J. Electrochem. Sci. Eng. **16** (2026) 3061; https://doi.org/10.5599/jese.3061

Open Access : : ISSN 1847-9286
https://pub.iapchem.org/ojs/index.php/JESE

Original scientific paper

Sustainable electrochemical sensor for green analysis of diaveridine HCl and sulphaquinoxaline Na in food and pharmaceutical samples

Mai Abd El-Aziz[1], Hadeer F. Manie[2], Heba T. Elbalkiny[3], Marwa I. Helmy[4,5], Dalia Mohamed[4] and Lobna M. Abd El Halim[2,✉]

[1]*Pharmaceutical Analytical Chemistry Department, Misr University for Science & Technology, College of Pharmaceutical Sciences and Drug Manufacturing, 6th of October City, Giza, 12566, Egypt*
[2]*Pharmaceutical Analytical Chemistry Department, Egyptian Drug Authority, Giza, Egypt*
[3]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Art, Egypt*
[4]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, Ain Helwan,11795, Egypt*
[5]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ahram Canadian University, 6th of October City, 12566, Cairo, Egypt*

Corresponding Authors: ✉Lobna_nodcar@yahoo.com; Tel.: +201229592032

Received: September 17, 2025; Accepted: November 20, 2025; Published: December 13, 2025

Abstract

In veterinary medicine, diaveridine hydrochloride (DVH) is a common antiprotozoal medication, and sulfaquinoxaline sodium (SQL) is an antibacterial used to treat intestinal infections, including coccidia. They are added to poultry feed as anticoccidials and their residues might be retained in chicken tissues. Therefore, there is an urgent need for fast determination of the lowest possible DVH and SQL concentrations to guarantee food safety. This study used cyclic voltammetry and square-wave voltammetry. A sensitive electrochemical sensor was developed for fast and selective simultaneous determination of DVH and SQL in drug substances, chicken meat and pharmaceutical formulations. Copper oxide nanoparticles (CuO NPs) served as the foundation for the created tailored sensor. An excellent linearity and high correlation (0.9995 for DVH and 0.9994 for SQL) were identified in the linear responses produced for DVH and SQL utilizing CuO NPs at carbon paste electrode (CPE), which were over the range of 15.00 nM to 120.00 μM for DVH and 90.00 nM to 100.00 μM for SQL. CuO NPs/CPE was used to determine residues in chicken meat as real samples, achieving outstanding recoveries due to its high sensitivity, superior repeatability, and wide linear range. The study also emphasizes the comprehensive environmental impact assessment facilitated by the synergistic application of Modified Complex GAPI (Complex MoGAPI) and AGREE tools.

Keywords

Coccidiosis treatment drugs; voltammetry techniques; modified electrodes; copper oxide nanoparticles; food safety; greenness assessment

Introduction

Diaveridine hydrochloride (DVH), the hydrochloride salt of 2,4-diamino-5-(3,4-dimethoxybenzyl) pyrimidine (Figure 1A) is a veterinary drug used to treat coccidiosis in its clinical form. Its primary usage is as an anticoccidial additive to chicken feed [1]. Coccidiosis is an intestinal tract parasite disease caused by protozoa of the genera *Eimeria* and *Isospora*, which cause intestinal haemorrhage and oedema, leading to weight loss or an increase in animal production and potentially significant financial consequences [2]. Sulfaquinoxaline sodium (SLQ), the sodium salt of 4-amino-N-(quinoxaline-2-yl)benzene-1-sulfonamide (Figure 1B), is the most widely used sulfonamide for the prevention and treatment of bacterial infections and to promote animal growth [3].

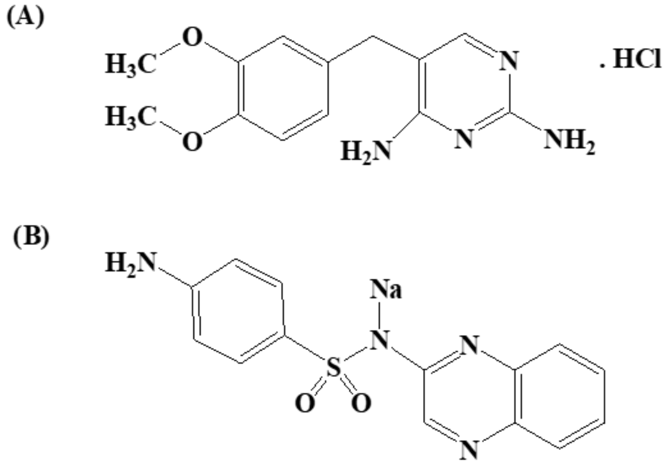


***Figure 1**. Structural formulae of A) diaveridine hydrochloride and B) sulfaquioxaline sodium*

DVH is typically taken with sulphonamides to prevent bacteria from producing dihydrofolate. DVH is a synthetic inhibitor of dihydrofolate reductase (DHFR), an essential enzyme in bacterial folate synthesis [4], while SQL interferes with the biosynthesis of folic acid in bacterial cells by competitively preventing para-aminobenzoic acid (PABA) from incorporation into the folic (pteroylglutamic) acid molecule [5]. Thus, the two distinct mechanisms by which this traditional medication combination inhibits bacterial folate metabolism confer synergistic efficacy [6,7]. Therefore, SQL and DVH are frequently used together as antiprotozoal drugs for the prevention and treatment of chicken coccidiosis, fowl cholera, leucocytozoonosis, and swine coccidiosis [8].

Most medications given to animals that produce milk and meat may cause their residues to accumulate in the muscles and organs of the animal. Monitoring drug residues in animal tissues and products is therefore crucial to ensuring food safety, as consuming these residues can put consumers' health at risk of conditions such as hypersensitivity reactions, allergies, reproductive disorders, and the development of antibiotic-resistant bacteria [9].

Frequent use of coccidiostats often leads to the accumulation of veterinary drug residues in food products intended for human consumption, posing harmful health risks to consumers. To protect consumers, it is crucial to control veterinary drug residue [10]. Therefore, there is an insistent need to develop analytical techniques for determining these residues. American and European organizations have set maximum residue levels (MRLs) of 100 μg kg^{-1} for DVH and SQL in foodstuffs of animal origin to safeguard consumer health [10]. Inappropriate use of these medications, however, could lead to their remaining in animal tissue beyond the MRL range [3,11].

The literature survey revealed that various analytical techniques have been employed to determine DVH and SQL. Chromatographic methods have been reported for the quantitative analysis of DVH [12-16] and SQL [15-18]. Spectrophotometric techniques have also been developed for analysis of DVH [19,20] and SQL [19-21], while voltammetric approaches have also been described for the determination of DVH [22,23] and SQL [24-26].

Electrochemical techniques like square-wave voltammetry (SWV) and cyclic voltammetry (CV) are quick, easy, and sensitive, and usually regarded as suitable substitutes for traditional methods for identifying organic molecules that undergo oxidation or reduction reactions, such as medications and related molecules in pharmaceutical preparations and biological fluids [27,28].

Electrochemical sensors have been regarded as the most promising sensor candidate among all chemical sensors, accounting for about 58 % of the total, for various applications (such as food, oil, and biomedical industries, as well as environmental and agricultural analyses), as they offer an affordable and practical alternative for the detection of variable analytes. The major benefits of electrochemical sensing are experimental simplicity, miniaturizability, low cost and low theoretical detection limits that result from variations in the Faradaic and non-Faradaic currents, as well as the variability of the reporting signals, such as the voltage, current, overall power output, or electrochemical impedance [29,30].

Among the oxides of transition metals, CuO has attracted much attention because it is the basis of several high-TC superconductors. CuO is a monoclinic structure and a p-type semiconductor compound with a narrow band gap and used for photoconductive and photo-thermal applications [31]. Recently, there has been significant interest in copper oxide nanoparticles (CuO NPs) due to their wide potential window, excellent catalytic properties, effective surface areas, superior thermal conductivity, antimicrobial activity, photovoltaic properties, non-toxicity, good environmental acceptability, high chemical and physical stability, good biocompatibility, and effective electronic transfer. These unique features can be attributed to their high-surface area, minimal overvoltage, and enhanced electrode kinetics, leading to low detection limits, high sensitivity, and rapid responses [32-36].

To date, no voltammetric method has been described in the literature for the simultaneous determination of DVH and SQL residues in tissues and pharmaceutical formulations. Thus, our work aims to develop, investigate and optimize the experimental conditions for the simultaneous determination of DVH and SQL in different matrices using a new sensor, CuO NPs/CPE, as a rapid and sensitive method. Additionally, the study aims to assess the environmental sustainability of the proposed method by evaluating its alignment with the principles of green analytical chemistry (GAC) [37]. This includes a comprehensive greenness assessment using multiple evaluation tools such as the Complex MoGAPI [38] and AGREE [39] to critically examine the ecological footprint and overall environmental compatibility of the developed method.

Experimental

Instrumentation

Electrochemical experiments were conducted on a PalmSens4 potentiostat operated with PSTrace 5.0 software (PalmSens, The Netherlands). The electrochemical operating cell consisted of carbon paste electrode (CPE), which was used as a working electrode, a counter electrode of platinum, and a reference electrode of Ag/AgCl.

A Cyberscan 500 digital pH-meter with a glass combination electrode was used for pH measurements (EUTECH Instruments, USA).

Electrochemical impedance spectroscopy measurements were performed over a frequency range of 100 kHz to 0.1 Hz at a dc potential of 0.23 V. All electrochemical experiments were carried out at a temperature of 25 °C.

Transmission electron microscopy (TEM) was carried out at an accelerating voltage of 200 kV using a JEOL JEM-2100 high-resolution transmission electron microscope (JEOL Ltd, Japan).

The XPERT-PRO powder diffractometer was used to obtain an X-ray diffraction (XRD) pattern at a wavelength Kα = 1.54614 nm with 2θ = 20 to 80°, and a minimum step size 2θ = 0.001 (Malvern Panalytical, Netherlands).

Fourier-transform infrared (FT-IR) spectra were obtained using Thermo-Scientific's NicoletTM iS50 (Thermo Fisher Scientific, USA).

Materials and reagents

DVH and SQL were supplied by Nanhai Beisha Pharmaceutical Co. The purity was reported as 99.20 % for DVH and 99.63 % for SQL.

SULPHACOX W.S.P (SQL 16 wt.% and DVH 2.5 wt.%), (B.N. 2184329), was purchased from the market. Chicken meat was purchased from the local market. Copper oxide nanoparticles were obtained from Nano Gate, Egypt.

Graphite powder with a particle size of 50.0 µm was supplied by Sigma-Aldrich, Germany. Paraffin oil (Merck Co., Germany) was used as the pasting liquid in the production of the paste electrodes. 0.04 M Britton-Robinson buffer (B-R buffer) was utilized as a supportive electrolyte. Phosphoric, acetic, and boric acids (Sigma-Aldrich, Germany) were mixed with 0.20 M NaOH to reach the required pH (2.0-10.0) [40].

Double-distilled water from the Human Corporation's New Human Power I water purification system (Seoul, South Korea) was used. All reagents were used as obtained from suppliers without further purification and the solutions were prepared in double-distilled water.

Preparation of bare carbon paste electrode

A carbon paste electrode (CPE) was prepared by mixing 0.50 g of graphite powder with 0.30 mL of paraffin oil in a glass mortar. The obtained paste was used in packing the cavity of the electrode and filter paper was used to smooth the electrode surface till obtaining the shiny appearance of the electrode.

The diameter of CPE was 2.0 mm, giving a geometric area of 0.0314 cm^2 and the diameter of the glassy carbon electrode (GCE) was 3.0 mm, giving a geometric area of 0.07 cm^2. Before each electrochemical experiment, the glassy carbon electrode was manually polished with 0.5 µm alumina powder on a smooth polishing cloth to increase the sensitivity and resolution of the voltammetric peaks. Following a thorough rinsing with methanol and double-distilled water, it was gently dried with tissue paper.

Preparation of ZnO nanoparticles/carbon paste electrode

A mixture of 980.00 mg of graphite powder and 20.00 mg of zinc oxide nanoparticles (ZnO NPs) was used to fabricate the sensor. Using a mortar, the mixture was gently homogenized for 45.0 min. The paste was then made by adding 0.60 mL of paraffin oil. The paste was loaded into the electrode's cavity and smoothed with filter paper until it gleamed. By removing the surplus paste from the syringe and polishing it with weighing paper, a new surface was obtained.

Preparation of CuO nanoparticles/carbon paste electrode

To prepare the enhanced sensor, 920.00 mg of graphite powder and 80.00 mg of CuO NPs were mixed. The mixture was gently homogenized for 45.00 minutes using a mortar. The paste was then made by adding 0.60 mL of paraffin oil. After loading the paste into the electrode's cavity, it was polished to a shine using filter paper. A fresh surface was achieved by removing the excess paste from the syringe and polishing it with weighing paper.

The redesigned electrode was dubbed CuO NPs/CPE. The same method was used to create a variety of modified carbon pastes with different proportions of CuO NPs (2.00, 5.00, 8.00 and 10.00 wt.%).

Electrochemical properties of diaveridine hydrochloride and sulfaquinoxaline sodium

To compare the bare GCE, bare CPE, ZnO NPs/CPE and CuO NPs/CPE responses to DVH and SQL, CVs were recorded to measure the current of 0.10 mM DVH and SQL, separately, in 0.04 M B-R buffer, pH 7.0, with a scan rate of 100 mV s^{-1}.

Standard and working solutions

Stock solutions (0.01 M) of DVH and SQL were prepared separately by dissolving accurately weighed quantities of 296.75 and 322.32 mg, respectively, in distilled water and diluting to 100 mL in volumetric flasks. The stock standard solutions were diluted with the same solvent to provide working solutions for DVH and SQL, with concentrations ranging from 1.00 mM to 10.0 µM. These solutions could be stored for about a week at 4 °C.

Experimental procedure

Prior to performing any voltammetric measurements, the modified electrode CuO NPs/CPE was repeatedly cycled between 0 and 1400 mV at a scan rate of 100 mV s^{-1} in a 0.04 M B-R buffer solution of pH 7.0, until a reproducible response was obtained. Then, the modified CuO NPs/CPE electrode was transferred to a separate cell containing the appropriate amounts of DVH or SQL and 0.04 M B-R buffer, pH 7.0. CVs were recorded between 0 and 1400 mV at a scan rate of 100 mV s^{-1}.

Recommended procedure for calibration curves

DVH aliquots equivalent to 15.00 nM to 120.00 µM and SQL aliquots equivalent to 90.00 nM to 100.00 µM were transferred separately using a micropipette into a series of 10 mL volumetric flasks, and the volume was adjusted to the mark using 0.04 M B-R buffer, pH 7.0. After transferring the solution to the electrolytic cell, a square wave voltammogram (SWV) was recorded. The scan rate used in this investigation was 100 mV s^{-1}. Pulse height, pulse width, step height, and scan rate were finally chosen to be 10, 25, 50 and 100 mV s^{-1} to get relatively high and narrow peaks. According to the requirements of the International Conference on Harmonisation (ICH), the determination of DVH and SQL by the SWV analytical technique was validated [41].

Application to pharmaceutical formulation

A precise amount of 1 g of SULPHACOX W.S.P.® powder, which was claimed to contain 25.00 mg of DVH and 160.00 mg of SQL, was transferred into a 100 mL volumetric flask. After extracting the powder for 20 minutes using a vortex shaker and 30.0 mL of distilled water, the mixture was filtered through 0.45 µm filter paper and transferred into a 100 mL volumetric flask. Then the volume was completed to the mark with water. Further dilutions were carried out to obtain final concentrations of 8.42 µM for DVH and 49.64 µM for SQL. One mL was precisely transferred into a 10 mL volumetric flask, and the volume was completed to the mark with 0.04 M B-R buffer, pH 7.0. Then the proposed method was applied.

Application to chicken meat

5.00 g of chicken meat was weighed and then homogenized for 5 min. The homogenized tissues were spiked with 1.00 mL of a stock solution (10.00 mM) containing a mixture of 296.75 mg DVH and 322.32 mg SQL. The spiked sample was mixed with 15 mL of acetonitrile and homogenized again at 5000 rpm for 5 min. After 15 minutes of sonication in an ultrasonic bath, the homogenate was centrifuged for 5 minutes at 3000 rpm. The mixture was re-extracted twice, adding 10 mL of acetonitrile each time. In 50-mL volumetric flasks, the extracts were combined and distilled water was used to complete the volume [42,43] and filtered through a 0.45 µm syringe filter. Then aliquots of the filtrate equivalent to (90 nM to 80 μM) for both drugs were added to 10 ml volumetric flask and completed to the mark with 0.04 M B-R buffer of pH 7.0 to be measured. Then the proposed method was applied.

Results and discussion

Characterization of CuO nanoparticles

Transmission electron microscope

According to the TEM image of the CuO, the synthesized copper oxide exhibited a spherical shape with an average size of 30±5 nm. Spherical CuO NPs having a large surface area lead to higher reactivity [44] (Figure 2).

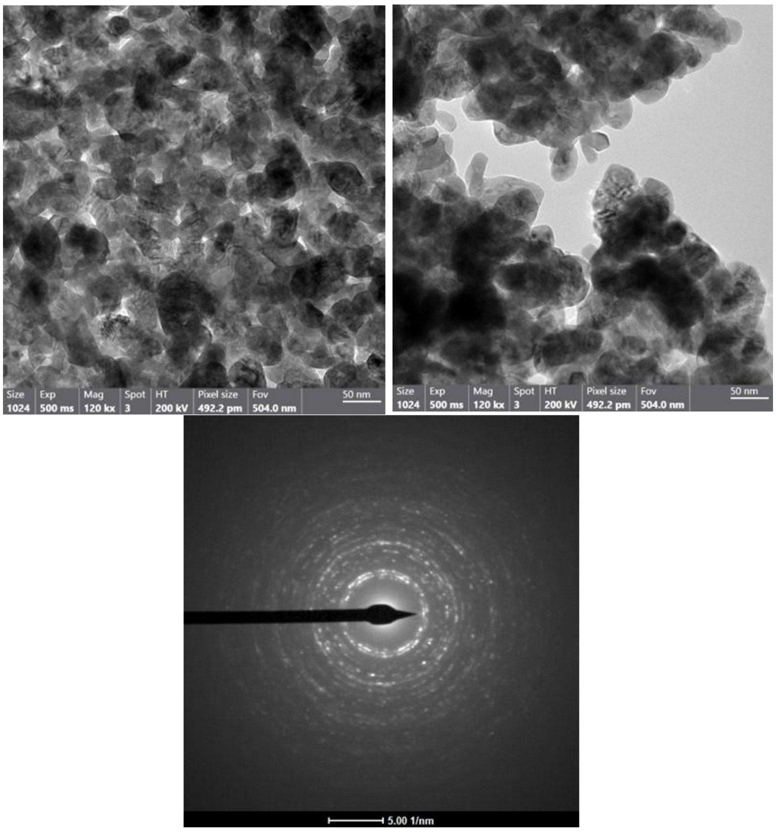


Figure 2. *TEM images and SAED pattern of CuO NPs*

X-ray diffraction analysis

CuO NPs with a purity of ≥ 99 % exhibit a strong match with the sample (Figure 3).

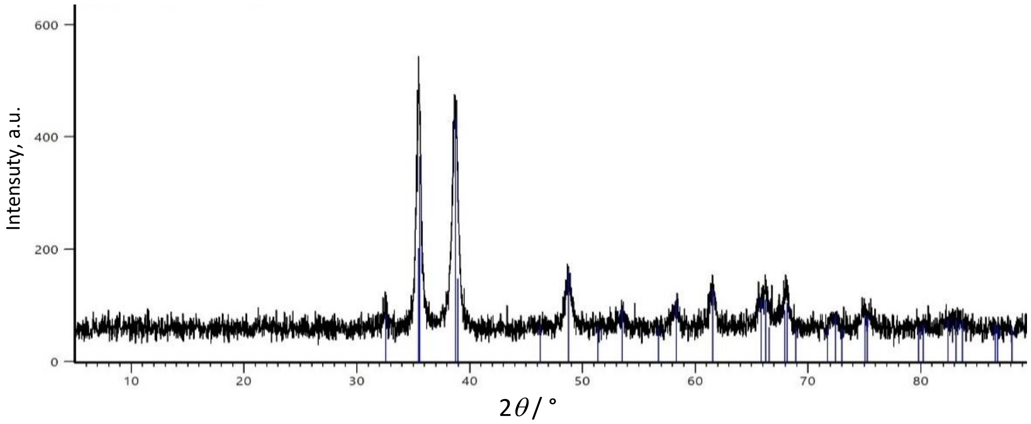


Figure 3. *The XRD pattern of CuO NPs*

Fourier-transform infrared spectra analysis

The FTIR spectrum of CuO (Figure 4) shows no peaks around 3500 cm^{-1}, indicating formation of CuO without the formation of the hydroxide phase, while the strong absorption peaks observed in the range of 500 to 800 cm^{-1} are due to the stretching vibrational modes of CuO [45]. These findings confirm the purity and successful formation of CuO in a pure phase.

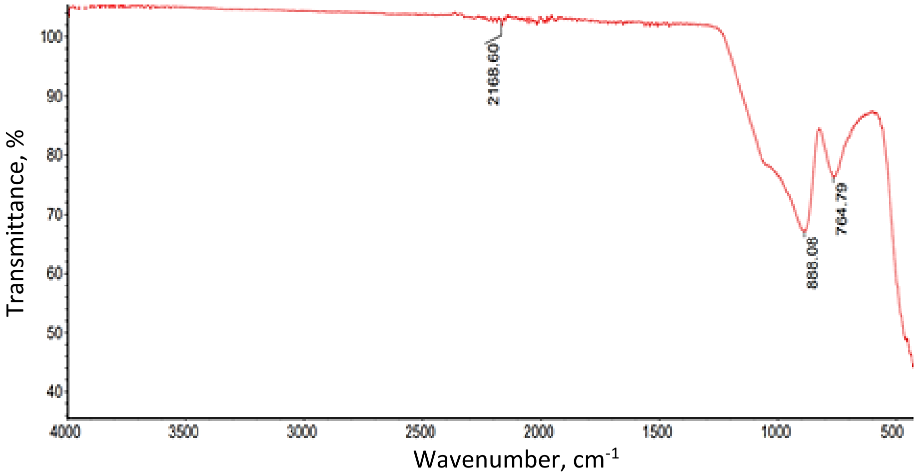


Figure 4. *FTIR spectrum of CuO NPs*

Electrochemical behaviour of the developed electrode

The surface area of CPE and the constructed sensor electrode of 8.00 wt.% CuO NPs/CPE was determined using the Randles-Ševčik equation [46] and measuring CVs in the presence of 5.00 mM $K_3[Fe(CN)_6]$ probe at different scan rates (Figure 5). CPE and 8.00 wt.% CuO NPs/CPE had surface areas of 0.055 and 0.293 cm^2, respectively. The gathered information demonstrated that using copper oxide nanoparticles in the CuO NPs/CPE sensor increases the electrode area and the electrochemical activity [47].

The CVs of bare GCE, bare CPE, ZnO NPs/CPE and CuO NPs/CPE were examined using 5.00 mM $K_3[Fe(CN)_6]$ as the electrochemical probe to better understand the electrochemical behaviour of the electrodes. Figure 6A shows that, among the other sensors, the highest anodic peak current was obtained at 8.00 wt.% CuO NPs/CPE, which may be ascribed to its greater surface area and good electrochemical conductivity owing to the integration of CuO nanoparticles. CuO NPs/CPE has 3.19 times more intense redox peaks than CPE.

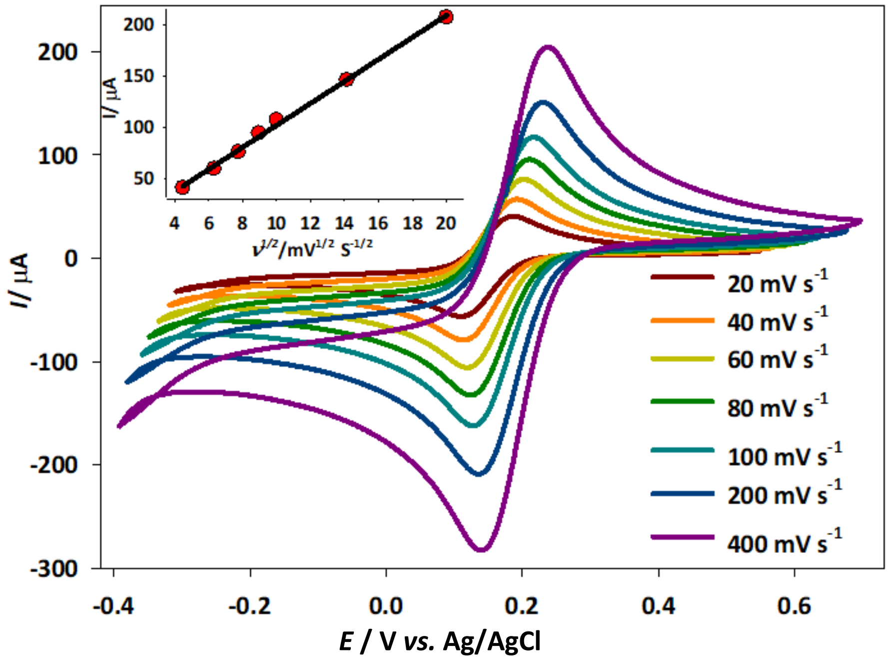


Figure 5. *CVs of CuO NPs/CPE in 5.00 mM $K_3[Fe(CN)_6]$ solution in 0.10 M KCl at different scan rates. The inset shows the anodic I vs. $v^{1/2}$ plot*

Furthermore, CV responses in the presence of 0.10 mM DVH and SQL, separately, in 0.04 M B-R buffer, pH 7.0, were used to analyse the modified sensor's electrochemical behaviour. The electrochemical oxidation of DVH and SQL are represented by the anodic peak current were 28.30 μA at 1134.52 mV for DVH (Figure 6C) and 26.00 μA at 885.22 mV for SQL (Figure 6D) when using bare CPE, whereas the current magnitude of DVH and SQL increased to 103.93 μA at 1085.31 mV for DVH and 92.06 μA at 821.29 mV for SQL when using 8.00 wt.% CuO NPs/CPE. Comparing the electrode responses, the CuO NPs/CPE have the highest anodic current. As a result of their strong catalytic activity, CuO NPs act as a mediator, increasing the rate of electron transfer.

Electrochemical impedance spectroscopy (EIS) was used to obtain a convenient determination of both mass-transport and kinetic parameters, as well as the charge transfer coefficient [48]. Figure 6B depicts the EIS response for bare GCE, bare CPE, ZnO NPs/CPE and CuO NPs/CPE. EIS measurements were performed in a 5.0 mM $K_3[Fe(CN)_6]$ solution in 0.1 M KCl. As shown in Figure 6B, a semi-circle was verified at the region of high frequencies and as is commonly known, the diameter of this semi-circle is equivalent to the charge transfer resistance across the electrode interface. Thus, as a preliminary qualitative analysis of the Nyquist plots, a decrease in the semi-circle diameter for the modified CPE with CuO NPs is observed, indicating an improvement in charge transfer at this electrode compared to the bare CPE.

The equivalent circuit model (inset of Figure 6B) was selected to fit the acquired impedance data for the electron transfer process (modelled as a charge-transfer resistance, RCT) and the diffusion process (modelled as a Warburg impedance, W). The R_{CT} and W are both in parallel with the interfacial capacitance (C). By fitting the data, the R_{CT} was estimated to be 5546 Ω at the bare CPE, decreasing to 1420 Ω for CuO NPs/CPE, indicating the significantly lower electron-transfer resistance of CuO NPs/CPE compared with other electrodes. The results are consistent with the CV results, suggesting that the CuO NPs/CPE composite may provide higher electron-conducting pathways.

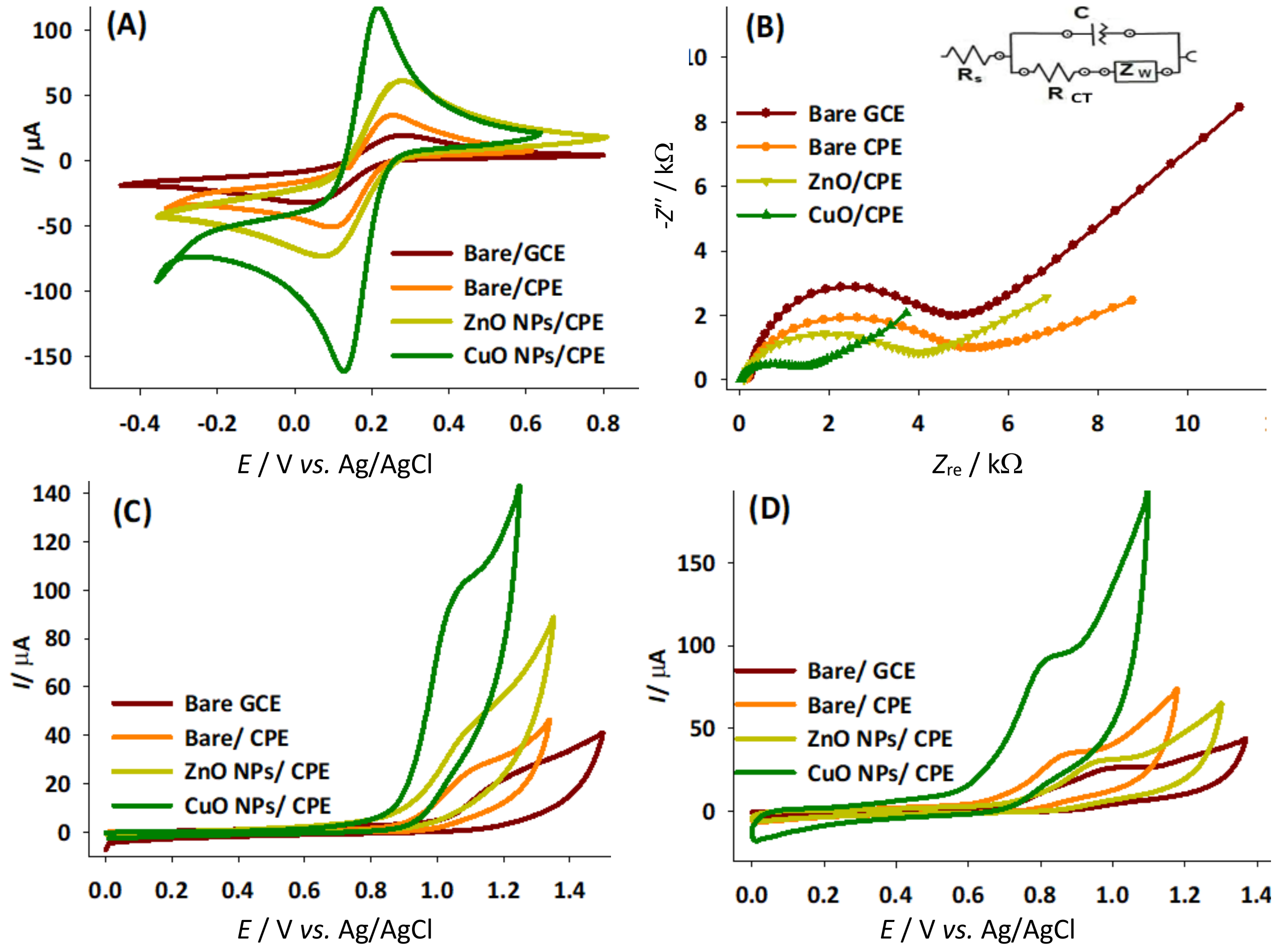


Figure 6. *(A) CVs at a scan rate of 100.0 mV s^{-1} and (B) impedance plots of 5.00 mM $K_3[Fe(CN)_6]$ in 0.10 M KCl solution. CVs of 0.10 mM of (C) DVH and (D) SQL in 0.04 M B-R buffer pH 7.0, recorded at bare GCE, bare CPE, ZnO NPs/CPE and CuO NPs/CPE electrodes*

Effect of CuO nanoparticles content

The content of CuO NPs used in sensor manufacture determines the electrochemical behaviour of CuO NPs/CPE. Using ratios ranging from 2.0 to 10.0 % as a function of CuO NPs, the anodic peak current for DVH and SQL oxidation at the CuO NPs/CPE electrode was investigated (Figure 7).

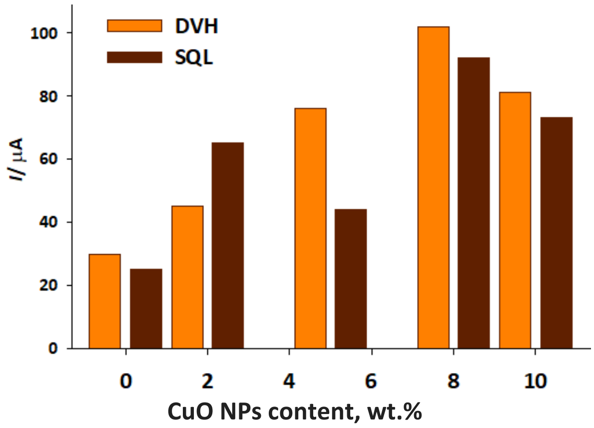


Figure 7. *Effect of different CuO NPs percentages used to fabricate CuO NPs/CPE on anodic peak current values of 0.10 mM DVH and 0.10 mM SQL in 0.04 M B-R buffer pH 7.0*

The optimal anodic peak current was obtained when the CPE was modified with 8.0% CuO NPs. The background current increased with increasing CuO NP concentration, reducing the reproduceibility of the DVH and SQL responses. The emergence of non-Faradaic current is the term used to describe the increase in background current. In basic voltammetric research, experimental setups are typically designed to have very low non-Faradaic contributions [49].

pH optimization

The electrochemical behavior of DVH and SQL depends on the buffer solution pH. In the pH study, CV was performed on the CuO NPs/CPE using 0.1 mM of DVH and SQL separately over a pH range of 2.0 to 10.0 in a 0.04 M B-R buffer solution at a scan rate of 100 mV s^{-1}. This scan rate was selected because it yielded relatively high sensitivity and well-shaped voltammetric curves with a narrow peak width. Figure 8A and Figure 8B represent the behavior of DVH and SQL, respectively, at different pH. It can be seen that peak potentials (E_p) shift towards more negative values with increasing pH (indicating that the oxidation of DVH and SQL is pH-dependent). The maximum anodic peak current was observed at pH 7.0 and was therefore selected as the optimum pH for the sensitive simultaneous determination of DVH and SQL. Examination of E_p values shows a linear relationship with buffer solution pH, which can be represented as: E_p = 1.40 - 0.050 pH (R^2 = 0.9991) for DVH, and E_p =1.17 - 0.054 pH (R^2 = 0.9992) for SQL. The slopes of the linear E_p *vs.* pH relationship are 50.0 and 54 mV pH^{-1} for DVH and SQL, which obey the Nernst equation, indicating that the electrochemical oxidation process involves an equal number of electrons and proton transfer reaction [48]. The proposed mechanisms for the oxidation of DVH and SQL are-revealed in Scheme 1.

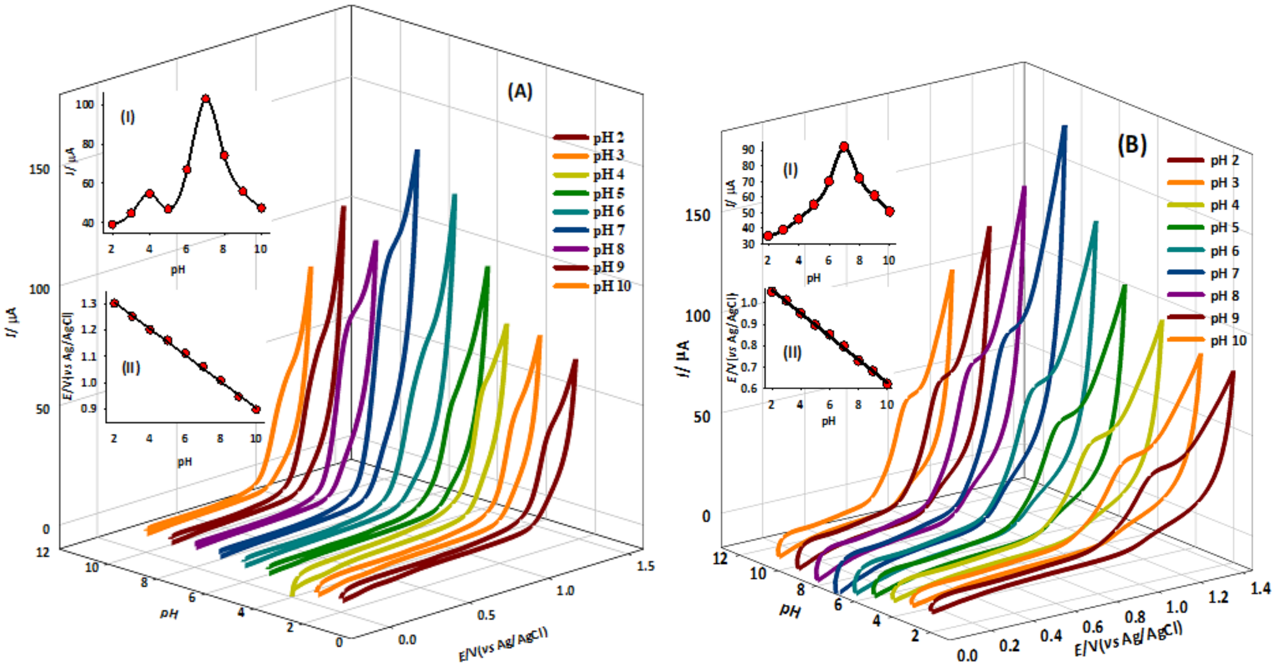
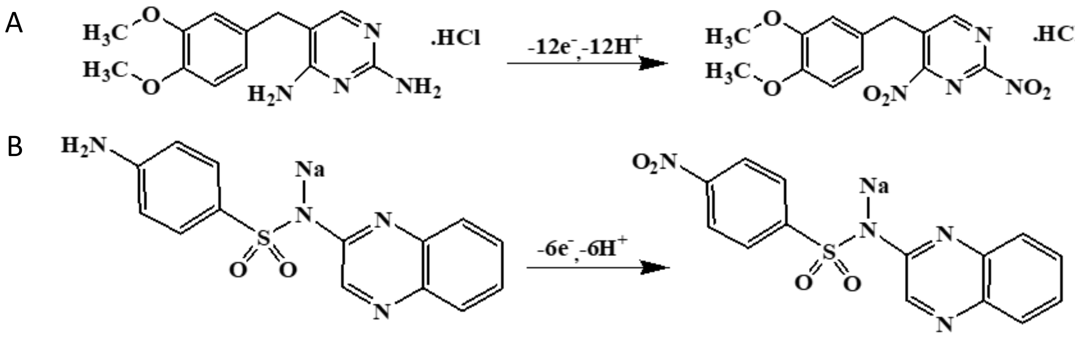


Figure 8. *CV responses of (A) 0.10 mM DVH and (B) 0.10 mM SQL at different pH values using CuO NPs/CPE, at scan rate 100.00 mV s^{-1}. The insets show plots of anodic peak (I) current, and (II) potential versus pH*



Scheme 1. *The suggested electrochemical oxidation mechanism of A) DVH and B) SQL*

Scan rate impact

To explore the process behaviour at the sensor surface and to evaluate the electrochemical strategy of the sensor reaction, CVs of a constant concentration (0.10 mM for both drugs) at pH 7.0 were collected at varied scan rates (Figure 9).

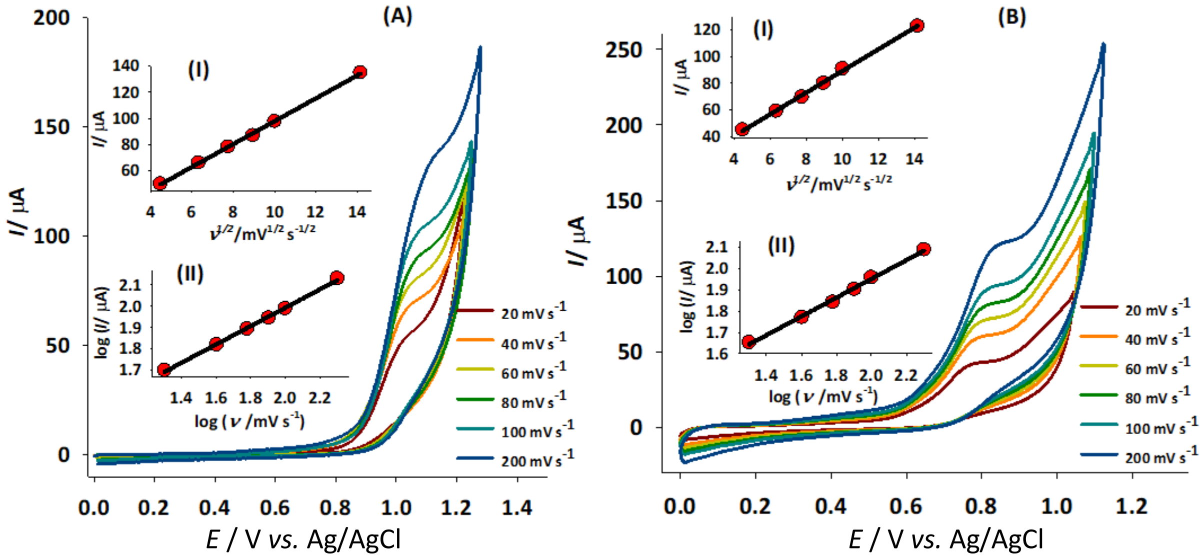


Figure 9. *CVs of (A) 0.10 mM DVH and (B) 0.10 mM SQL in 0.04 M B-R buffer pH 7.0 using CuO NPs/CPE sensor, recorded at various scan rates (20.0 to 200.0 mV s^{-1}). Insets: (I) plots of peak current vs. the square root of scan rate, and (II) plots of log peak current vs. log scan rate*

The increase in scan rate was accompanied by higher peak currents (I). As shown in insets (I) of Figure 9A and 9B, the I values for both drugs increased linearly with the square root of the scan rate, indicating that the process is diffusion-limited. For the oxidation process of DVH, the appropriate linear equations were $I = 8.75\ \nu^{1/2}(\text{mV}^{1/2}\ \text{s}^{-1/2}) + 10.39$, $R^2 = 0.9996$, while for SQL, $I = 8.16\ \nu^{1/2}(\text{mV}^{1/2}\text{s}^{-1/2}) + 7.70$, $R^2 = 0.9992$. The processes at the working electrode surface are clearly diffusion-controlled, as indicated by the foregoing findings.

Insets (II) of Figure 9A and B, show direct proportionality between log of peak current and log of scan rate in the 20 to 200 mV s^{-1} range, defined by $\log I = 0.43 \log \nu + 1.13$, $R^2 = 0.9992$ for DVH and $\log I = 0.44 \log \nu + 1.07$; $R^2 = 0.9984$ for SQL. The slope of the derived linear relationships is less than 0.5, indicating that the electroactive constituents of the two drugs are transported via a diffusion-controlled mechanism, and that the modified electrode, CuO NPs/CPE, plays a substantial role [49].

Chronoamperometric measurements

Cottrell's law [50] describes the current corresponding to the electrochemical reaction (under diffusion control) for an electroactive material with the diffusion coefficient (D) as Equation (1):

$$I = \text{n}FAC(D/\pi t)^{1/2} \qquad (1)$$

where A is the electrochemically active area, D is the diffusion coefficient, C is the analyte concentration, t is time, while the rest of the parameters have their standard definitions.

The diffusion coefficients of analytes were calculated for bare CPE and CuO NPs/CPE using the Cottrell equation [51]. From Figure 10, the diffusion coefficient values were found to be 0.0439 and 0.00130 mm^2 s^{-1} for DVH and SQL, respectively. The diffusion coefficient value of CuO NPs/CPE is greater than that of CPE, showing that the modified electrode has more diffusion capacity than the bare electrode and the CuO NPs facilitated the charge transfer and diffusion of ions through the electrode surface.

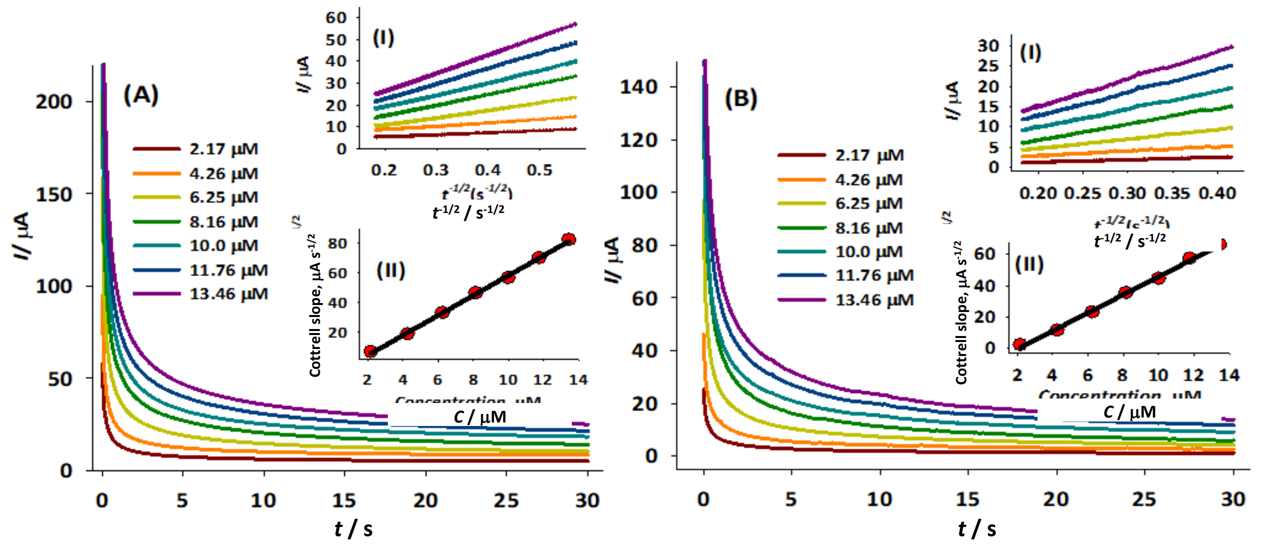


Figure 10. *Chronoamperograms for the oxidation of different concentrations of (A) DVH and (B) SQL at CuO NPs/CPE surface in B-R buffer, pH 7.0, for a potential step of 1085.31 mV for DVH and 821.29 mV for SQL vs. Ag/AgCl. Insets (I): I vs. $t^{-1/2}$(Cottrell equation) and insets (II): Cottrell slope of the straight lines against concentration of drugs*

Validation of the proposed CuO nanoparticles/carbon paste electrode

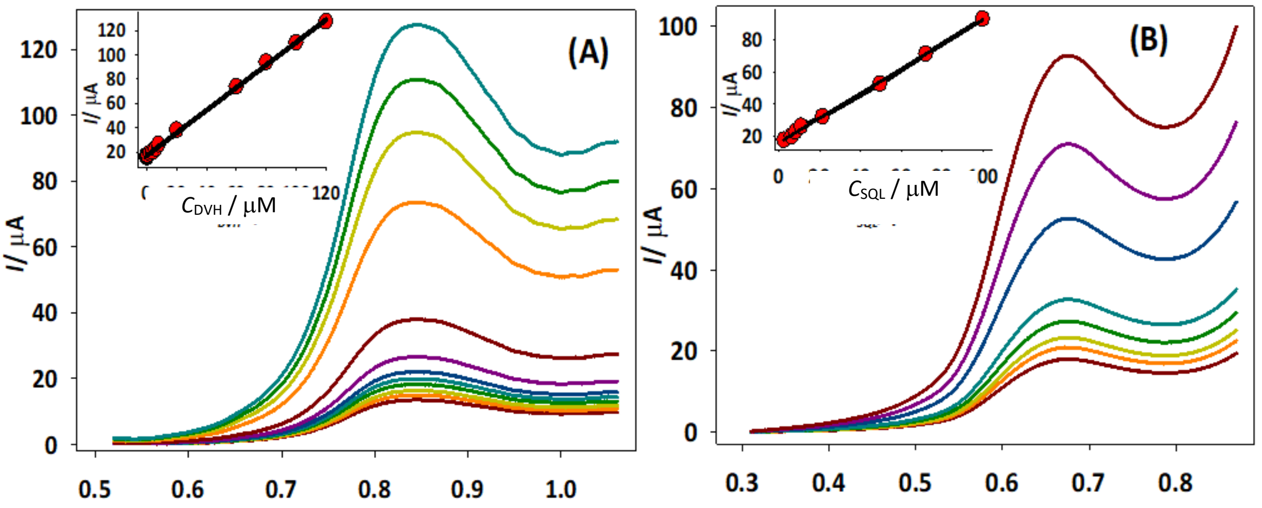
The linearity, selectivity, precision, and accuracy of the created electrochemical platform were tested in accordance with the International Conference on Harmonization (ICH) requirements [41].

Linearity

Using the CuO NPs/CPE, it was discovered that the peak currents related to the electrochemical oxidation of DVH are linearly dependent on DVH quantity over the range of 15.00 nM to 120.00 µM, while the regression equation is found to be: $I = 0.934\ C_{\mathrm{DVH}} + 17.01$; $R^2 = 0.9995$ (Figure 11A). On the other hand, the linearity range for SQL is found to be 90.00 nM to 100.00 µM and the regression equation is found to be: $I = 0.756\ C_{\mathrm{SQL}} + 16.34$; $R^2 = 0.9994$ (Figure 11B).

Detection and quantitation limits

The limit of detection (LOD) and the limit of quantification (LOQ) were determined to be 3.35 and 10.14 nM, respectively for DVH, and 12.5 and 37.80 nM, respectively for SQL by utilizing the formulas $10S/b$ and $3.3S/b$ (where S is defined as the standard deviation of the I and b is referring to the calibration curve slope).



E / V *vs.* Ag/AgCl *E* / V *vs.* Ag/AgCl

Figure 11. *SWVs using CuO NPs/CPE sensor in B-R buffer pH 7.0, corresponding to (A) 15.00 nM to 20.00 μM DVH and (B) 90.00 nM to 100.00 μM SQL. Insets: plots of peak current vs. concentration of drugs*

Accuracy

The percent recovery of the proposed electrochemical procedure was used to assess accuracy [41]. Five repeat assays were performed at varying concentrations of DVH and SQL within the quantification ranges. The accuracy of the disclosed technique for DVH and SQL assay was demonstrated with satisfactory results, as shown in Table 1.

Table 1*. Accuracy of the proposed SWV method for the determination of DVH and SQL in drug substance*

Amount taken, µM	Amount found, µM	Recovery, %
	DVH	
20.00	20.23	101.15
40.00	39.88	99.70
60.00	59.90	99.83
80.00	81.14	101.43
100.00	100.50	100.50
Mean ± RSD*		100.52 ± 0.77
	SQL	
10.00	9.83	98.30
20.00	19.73	98.65
60.00	59.67	100.92
80.00	80.64	101.04
100.00	99.70	99.70
Mean ± RSD		99.72 ± 1.26

*relative standard deviation

Repeatability and intermediate precision

The mean RSD, % was calculated after triplicate examinations of three concentrations of DVH and SQL (20.00, 80.00 and 100.00 µM), performed on the same day (intra-day test) or over three successive days (inter-day assay). As shown in Table 2, the estimated average RSD for the intra- and inter-day studies did not exceed 2 %, demonstrating acceptable precision. The stability of the CuO NPs/CPE was investigated over an 8-day storage period in air, with no change in the voltammetric peak current. Table 2 shows that the sensor maintained 98.75 to 101.29 % of its initial response after 14 days.

Table 2. *Repeatability and intermediate precision of the proposed SWV method for the determination of DVH and SQL in drug substances*

Amount taken, µM	Intra-day		Inter-day	
	Amount found ± SD*, µM	Precision (RSD, %)	Amount found ± SD*, µM	Precision (RSD, %)
		DVH		
20.00	20.11 ± 0.216	1.074	20.01 ± 0.129	0.645
80.00	80.14 ± 0.264	0.329	79.67 ± 0.670	0.841
100.00	100.45 ± 0.665	0.662	99.83 ± 0.791	0.792
		SQL		
20.00	19.84 ± 0.127	0.640	20.11 ± 0.246	1.223
80.00	79.00 ± 0.400	0.506	81.03 ± 0.351	0.433
100.00	100.65 ± 0.855	0.849	101.08 ± 0.288	0.285

*standard deviation

Statistical analysis

Student t-test and F value were used in data comparison and results showed that there are no significant differences between the proposed method and the manufacturer's or reported methods, concluding that the proposed method is accurate and precise as given in Table 3.

Table 3. *Statistical comparison between the proposed method and the manufacturer method for the determination of DVH and SQL*

Parameters	DVH		SQL	
	Proposed method	Manufacturer method[a]	Proposed method	Reported method[b] [18]
Mean[c]	100.52	99.67	99.72	99.07
Standard deviation	0.77	1.28	1.26	1.08
n	5	5	5	5
Variance	0.59	1.64	1.59	1.16
Standard error	0.34	0.57	0.56	0.48
t-value (2.306)[d]	1.26	-	0.88	-
F-value (6.390)[d]	2.74	-	1.37	-

[a]Manufacturer HPLC method for DVH using Eclipse PLUS C18 5 µm, 150×4.6 mm as column and methanol, phosphate buffer (13.61 g of potassium dihydrogen phosphate in 800 mL distilled water adjusted to pH 2.0 with phosphoric acid) then complete to 1000 mL with distilled water), in ratio; 30:70 (v/v) as mobile phase with flow rate 1.5 mL/min, injection volume 10.0 µL and UV detection at 230 nm.
[b]Reported HPLC method for SQL using SEA C18 5 µm, 250×4.6 mm as column and mixture of methanol: acetonitrile: distilled water, in ratio 20:20:60 (v/v) as mobile phase with flow rate 0.8 mL min^{-1}, injection volume 30 µL and UV detection at 230 nm.
[c]Mean of five determinations at four different concentrations.
[d]the values between parentheses are the theoretical values of *t* and *F* at (p = 0.05).

Analytical parameters involving LOD, LOQ, regression, accuracy and precision are summarized in Table 4.

Table 4. *Analytical parameters and validation results for the determination of DVH and SQL in drug substances in 0.04 M B-R buffer pH 7.0 at CuO NPs/CPE using the proposed SWV method*

Analytical parameters	DVH	SQL
Concentration linearity range, µM	0.015 to 120.000	0.090 to 110.000
Regression parameters		
Intercept, µA	17.01	16.34
Slope, µA µM^{-1}	0.93	0.76
Standard error of intercept, µA	0.481	0.456
Standard error of slope, nA µM^{-1}	2.63	9.53
Correlation coefficient	0.9995	0.9994
Accuracy		
Mean* ± RSD, %	100.52 ± 0.77	99.72 ± 1.26
Precision**		
Repeatability (RSD, %)	0.190	0.998
Intermediate precision (RSD, %)	0.230	0.377
LOD, nM	3.35	12.50
LOQ, nM	10.14	37.80

*Mean of five different determinations; ***n* = 9

Analytical application of CuO nanoparticles / carbon paste electrode

Simultaneous determination of diaveridine hydrochloride and sulfaquinoxaline sodium in chicken meat samples

Figure 12A shows SWVs of CuO NPs/CPE in a B-R buffer solution, pH 7.0, containing different concentrations of both drugs (DVH and SQL) in a spiked chicken meat sample. Well-defined anodic peaks at potentials of 951.00 and 714.00 mV correspond to the oxidation of DVH and SQL, respectively. As seen in Figure 12B and 12C, the electrode response to chicken meat is linear over the range 90.00 nM to 80 µM for both drugs, with correlation coefficients R^2 = 0.9995 for DVH and R^2 = 0.9993 for SQL. It is evident from the results that monitoring of DVH and SQL residues can be achieved by the investigated method, as according to the European Union, the maximum residue level (MRL) was stated to be 100 μg kg^{-1} for both drugs [10] in chicken meat (MRL limit is equivalent to 1.68 μM for DVH and 1.55 μM for SQL).

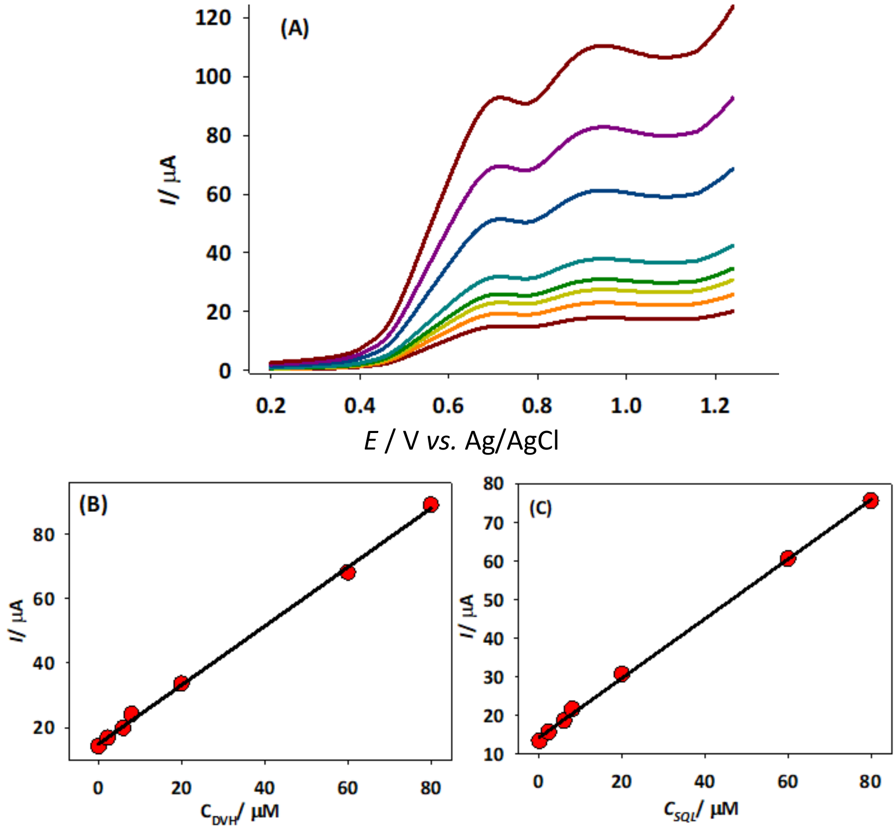


Figure 12. *(A) SWVs of CuO NPs/CPE in a B-R buffer solution, pH 7.0, containing 90 × 10^{-9}- 80.00 × 10^{-6} M of DVH and SQL in spiked chicken meat. Plots of I versus concentration of (B) DVH and (C) SQL*

The data obtained (Table 5) show satisfactory recoveries for DVH and SQL in chicken meat samples, with recovery ± RSD values 95.79 ± 1.34 and 94.10 ± 1.921 %, respectively.

Table 5. *Assay results for the simultaneous determination of DVH and SQL in chicken meat using the proposed method*

Sample	DVH		SQL	
Amount added, μM	Amount found, μM	Recovery, %*	Amount found., μM	Recovery, %*
8	7.54	94.25	7.33	91.63
20	19.04	95.20	18.86	94.30
60	57.85	96.42	57.79	96.32
80	77.83	97.29	75.33	94.16
Mean ± RSD		95.79 ± 1.34		94.10 ± 1.921

*Average of three determinations

Application to the simultaneous determination of diaveridine hydrochloride and sulfaquinoxaline in pharmaceutical formulation

According to the literature, there is no reported work for the simultaneous determination of DVH and SQL by electrochemical methods. Therefore, the main key feature of this work is the determination of DVH and SQL simultaneously in SULPHACOX W.S.P.® powder using CuO NPs/CPE platform to demonstrate the applicability of the proposed electrode. The validity of the proposed method was assessed by applying the standard addition technique.

The sensitivity of the modified electrode towards the oxidation of DVH and SQL in case of the single determinations was 0.93 and 0.76 μA μM^{-1}, respectively. These values are very close to those obtained for the mixture of DVH and SQL, which are 0.90 and 0.74 μA μM^{-1}, respectively. The results indicate that the oxidation processes of these compounds at the CuO NPs/CPE are independent, and therefore simultaneous determination of their mixtures is possible without significant interferences. The listed results in Table 6 show good precision and accuracy of the proposed method, which showed accurate results and there was no interference from excipients.

Based on the experimental results, it is clear that this sensor has significant potential for the determination of trace amounts of these compounds in pharmaceutical formulations.

Comparing our proposed electrode to the reported ultrafine urethane electrode for DVH determination [23], it is obvious that the present voltammetric work showed a wider dynamic range, while the range of the reported electrode was 1.68 to 16.4 μM.

Table 6. *Application of the standard addition method for simultaneous determination of DVH and SQL in SULPHACOX W.S.P.® powder*

Drug	Amount taken, μM	Amount of standard added, μM	Amount found, μM	Recovery, %*
DVH	8.42	20.00	20.27	101.35
		80.00	79.40	99.25
		100.00	99.80	99.80
Mean ± RSD			98.87 ± 0.697	100.13±1.09
SQL	49.64	10.00	9.91	99.10
		30.00	29.87	99.57
		50.00	49.63	99.26
Mean ± RSD			99.82 ± 0.882	99.31±0.241

*Average of three determinations.

Assessing the greenness of the developed method

Green analytical chemistry (GAC) requires a thorough assessment of all steps in an analytical procedure to ensure minimal environmental impact. Various evaluation tools exist, each offering unique strengths and limitations [52]. To achieve a well-rounded assessment, it is essential to utilize tools that address multiple dimensions of green chemistry.

In this study, a dual-tool approach was employed to evaluate the environmental sustainability of the developed method. The first tool, the modified green analytical procedure index (MoGAPI), provides a comprehensive assessment covering the entire process, including pre-analytical steps such as electrode fabrication. The second tool, AGREE, focuses on the analytical phase post-electrode creation and assesses the method’s compliance with the 12 principles of green analytical chemistry. Together, these tools provide an in-depth, balanced overview of the method's environmental impact.

Complex modified green analytical procedure index (complex MoGAPI)

The Complex MoGAPI tool provides a detailed visual and numerical evaluation of the method, using a series of pentagrams and a hexagonal diagram to represent the pre-analytical processes. It employs a color-coded system, red for high environmental risk, yellow for moderate impact, green for eco-friendly practices, and white for unavailable data to facilitate a quick and intuitive assessment [53]. Additionally, it provides a numerical score from 0 (least green) to 100 (most green) to quantify the method's alignment with GAC principles. In our case, as shown in Figure 13A, the method achieved a score of 85, indicating a high level of environmental friendliness, especially in sample preparation and procedural steps. The dominance of green areas in the MoGAPI pictogram also highlights the excellent eco-profile of the electrode fabrication process.

Analytical greenness

Analytical greenness AGREE evaluates adherence to the 12 principles of green analytical chemistry, offering both a visual clock-like diagram and a numerical score. The scale ranges from 0 (poor, shown in red) to 1 (excellent, shown in green), providing insight into the overall and individual environmental performance of the method. While MoGAPI gives a broad overview, AGREE offers a more detailed examination of how well the method aligns with each GAC principle [54]. In our evaluation, the developed voltammetric method showed strong alignment with green chemistry standards. Most segments appeared in green, with only three in other colour due to the use of a non-automated device, a non-biobased buffer, and some waste production. The final AGREE score was 0.77, indicating a high level of greenness. The full evaluation is illustrated in Figure 13B.

The greenness assessment using both complex MoGAPI and AGREE tools demonstrated that the proposed electrochemical method offers a sustainable and environmentally friendly approach for the simultaneous analysis of DVH and SQL. These findings highlight the method's potential as a green analytical approach for use in pharmaceutical formulations and chicken tissue matrices.

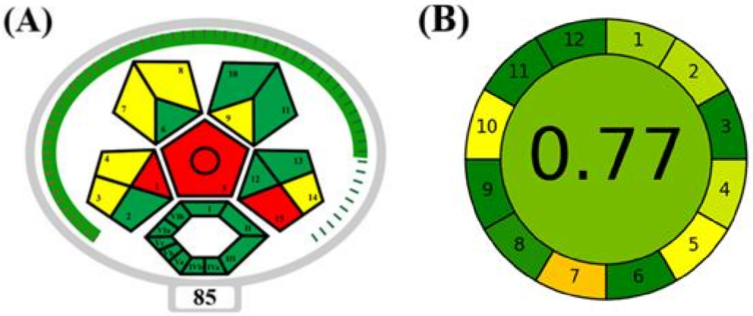


Figure 13. *Greenness evaluation of the developed voltammetric method for simultaneous determination of DVH and SQL using (A) Complex MoGAPI, (B) AGREE*

Conclusion

The proposed study introduces an innovative electrochemical protocol for the simultaneous determination of DVH and SQL utilizing a carbon paste electrode modified with CuO NPs. The voltammetric responses of the CV and SWV tests revealed effective electrochemical activity, high sensitivity, selectivity, and repeatability of the prepared CuO NPs/CPE. The technique was used to simultaneously measure DVH and SQL in drug substances, chicken meat and pharmaceutical formulations. The method's low detection limit, along with the convenience of electrode fabrication, makes it ideal for reliable drug determination in routine quality control work, and the findings demonstrate that the proposed sensor can detect residues of DVH and SQL in chicken meat to ensure food safety. Greenness assessment *via* Complex MoGAPI and AGREE confirms the method's sustainability for analysing DVH and SQL in pharmaceutical formulation and chicken meat.

Funding: *No funding was received for this study.*

Conflict of interest: *None*

References

[1] A. Brayfield, Martindale: The Complete Drug Reference, 39th ed., The Pharmaceutical Press, London, UK, 2017. ISBN: 9780857113092

[2] N. Broekaert, C. Van Peteghem, E. Daeseleire, D. Sticker, C. Van Poucke, Development and validation of an UPLC-MS/MS method for the determination of ionophoric and synthetic coccidiostats in vegetables, *Analytical and Bioanalytical Chemistry* **401** (2011) 3335-3344. https://doi.org/10.1007/s00216-011-5433-1

[3] L. Clarke, T.L. Fodey, S.R.H. Crooks, M. Moloney, J. O'Mahony, P. Delahaut, R. O'Kennedy, A review of coccidiostats and the analysis of their residues in meat and other food, *Meat Science* **97** (2014) 358-374. https://doi.org/10.1016/j.meatsci.2014.01.004

[4] Y.-J. Yang, X.-W. Liu, B. Li, S.-H. Li, X.-J. Kong, Z. Qin, J.-Y. Li, Simultaneous determination of diaveridine, trimethoprim and ormetoprim in feed using high performance liquid chromatography tandem mass spectrometry, *Food Chemistry* **212** (2016) 358-366. https://doi.org/10.1016/j.foodchem.2016.05.184.

[5] J. F. Prescott, Sulfonamides, diaminopyrimidines, and their combinations, *Antimicrobial Therapy in Veterinary Medicine* (2013) 279-294. https://doi.org/10.1002/9781118675014.ch17

[6] Z. L. Chen, *Veterinary Pharmacology* (3rd ed.). Agricultural Press, Beijing, China, 2009. ISBN: 9787109121317.

[7] S. Hawser, S. Lociuro, K. Islam, Dihydrofolate reductase inhibitors as antibacterial agents, *Biochemical Pharmacology* **71** (2006) 941-948. https://doi.org/10.1016/j.bcp.2005.10.052

[8] L. Wang, L. Wen, Y. Pan, Z. Liu, C. Zhang, Z. Yuan, L. Huang, Development of radioactive tracing coupled with LC/MS-IT-TOF methodology for the discovery and identification of diaveridine metabolites in pigs, *Food Chemistry* **363** (2021) 130200. https://doi.org/10.1016/j.foodchem.2021.130200 .

[9] C. Girardi, R. Odore, Pharmacological treatments and risks for the food chain, *Veterinary Research Communications* **32** (2008) 11-18. https://doi.org/10.1007/s11259-007-9001-2

[10] European Union. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, *Official Journal of the European Union* L 15 (2010) 1-72.

[11] V. Kant, P. Singh, P. K. Verma, I. Bais, M. S. Parmar, A. Gopal, V. Gupta, Anticoccidial drugs used in the poultry: an overview, *Science International* **1** (2013) 261-265. https://scialert.net/abstract/?doi=sciintl.2013.261.265.

[12] T. Yamaguchi, M. Okihashi, K. Harada, K. Uchida, Y. Konishi, K. Kajimura, K. Hirata, Y. Yamamoto, Rapid and easy multiresidue method for the analysis of antibiotics in meats by ultrahigh-performance liquid chromatography-tandem mass spectrometry, *Journal of Agricultural and Food Chemistry* **63** (2015) 5133-5140. https://doi.org/10.1021/acs.jafc.5b00170

[13] D. Mooney, C. Coxon, K. G. Richards, L. W. Gill, P.-E. Mellander, M. Danaher, A new sensitive method for the simultaneous chromatographic separation and tandem mass spectrometry detection of anticoccidials, including highly polar compounds, in environmental waters, *Journal of Chromatography A* **1618** (2020) 460857. https://doi.org/10.1016/j.chroma.2020.460857

[14] F. Belal, S. A. El-Razeq, M. Fouad, S. Zayed, F. Fouad, Simultaneous determination of five coccidiostats in veterinary powders, feed premixes, and baby food by micellar electrokinetic chromatography: application to chicken tissues and liver, *Food Analytical Methods* **11** (2018) 3531-3541. https://doi.org/10.1007/s12161-018-1330-y

[15] T. Sakano, S. Masuda, T. Amano, Determination of residual diaveridine and sulfaquinoxaline in hen's egg, chicken plasma and tissues by high-performance liquid chromatography, *Chemical & Pharmaceutical Bulletin (Tokyo)* **29** (1981) 2290-2295. https://doi.org/10.1248/cpb.29.2290

[16] M. Y. Fares, S. E. A. Aziz, I. A. Khalil, A. O. El Demerdash, N. M. Habib, Development and validation of a stability-indicating HPLC method for the simultaneous determination of anticoccidial drugs in veterinary formulations: greenness and whiteness assessment, *Scientific Reports* **15** (2025) 5190. https://www.nature.com/articles/s41598-024-84849-4#Abs1

[17] S. A. A. Razeq, S. E. A. Aziz, N. S. Ahmed, TLC-Densitometry and UHPLC methods for simultaneous determination of amprolium HCl, ethopabate, and sulfaquinoxaline-Na in their

new combined dosage form, *Chromatographia* **85** (2022) 563-574. https://doi.org/10.1007/s10337-022-04163-x

[18] A. M. Michael, Y. M. Fayez, H. H. Monir, C. K. Nessim, A thorough HPLC assay of quaternary veterinary formulation coupled with environmental assessment tool, *Journal of Chromatographic Science* **59** (2021) 776-784. https://doi.org/10.1093/chromsci/bmaa135

[19] Y. M. Fayez, A. M. Michael, H. H. Monir, H. M. Lotfy, C. K. Nessim, Comprehensive comparative study of eco-friendly univariate and multivariate methodological approaches on processing multi-component formulation quality, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **243** (2020) 118816. https://doi.org/10.1016/j.saa.2020.118816

[20] M. M. Abbas, A. M. El-Kosasy, L. A. E. A. Hussein, N. M. Hanna, Universal procedures for spectrophotometric determination of anticoccidial drugs; application to multi-ingredient veterinary formulation and computational investigations for multivariate analysis, *European Journal of Chemistry* **12** (2021) 368-376. https://doi.org/10.5155/eurjchem.12.4.368-376.2144

[21] S. Abdelaziz, S. Abdel Razeq, N. Ahmed, Smart UV-Spectrophotometric methods for the simultaneous determination of amprolium-HCl, ethopabate and sulfaquinoxaline-Na in combined dosage forms, *Azhar International Journal of Pharmaceutical and Medical Sciences* **1** (2021) 63-71. https://doi.org/10.21608/aijpms.2021.62019.1046

[22] M. A. Kassem, H. H. El-Feky, The impact of ultrasonication on SDS-stabilized silver nanoparticles as a probe for trace determination of diaveridine, *Plasmonics* **18** (2023) 395-405. https://doi.org/10.1007/s11468-022-01765-x

[23] M. A. Kassem, M. Morad, M. I. Awad, Voltammetric analysis of diaveridine at electrochemically pretreated ultrafine urethane electrode, *Current Physical Chemistry* **7** (2017) 204-209. http://dx.doi.org/10.2174/1877946807666170130142719

[24] S. Li, B. He, Y. Liang, J. Wang, Q. Jiao, Y. Liu, R. Guo, M. Wei, H. Jin, Sensitive electrochemical aptasensor for determination of sulfaquinoxaline based on AuPd NPs@UiO-66-NH_2/$CoSe_2$ and RecJf exonuclease-assisted signal amplification. *Analytica Chimica Acta* **1182** (2021) 338948. https://doi.org/10.1016/j.aca.2021.338948

[25] A. Soleymanpour, S. A. Rezvani, Development of a novel carbon paste sensor for determination of micromolar amounts of sulfaquinoxaline in pharmaceutical and biological samples, *Materials Science and Engineering C* **58** (2016) 504-509. https://doi.org/10.1016/j.msec.2015.08.034

[26] X. Chen, L. Yang, J. Tang, X. Wen, X. Zheng, L. Chen, J. Li, Y. Xie, T. Le, An AuNPs-based fluorescent sensor with truncated aptamer for detection of sulfaquinoxaline in water, *Biosensors* **12** (2022) 513. https://doi.org/10.3390/bios12070513

[27] M. G. Ghoniem, M. A. Mohamed, G. M. G. Eldin, A. Errachid, Sensitive electrochemical strategy via the construction of functionalized carbon nanotubes/ionic liquid nanocomposite for the determination of anaesthetic drug cinchocaine, *Measurement* **185** (2021) 110071. https://doi.org/10.1016/j.measurement.2021.110071

[28] M.A. Mohamed, G.M.G. Eldin, S.M. Ismail, N. Zine, A. Elaissari, N. Jaffrezic-Renault, A. Errachid, Innovative electrochemical sensor for the precise determination of the new antiviral COVID-19 treatment Favipiravir in the presence of co-administered drugs, *Journal of Electroanalytical Chemistry* **895** (2021) 115422. https://doi.org/10.1016/j.jelechem.2021.115422

[29] K. Ota, G. Kreysa, and R. F. Savinell, Encyclopedia of applied electrochemistry. Springer New York, 2014. https://link.springer.com/referencework/10.1007/978-1-4419-6996-5

[30] M. Ghalkhani, N. K. Bakirhan, S. A. Ozkan, Combination of efficiency with easiness, speed, and cheapness in development of sensitive electrochemical sensors, *Critical Reviews in Analytical Chemistry* **50** (2020) 538-553. https://doi.org/10.1080/10408347.2019.1664281

[31] A. E. Rakhshani, Preparation, characteristics and photovoltaic properties of cuprous oxide, *Solid-State Electronics* **29** (1986) 7-17. https://doi.org/10.1016/0038-1101(86)90191-7

[32] S. Shahrokhian, R. Kohansal, M. Ghalkhani, M.K. Amini, Electrodeposition of copper oxide nanoparticles on precasted carbon nanoparticles film for electrochemical investigation of anti-HIV drug nevirapine, *Electroanalysis* **27** (2015) 1989-1997. https://doi.org/10.1002/elan.201500027

[33] M. Ghalkhani, A. A. Firooz, M. Ghanbari, M. Ghanbari, B. Patanair, S. Thomas, Green synthesis of nonprecious metal-doped copper hydroxide nanoparticles for construction of a dopamine sensor, *Future Medicinal Chemistry* **13** (2021) 715-729. https://doi.org/10.4155/fmc-2020-0333

[34] F. Rasi, S. Sadeghi, M. Ghalkhani, Graphitic Carbon Nitride/Ni Doped Copper Hydroxide Nanocomposite Based Electrochemical Sensor for Trace Level Detection of Ponceau 4R Food Colorant, *Journal of The Electrochemical Society* **169** (2022) 127513. https://iopscience.iop.org/article/10.1149/1945-7111/aca837/meta

[35] F. Kardaş, M. Beytur, O. Akyıldırım, H. Yüksek, M. L. Yola, N. Atar. Electrochemical detection of atrazine in wastewater samples by copper oxide (CuO) nanoparticles ionic liquid modified electrode, *Journal of Molecular Liquids* **248** (2017) 360-363. https://doi.org/10.1016/j.molliq.2017.10.085

[36] V. B. Patil, S. J. Malode, S. N. Mangasuli, S. M. Tuwar, K. Mondal, N. P. Shetti, An electrochemical electrode to detect theophylline based on copper oxide nanoparticles composited with graphene oxide, *Micromachines* **13** (2022) 1166. https://doi.org/10.3390/mi13081166

[37] M. Locatelli, M. Perrucci, I. Ali, H.I. Ulusoy, F.R. Mansour, V.F. Samanidou, A. Kabir, *Green analytical methods and principles of green analytical chemistry,* in *Green Analytical Methods and Miniaturized Sample Preparation Techniques for Forensic Drug Analysis*, Elsevier, Amsterdam, Netherlands, 2025, 3-21. https://doi.org/10.1016/B978-0-443-13907-9.00002-4.

[38] F. R. Mansour, K. M. Omer, J. Płotka-Wasylka, A total scoring system and software for complex modified GAPI (ComplexMoGAPI) application in the assessment of method greenness, *Green Analytical Chemistry* **10** (2024) 100126. https://doi.org/10.1016/j.greeac.2024.100126

[39] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE—Analytical GREEnness metric approach and software, *Analytical Chemistry* **92** (2020) 10076-10082. https://doi.org/10.1021/acs.analchem.0c01887

[40] J. Heyrovský, P. Zuman, *Practical Polarography*, Academic Press, London and New York, UK, 1968. ISBN-10: 0123466563.

[41] Q2(R1) Validation of Analytical Procedures: Text and Methodology Guidance for Industry, 2005. [Online]. https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances.

[42] S. A. Atty, H. E. Zaazaa, F. A. Morsy, D. M. Naguib, G. A. Sedik, Nano green voltammetric determination of imidocarb dipropionate and its residues in bovine meat, milk and urine samples, *Journal of The Electrochemical Society* **167** (2020) 047510. https://doi.org/10.1149/1945-7111/ab7115

[43] F. Belal, S. A. A. El-Razeq, M. M. Fouad, S. Zayed, F. A. Fouad, Determination of menbutone in bovine milk and meat using micellar liquid chromatography: application to injectable dosage forms, *Food Analytical Methods* **9** (2016) 638-645. https://doi.org/10.1007/s12161-015-0230-7

[44] A. Menichetti, A. Mavridi-Printezi, D. Mordini, M. Montalti, Effect of size, shape and surface functionalization on the antibacterial activity of silver nanoparticles, *Journal of Functional Biomaterials* **14** (2023) 244. https://doi.org/10.3390/jfb14050244

[45] V. Sudha, G. Murugadoss, R. Thangamuthu, Structural and morphological tuning of Cu-based metal oxide nanoparticles by a facile chemical method and highly electrochemical sensing of sulphite, *Scientific Reports* **11** (2021) 3413. https://doi.org/10.1038/s41598-021-82741-z

[46] A. J. Bard, L. R. Faulkner, H. S. White, *Electrochemical Methods: Fundamentals and Applications*, John Wiley & Sons, Hoboken, NJ, USA, 2022. ISBN-13: 978-1119334064.

[47] E. P. Randviir, C. E. Banks, Electrochemical impedance spectroscopy: an overview of bioanalytical applications, *Analytical Methods* **5** (2013) 1098-1115. https://doi.org/10.1039/C3AY26476A

[48] N. Sato, Y. Ohta, M. Haruta, H. Takehara, H. Tashiro, K. Sasagawa, O. Jongprateep, J. Ohta, Electrochemical activities of Fe_2O_3-modified microelectrode for dopamine detection using fast-scan cyclic voltammetry, *AIP Advances* **13** (2023) 025026. https://doi.org/10.1063/5.0123865

[49] P. H. Rieger, *Electrochemistry*, Prentice-Hall International, Inc., New Jersey, USA, 1987, pp. 216-219. ISBN-13: 978-0132491389.

[50] E. Laviron, L. Roullier, C. Degrand. A multilayer model for the study of space distributed redox modified electrodes: Part II. Theory and application of linear potential sweep voltammetry for a simple reaction, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **112** (1980) 11-23. https://doi.org/10.1016/S0022-0728(80)80003-9

[51] A. L. Yarin, B. Pourdeyhimi, S. Ramakrishna, *Fundamentals and Applications of Micro- and Nanofibers*, Cambridge University Press, Cambridge, UK, 2014. ISBN-13: 978-1-107-06029-6.

[52] S. S. Saleh, R. H. Obaydo, M. A. El Hamd, Y. Rostom, D. Mohamed, H. M. Lotfy, Guidelines for accurate application of green and white analytical concepts: Merits versus demerits with insights of significant milestones of assessment tools applied for antiviral drugs, *Microchemical Journal* **199** (2024) 109917. https://doi.org/10.1016/j.microc.2024.109917

[53] M. I. Helmy, M. A. El Hamd, R. H. Obaydo, D. Nashed, C. K. Nessim, Bridging Pharma and Sustainability: Green Electrochemical Analysis of Antiparkinsonian Drug in Pharmaceuticals and Plasma, Aligned with United Nations Goals via the NQS Index, *Journal of The Electrochemical Society* **171** (2024) 077512. https://doi.org/10.1149/1945-7111/ad60f9

[54] S. A. Boltia, E. M. Morgan, R. H. Obaydo, Y. M. Fayez, M. Abdelkawy, H. M. Lotfy, Green Electrochemical Sensing: Novel Ion-Selective Electrode Method for Precise Determination of Dimenhydrinate and its Metabolite along with Cinnarizine in Pharmaceutical and Plasma Samples, *Journal of The Electrochemical Society* **171** (2024) 057508. https://doi.org/10.1149/1945-7111/ad4ba4.