Highly Sensitive Voltammetric Sensor Using Carbon Nanotube and an Ionic Liquid Composite Electrode for Xylazine Hydrochloride

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Electrochemical techniques were used for estimating xylazine HCl (XLZ) in bulk powder, medicinal manufacturing and human serum. Electro-oxidation of XLZ at carbon multiwalled nanotube (MWCNT), 1-n-butyl-3-methylpyridinium hexafluorophosphate ion crystal (BMH) and sodium dodecyl sulfate (SDS) MWCNT-BMH-SDS electrode in 0.04 M Britton-Robinson buffer (BR) with pH 7.0, was studied in numerous buffer structures and at different pH values. The experimentation and instrumental parameters to assessable commitment of XLZ had been optimized, and a detection limit was observed as 4.80 nM. The precision and accuracy for the recognized method was tested by retrieval studies with good repeatability and reproducibility of the estimated method. The projected method was practiced successfully to the dosage form and spiked serum.

Keywords Ionic crystal, electroanalysis, xylazine HCl, surfactant, carbon paste electrode, biological fluids

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Introduction

Xylazine (XLZ), a veterinary medicine, is a potent α2-adrenergic agonist used for sedation, analgesia, muscle, and relaxation.12 XLZ (C12H16N2S) (Scheme 1), a thiazine derivative, is registered as veterinary medicine for use in farm animals such as cattle, horses3 and also used in other animals4 such as sheep, goats, llamas, and cervidae. The recommended dose depends on the sensitivity of the animal species.5,6 The pharmacodynamic effect and metabolic pathway of XLZ was studied.7 Xylazine is generally metabolized rapidly in all animals, with a biological half-life of less than 3 h.8 Until now, limited established methods for XLZ analysis have included gas chromatography (GC),9 high-performance liquid chromatography (HPLC),10 GC-mass spectrometry,11,12 ultra high-performance liquid chromatography coupled with quadrupole-time of flight mass spectrometry (UHPLC-QTOF)13 and LCMS.14

The necessity to develop a highly sensitive method for analyzing XLZ products is increased as its residue in animal-derived food may cause food safety problems. The main xylazine biotransformation pathway is most likely thiazine ring break down, and the main product is most likely 2,6-dimethylaniline (DMA).15 Many intoxication cases in humans have been identified in the past years.13 The most important analytical advantages include excellent chemical species selectivity and sensitivity with a low detection range (10–12 to 10–1 M) and rapid analysis time (seconds),16,17 which give it a value position to be used in advanced analysis. The carbon paste electrode (CPE) has been broadly utilized because of extensive benefits, such as growing of peak current. The CMCPEs mechanism depends on the modifier possessions;21 several modifiers can be used as transition metal complexes,22,23 carbon nano tube,24 polymer composite25 and organic compounds26 for their additional benefits, like high conductivity and sensibility. Recently, ionic liquid crystals have gained attention owing to enhancing drug determination in voltammetry. Carbon ionic liquid electrodes (CILEs) display respectable electrocatalytic activities, and are used for various purposes.27–31 For example, the anionic surfactant sodium dodecyl sulfate (SDS) has been used to increase the detection limits in electrochemical studies.32–35 It is appropriate for surface immobilization, acting as a minute conduction center; and allowing electron transmission.36–39

To the best of our knowledge, there are no reported electrochemical methods available for the determination of XLZ. Estimation of XLZ using a carbon multiwalled nanotube (MWCNT), 1-n-butyl-3-methylpyridinium hexafluorophosphate ion crystal (BMH) and sodium dodecyl sulfate (SDS) reformed modified graphite electrode reports are not available. A description for a novel approach of the electrocatalytic modified electrode with MWCNT, BMH and SDS has been performed for XLZ revealing in bulk powder and pharmaceutical dosage form.

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Scheme 1 Chemical structure of XLZ.
Experimental

Reagents and chemicals

XLZ was delivered from Prodivet pharmaceutical company. The potency was certified to be 100.5%.

ProxylaZ® 2% injectable solution was purchased from a local market, and each milliliter contains 20 mg of xylazine base from Prodivet pharmaceutical company, Belgian.

A stock solution of 20 μg/mL XLZ was made with bidistilled water and stored in a refrigerator at 4°C. A carbon multiwall nanotube (MWCNT), 3 – 20 nm o.d., 1 – 3 nm i.d., 0.1 – 10 micron long 95% powder and (BMH) and (SDS), were all obtained from Sigma Aldrich Egypt.

B-R of concentration 0.04 M was organized by mingling phosphoric acid, acetic acid and boric acid\(^\text{46}\) with 0.2 M NaOH to attain the anticipated pH range 2 – 10. All solutions were synthesized using deionized water at room temperature (25 ± 1°C).

Apparatus

Voltammetry measurement. A voltammetric investigation was accomplished by utilizing Metrohm electro analyzers Model 797TA Computrace, Ver. 1.3.1. The employed electrodes were Ag/AgCl (3 M KCl) electrode, a reference electrode, and a platinum wire, an auxiliary electrode. A JENWAY 3510 pH meter (England) was used.

Impedance spectroscopy measurements. Electrochemical impedance spectroscopy was achieved by a Gamry-750 system. The software given by the instrument for data analysis applied a non-linear least-squares fitting by the Levenberg–Marquardt method using calibration formula.

Analysis of pharmaceutical dosage form. A portion of a 1-mL suspension of PROXYLAZ® 2% injectable solution was carefully transferred in a 100-mL volumetric flask and diluted to the mark with double-distilled water, and then filtered to organize a stock of 20 μg/mL. The amount of XLZ was appraised by the linear-regression formula attained from the calibration curve of pure XLZ.

Standard addition technique. The standard addition technique was employed by adding different additions of standard solution to a stable concentration of a drug dosage solution. The quantity of XLZ was gauged by using the linear regression formula attained from the calibration curve of pure XLZ.

Application to human spiked serum samples. Drug-free human blood samples obtained from healthy collaborators after their inscribed acquiescence. It was centrifuged (4000 rpm) for 15 min and detached samples were retained frozen. After softening, an appropriate volume of sample was fortified with XLZ melted in doubly distilled water to required concentration and preserved with 0.5 mL of acetonitrile as serum protein triggering agent and then accomplished to 2 mL with serum. The tubes were vortexed for 30 s and centrifuged 5 min at 4000 rpm to get rid of protein remainders. The supernatant was taken cautiously and a proper volume was shifted in the voltammetric cell holding the supporting electrolyte. The preeminent outcomes were attained with 0.5 mL acetonitrile. The concentration of XLZ was changed in the range of 4 – 10 μg/mL in serum samples. Quantifications were achieved by calibration curve method using calibration formula.

Results and Discussion

Electrodes surface morphology

Figures 1A – 1C illustrate the SEM of CPE, MWCNT/CPE and MCNT-BMH-SDS/CPE, respectively. The SEM of CPE electrode displayed discrete asymmetrical graphite peel (Fig. 1A). Upon modification with MWCNT/CPE, smooth superficial boundaries appeared with flowery formed nanostructures (Fig. 1B). The modest viscosity and decent high conductivity of the MCNT-BMH-SDS/CPE enabled its delivery into the spaces among the past flakes improving its dispersal with the modified graphite paste.\(^{42,43}\) The breakthrough of the exceedingly conductive ionic liquids crystal (ILC) among the graphite flakes caused in a well-ordered films influencing on paste conductivity because of the solid formal assembly of ILC with its molecular orientation. In case of the occurrence of SDS, squishy films of SDS were collected at surface of electrode (Fig. 1C). These films caused in the assistance of the growth of XLZ drug on the surface and mediocrity of electron transference kinetics.\(^{44}\) As observed in the SEM images, the improvement effective surface area enhance the microscopic area of MCNT-BMH-SDS/CPE surface and finally, sensitivities of the response in voltammetric determinations; this is observed also in the electrode area as determined using the Randles Sevcik equation described below.

Electrochemical impedance spectroscopy (EIS) studies

Figure 2 displays the Nyquist designs of K\(_4\)[Fe(CN)\(_6\)] as a semicircle (related to charge transfer resistance) at high frequency and a linear part (related to a diffusion) at low frequency. The well model applied to fitting the experimentations (inset in Fig. 2) is a two time steady model\(^{44,49}\) involves R\(_1\) (solution resistance), R\(_2\), R\(_3\) (resistances of exterior and interior layers, respectively), W (Warburg impedance) and C\(_2\), C\(_3\) (corresponding capacitances of exterior and interior layers, respectively). The data simulated with a 2% average error.
Thus, the reaction is governed by both charge transfer and diffusion process. Table 1 lists the fitting parameters. The MWCNT-BMH-SDS electrode shows higher value for the internal capacitance ($5.11 \mu F$), Warburg impedance ($1158 \Omega \cdot s^{-1/2}$) and lower values for the charge transfer resistance ($1002 \Omega$) indicating a higher conductivity compared to other performed electrodes. These results indicate well the highest peak of oxidation current obtained from CV’s results to MWCNT-BMH-SDS electrode.

Electrochemical performance of XLZ

The electrooxidation performance of XLZ was documented as CV, in the range of 500 to 1400 mV (Fig. 3), in B-R buffer pH 7.0, at a scan rate of 100 mV s^{-1} for the tested electrodes: (bare CPE), (CPE-SDS), (MWCNT-SDS), (BMH-SDS) and (MWCNT-BMH-SDS). For bare CPE, the anodic peak oxidation of XLZ appeared at $E_p = 1250$ mV with a current value of $0.711 \mu A$. The peak current increases upon adding SDS, and then on adding MWCNT, this designated that the SDS and MWCNT have increased the sensor surface area. The BMH can promote the electron allocate among the drug and the bulk electrode surface. Finally, for MWCNT-BMH-SDS, the anodic peak current extended to a highest value of $66 mA$. This may be attributed to an electrostatic attraction amongst the cationic XLZ and the anionic BMH forming hydrogen bond that endorses faster electron transfer developing well the diffusion of XLZ among the surface. The schematic demonstration for this contact is explained in Fig. 3 inset.

Influence of pH

The influence of the electrolyte pH on the oxidation of XLZ at MWCNT-BMH-SDS is represented in Fig. 4 in B-R buffer (pH = 2 - 12). The anodic peak potentials goes negatively with pH implicating that NPF oxidation is a pH-reliant reaction corresponding to the linear-regression formula $E(V) = 1.3539 - 0.0560 pH$, with a correlation coefficient $r^2 = 0.9859$ at room temperature $(25 \pm 1 \degree C)$ and a slope near to a theoretical value of $59$ mV ($56.0$ mV per pH). Thus, the XLZ deprotonation step occurs prior to its electron transfer stage, and the protons and electrons number are equal. A higher anodic current was accomplished for XLZ at pH 7, So, pH 7.0 was chosen as the working pH for XLZ determination to attain higher sensitivity. XLZ is N-(2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine hydrochloride. Nitrogen atom in amino group was found to have an electrically negative charge, owing to lone pair of free electrons on it; so, it is the highest candidate to lose electrons with the rest of the atoms in the molecule. The other nitrogen atom holds to the thiazin ring had the effect of

| Electrode          | $R_1/\Omega$ | $R_2/\Omega$ | $R_3/\Omega$ | $C_2/\mu F$ | $C_3/\mu F$ | $W/\Omega \cdot s^{-1/2}$ |
|-------------------|--------------|--------------|--------------|-------------|-------------|---------------------------|
| CPE               | 220          | 505          | 20400        | 0.37        | 0.16        | 760                       |
| CPE-SDS           | 233          | 389          | 17320        | 1.75        | 1.32        | 909                       |
| MWCNT-SDS         | 245          | 221          | 15411        | 2.81        | 2.30        | 1002                      |
| BMH-SDS           | 235          | 144          | 13200        | 4.81        | 3.18        | 1091                      |
| MWCNT-BMH-SDS     | 184          | 76.0         | 10084        | 5.11        | 4.74        | 1158                      |

Fig. 1 SEM micrographs of: (A) CPE, (B) MWCNT/CPE and (C) MCNT-BMH-SDS/CPE.

Fig. 2 Nyquist plots in [Fe(CN)$_6$]$^{3-/-4-}$ solution (inset: equivalent circuit model).

Table 1 Electrochemical impedance spectroscopy fitting data of Fig. 1
Impact of scan rate

The impact of scan rates on the current response of 1.0 to $10^{-3}$ M XLZ at MWCNT-BMH-SDS in 0.04 M B-R buffer (pH 7.0) was estimated up to a scan rate of 300 mV s$^{-1}$ (Fig. 5A). The positive shift in the peak potential commends that the oxidation process is irreversible. The relation between the peak current versus the square root of scan rate was constructed (Fig. 5B), giving a straight line with an equation of $I_p(\mu A) = 1.725\sqrt{v} + 0.092$; $r^2 = 0.9934$, which indicates mass transfer-controlling process of oxidation via diffusion. 

Validation of the anticipated technique

Under guidelines of International Conference on Harmonization (ICH)$^{56}$ for method justification were followed. DPV mode hired to obtain sharper peaks under optimal conditions, it was achieved using MWCNT-BMH-SDS electrode in 0.04 M B–R buffer pH 7.0 solution with several concentrations of XLZ (Figs. 6A and 6B). The calibration graph was carried out through consideration of the essential practical series, to the XLZ concentration contemporaneous in the pharmaceutical merchandise, to contribute precise, preciseness and rectilinear consequences. The calibration range is displayed that the peak height currents of XLZ oxidation at the surface of MWCNT-BMH-SDS were in linear correlation depending on the XLZ concentration, over the range of 13.3 – 320.0 ng/mL with a slope 2.6173 ng/A and correlation coefficient 0.999.

As identified by International Conference on Harmonization (ICH) approvals,$^{56,57}$ the recognition (LOD) and quantitation limits (LOQ) was estimated to be 4.8 and 14.56 nM, respectively. These precise low values can be attributed to the existence of MWCNT and BMH in the configuration structure of the improved modified electrode.

The interfering effect of excipients in the pharmaceutical form
was estimated by the suggested technique. Intended for this persistence, the standard addition technique was measured to the marketable pharmaceutical preparation form of XLZ. The mean retrievals percentage and its standard deviations for the measurement of the planned procedures are estimated in Table 2, compared to the reference method. Decent exactitude and accurateness were noticed for this technique. Subsequently, the excipients do not have any effect in the estimation of XLZ in its pharmaceutical form. A reliability has been examined, where, the peak current is invariable after storing in air for one week. Also, the modified electrode reserved 98% of its initial response up to 1 month when placed in a refrigerator.

Analytical application
Analysis of PROXYLAZ® 2% injectable solution. It was realized to the XLZ determination in commercial PROXYLAZ® 2% injectable solution (which nominally contain 20 mg XLZ per 1 mL). The XLZ contented is 200.1 ng/mL with a SD of 0.129% (n = 3) per ml close to 200 ng (measured by investigated method). To validate the applicability of the planned electrode for real samples examination, which displayed accurate results with no interfering from excipients. Statistical calculations were made to check the confidence and correlation between the suggested procedures and the reported method. From the calculated t- and F-values at the 95% confidence level, the results obtained by the established method are in good agreement with those obtained by a reported method, as shown in Table 3.

Assay of XLZ in in human serum samples. This method was successfully applied in the determination of xylazine and in human serum that a woman died of xylazine poisoning. The optimized applicability procedure of the projected technique for quantitative estimation of XLZ concentration in human serum was successfully investigated (n = 3); acetonitrile and methanol were investigated as the serum hastening agents. The best effects were found with acetonitrile. So, acetonitrile was exploited for the subsequent studies. The measurements of XLZ in serum samples were achieved as designated in the investigation of spiked serum samples. For the applicability of the suggested way to the human serum, the calibration formula was successfully applied in the determination of xylazine and in spiked serum samples. The results illustrate that the oxidation of XLZ is catalyzed at pH 7.0. The modified electrode demonstrated higher selectivity in voltammetric measurements of pure form, pharmaceutical and in spiked serum samples.

Conclusions
This work validates the direct measurement of an XLZ at a modified carbon MWCNT-BMH-SDS electrode by the incorporation of 1-n-butyl-3-methylpyridinium hexafluoro-phosphate ion crystal as modifying species in pharmaceutical preparations and human serum samples. It was found that the results are convenient for XLZ quantitative determination in conventional supporting electrolytes. The carbon nanotube paste electrode adapted with MWCNT-BMH-SDS was considerably stable up to 1 month on placing in refrigerator. This electrode preparation is easy, simple and easy to renewal surface. The results illustrate that the oxidation of XLZ is catalyzed at pH 7.0. The modified electrode demonstrated higher selectivity in voltammetric measurements of pure form, pharmaceutical and in spiked serum samples.

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