range	20.00–2000.00 ng mL^{-1}	2.00–20.00 µg mL^{-1}	20.00–2000.00 ng mL^{-1}	2.00–20.00 µg mL^{-1}
Correlation coefficient (r)	0.9998	0.9995	0.9994	0.9997
Slope	0.0039 ng mL^{-1}	0.2835 µg mL^{-1}	0.0059 ng mL^{-1}	0.5143 µg mL^{-1}
Intercept	1.653 ng mL^{-1}	8.9137 µg mL^{-1}	3.068 ng mL^{-1}	16.886 µg mL^{-1}
Standard error of slope	3.42×10^{-5}	0.0046	9.4×10^{-5}	0.0067
Standard error of intercept	0.0384	0.0577	0.1054	0.0847
Accuracy [a] (mean ± RSD%) Precision [b]	99.99 ± 0.302	98.83 ± 0.364	100.47 ± 0.177	99.79 ± 0.473
Repeatability	0.274	0.194	0.207	0.219
Intermediate	0.121	0.307	0.231	0.259
LOD [c]	0.03 ng mL^{-1}	0.67 µg mL^{-1}	0.05 ng mL^{-1}	0.54 µg mL^{-1}
LOQ [c]	0.09 ng mL^{-1}	2.04 µg mL^{-1}	0.15 ng mL^{-1}	1.62 µg mL^{-1}

[a] $n = 3$

[b] $n = 9$

[c] LOD and LOQ were calculated from the standard deviation (s) of the response and the slope of the calibration curve (S) according to the following equations: LOD = 3.3(s/S) and LOQ = 10(s/S)

storage at room temperature for the ZrO$_2$/MWCNT/MCPE, the stability of the modified electrode might be influenced by the SD of the peak current, which is 2.5%. As a result, the utilized electrode was appropriate for the analysis of PYR and DOX in pharmaceutical formulation.
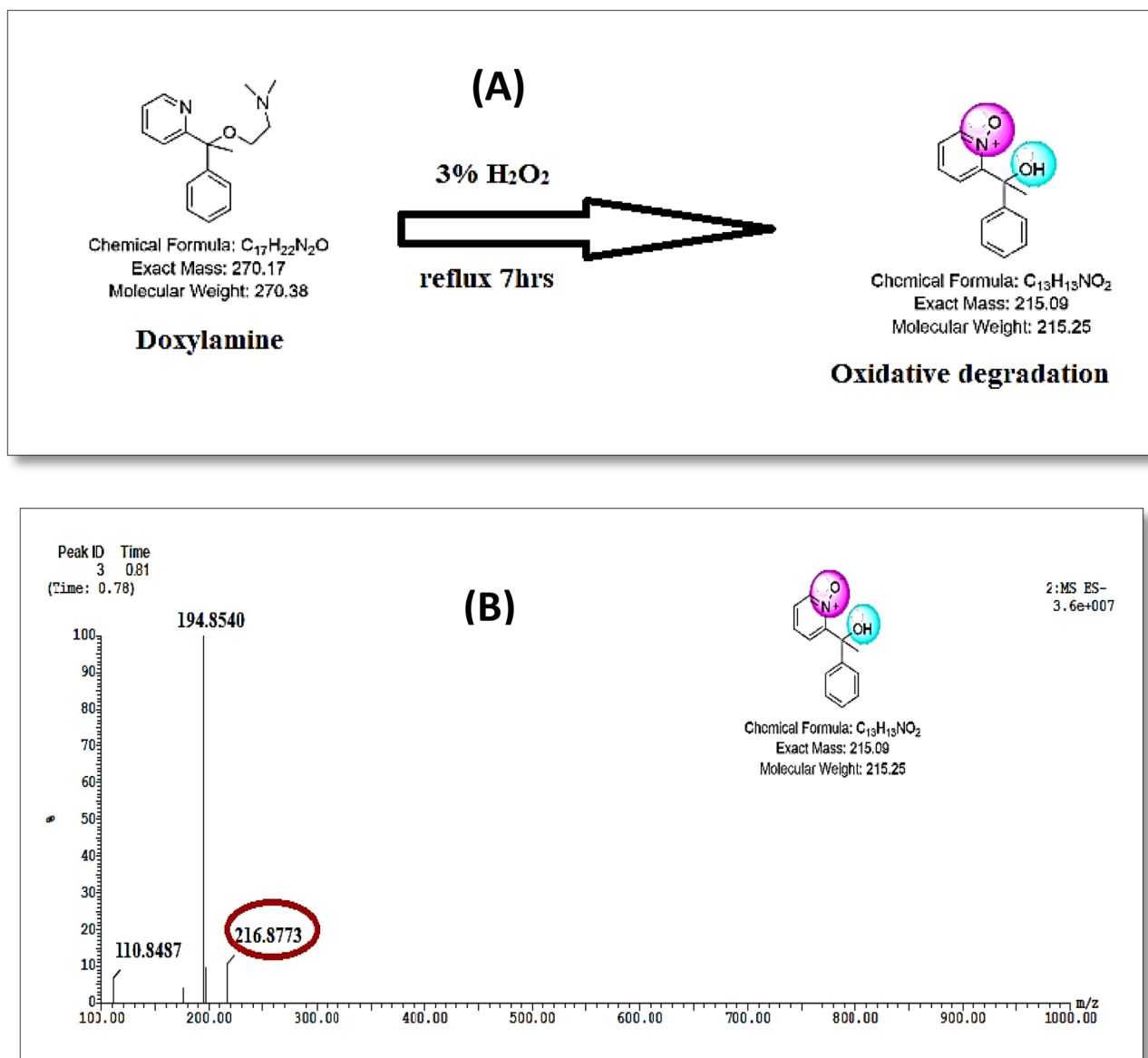
Degradation Behavior of DOX

The DOX solution was oxidized as described previously. According to the proposed degradation process, an alcohol derivative was formed by changing an ether group into an alcoholic one. Furthermore, oxidation of the nitrogen atom in the pyridine ring was observed [51,

Table 2 The effect of interference material on the electro-analytical determination of PYR and DOX by SWVs in acetate buffer (pH 7.0) at ZrO$_2$/MWCNT/MCPE

Interference material 100 (ng mL^{-1})	RSD % of peak current
Cellulose	1.810
Starch	2.057
Glucose	1.932
Sucrose	2.161
Na, Mg, K ions	2.230

52], as illustrated in Scheme 2A. DOX DEG was evaluated by spotting on a TLC plate (the developing system was acetone–chloroform–methanol–25% ammonia solution (7:1.5:0.3:1.2, *v/v*)), which demonstrated the formation of a new spot distinct from DOX. After 7 h, the product of oxidation was determined using a XEVO TQD triple quadrupole instrument from Waters Corporation in Milford, MA01757, USA, a mass spectrometer, an ACQUITY UPLC—BEH C18 1.7 m–2.1 50 mm column, and a mobile phase system consisting of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. The computed molecular weight (M. wt.) of intact DOX was 270.38 m/z, while the structure of DOX DEG was validated by LC–MS analysis in a negative (-ve) ionization mode utilizing ESI–MS negative ion acquisition mode and it had M. wt. of 215.09 m/z (Scheme 2B).

The occurrence of three elements (C, H, and N) where the principle heavier isotope is one mass unit heavier than the most frequent isotope may explain the rise in m/z over the calculated one. When these elements are present in a compound, they produce a tiny isotopic peak with a one-unit mass larger than the molecular ion (M$^+$ + 1) [53]. In terms of SAR of doxylamine pure drug, its pharmacological activity (significant H1 receptor affinity) is influenced by the presence of two phenyl rings and an oxygen atom that work as a connecting atom or spacer group for the needed pharmacophore. Also, in the expanded

Scheme 2 **A** Schematic diagram showing the suggested degradation pathway of DOX and **B** LC-mass spectra showing M. WT of full oxidative degradation of DOX

conformation, the carbon chain between the central point of the diphenyl rings and the terminal nitrogen atom must be 2 to 3 atoms in the range of 5–6 ^{0}A, and the terminal N-atom should be tertiary amine for best activity [54] (Scheme 2). When DOX DEG was detected in the presence of the two additional medications using the prior voltammetry condition, there was no oxidation peak as shown in Fig. 6.

Analytical Eco-Scale Greenness Evaluation of the Proposed Method Versus the HPLC Method

The eco-scale of an analytical method is a semi-quantitative ecological metric system for evaluating analytical procedures, allowing for comparison and selection of the greenest option [55]. The eco-scale tool uses a penalty point system with a starting point of 100 (the optimum score of a green

Fig. 6 Voltammogram of PYR, DOX, and DOX DEG in acetate buffer (pH 7.0) at scan rate 20.0 mV s.$^{-1}$ using ZrO$_2$ 5%/ MWCNT 0.5%/MCPE
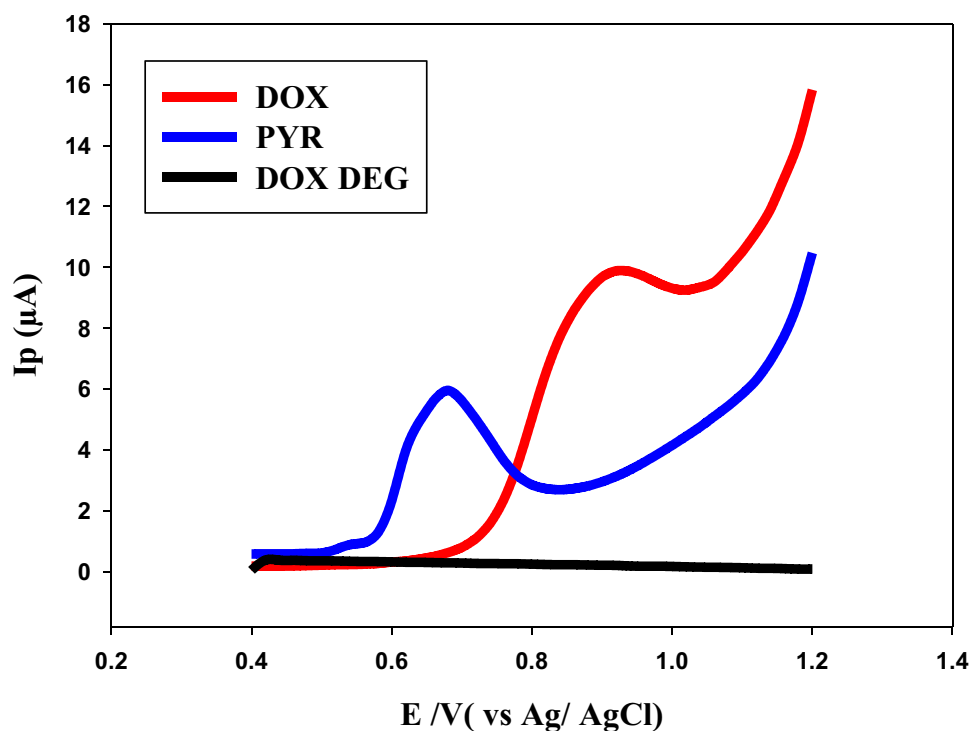
analytical procedure). For each parameter of the analytical procedure (reagent quantity and quality, occupational hazard, energy consumption, and waste generated), penalty points are calculated and subtracted from 100 [56]. The greener and more cost-effective the analytical procedure is, the better the score. The results of the computations are ranked on a scale where a score of > 75 indicates an excellent green analysis, a score of 75 to 50 indicates an acceptable green analysis, and a score of 50 indicates an unsatisfactory green analysis [3, 57]. The analytical eco-scale for the developed voltammetric method and the published HPLC method were computed, and the findings showed that the proposed method excelled over the reported one (Table 3).

Table 3 Penalty points (PPs) for the proposed voltammetric method and reported HPLC methods

Parameters	Penalty points (PPs)	
	Proposed method	**Reported method**
Reagents		
Acetonitrile	-	4
Acetate buffer	0	-
Phosphate buffer	-	0
Instrument		
Energy (˃0.1kWh per sample)	0	1
Occupational hazard	0	3
Waste	3	5
Total PPs	Σ 3	Σ 13
	97	**87**
Analytical eco-scale score	**Excellent green analysis**	**Excellent green analysis**

*HPLC method: phosphate buffer pH 3.5 and acetonitrile (30:70% *v/v*) at flow rate of 1 mL/min, detection at 254.0 nm 28

Table 4 Determination of PYR and DOX in a pharmaceutical formulation using the new proposed sensing protocol

Sample	Amount added standard PYR (µg mL^{-1})	Amount added standard DOX (µg mL^{-1})	Apparent recovery % PYR	Apparent recovery % DOX
Vomibraek tablets (10.00 mg PYR and 10.00 mg DOX)	5.00	5.00	100.76	101.84
	10.00	10.00	99.18	99.50
	20.00	20.00	99.28	101.24
Recovery% ± RSD			99.74 ± 0.881	100.86 ± 1.204

Table 5 Statistical comparison of the results obtained from the proposed methods and the reported method for determination of PYR and DOX

Parameters	Proposed voltammetric method for PYR		Proposed voltammetric method for DOX	
	ZrO$_2$/MWCNT/MCPE	Reported method [b]	ZrO$_2$/MWCNT/MCPE	Reported method [b]
Mean(%)	99.74	102.00	100.86	101.00
SD	0.88	0.72	1.20	0.33
N	6	6	6	6
Variance	1.190	0.525	0.538	0.117
Student *T* test (2.23)[a]	0.140	-	0.110	-
F-value calculated (5.05)[a]	2.251	-	4.702	-

[a]Values between parenthesis are the theoretical values of *t* and *F* at $p = 0.05$

[b]HPLC: A symmetry C18 (4.6×150 mm, 5 µm, phosphate buffer pH 3.5 and acetonitrile 30:70, *v/v*) at flow rate of 1 ml min.$^{-1}$, detection at 254 nm 20

Application to a Pharmaceutical Preparation

Table 4 shows how the suggested method was used to determine PYR and DOX in pharmaceutical manufacture. The proposed method was statistically tested and found to have no significant differences when compared to a previously described HPLC method [26], indicating that the created method is precise and accurate (Table 5).

Conclusion

The emergence of the green chemistry concept has compelled researchers and chemists from all disciplines to consider the environmental impact of the chemicals used in their methods and to assess the greenness of their processes. Voltammetric methods are widely employed in a wide range of applications, including fundamental research of oxidation and reduction procedures in a variety of media. In this study, multi-walled carbon nanotubes and zirconium dioxide were employed as modifier materials on carbon paste electrodes to create a novel electrochemical sensor for green voltammetric detection of PYR and DOX in pharmaceutical tablets or the presence of an oxidized DOX DEG. The modified electrode demonstrated high selectivity, a low detection limit, and a broad linear range. Furthermore, the proposed sensor demonstrated good repeatability, reproducibility, and stability. The developed electrode offered an efficient method for simultaneous determination of PYR and DOX in the presence of glucose, sucrose, starch, cellulose, NaCl, Mg, K, and Na ions in the presence of glucose, sucrose, starch, starch, cellulose, NaCl, Mg, K, and Na ions which around no interference from common excipients expected to be present in pharmaceutical formulation. Nowadays, we are developing the previous voltammetric method to be suitable for application to the plasma of healthy volunteers.

Declarations

Conflict of interest The authors declare no competing interests.

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