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Original scientific paper

Novel and simple electrochemical sensing platform based on bimetallic nanoparticles/screen-printed carbon electrode for sensitive determination of metronidazole

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Abstract

A straightforward method for determining metronidazole (MNZ) using voltammetry is presented in this paper. This approach was developed by modifying screen-printed carbon electrodes (SPCE) with Co-Ce nanoparticles (CC-SPCE). Compared with unmodified SPCE, cyclic voltammetry showed an improved peak current response ($-16 \mu\text{A}$) at the surface of CC-SPCE and a clearly defined reduction peak for MNZ at -630 mV . Differential pulse voltammetry was used to quantify MNZ electrochemically. The CC-SPCE responded well to MNZ in ideal circumstances. With a low detection limit of $0.005 \mu\text{M}$, the CC-SPCE showed a linear concentration range for MNZ (0.008 to $450.0 \mu\text{M}$). Ultimately, the presented sensing platform may offer an alternative approach to pharmaceutical product analysis.

Keywords

Nitroimidazole derivatives; antimicrobial properties; carcinogenicity

Introduction

One nitroimidazole derivative well known for its antibacterial properties is metronidazole (MNZ). MNZ has long been used to treat bacterial infections, dental abscesses, suppositories, and skin infections in both people and animals, including rosacea, acute ulcerative gingivitis, and dracunculiasis [1]. MNZ is also used for whipworm infections, pelvic inflammation, and insect stings.

MNZ is mostly used to treat trichomoniasis and histomoniasis infections in poultry, as well as hemorrhagic enteritis in domestic animals [2].

Despite its effective antibacterial properties, MNZ is considered extremely dangerous to human and wildlife health [3]. As a result, several nations have prohibited MNZ. Some state that although there is insufficient evidence to demonstrate that MNZ causes carcinogenicity in humans, it has been reported that MNZ caused cancer in some animals when used as a supplement in animal feed [4]. For patients taking MNZ long term, monitoring MNZ concentration is therefore crucial. Meanwhile, because of its high solubility, limited biodegradability, and hydrolytic and photolytic stability, MNZ can accumulate in the environment. As a result, there is a growing need to investigate scientific approaches to eliminate and break down this medication from different environmental sources [4].

Analytical approaches that are commonly used for the detection of MNZ include high-performance liquid chromatography (HPLC) [5], colorimetry [6], gas chromatography (GC) [7], spectrophotometry [8], fluorescence [9], a chemiluminescence assay [10] and capillary zone electrophoresis (CZE) [11]. Although many of these approaches have fairly high detection efficiency, the overall measurement routine is time-consuming and somewhat convoluted; instrument costs are also very high, and they often require laborious steps before readout [5]. In contrast, electrochemical methods offer great potential, mainly because sample handling is easier, detection is relatively quick, the equipment is not that expensive, and miniaturization is comparatively straightforward [12,13].

Because of their small format and ability to connect to portable devices for on-site analyte detection, screen-printed electrodes (SPEs) fabricated with microfabrication technology feature three electrodes printed on a single strip [14]. These devices can also be surface-modified with different compositions, making their design versatile rather than rigid [15]. SPEs align with green chemistry principles, such as creating safer products, and are low-cost, easy to build, and suitable for large-scale production. SPEs can also deliver good sensitivity, low energy usage, a linear response, and efficient operation at room temperature [16].

The aromatic nitro group found in MNZ is easily reduced electrochemically at different electrode systems. However, during the redox process, MNZ and its reduction products can adsorb on bare electrodes, causing electrode surface fouling. In addition to impeding electron transfer and electrochemical reactions, this surface pollution can negatively impact the electrode's analytical performance, specifically its sensitivity, selectivity, and repeatability. Additionally, bare electrodes often require high overpotentials to reduce MNZ and show poor stability across a wide range of electrochemical solutions [12-16].

Modifying the electrode surface with appropriate catalytic materials is a promising way to improve electrochemical discrimination of target biomolecules and increase selectivity and sensitivity, overcoming these limitations. By reducing charge-transfer resistance, adding suitable modifier materials can increase the electrochemically active surface area and promote electron transfer. As a result, the modified electrode can offer improved electrochemical reactions, a lower limit of detection (LOD), increased sensitivity, and a wider linear detection range [17]. Nanoparticles (NPs) have emerged as a crucial component in the development of contemporary technologies, including sensors, medicine delivery, water treatment, batteries, fuel cells, and more [18].

Industry's quest to enhance device performance has driven efforts to create nanomaterials with predicted attributes. Furthermore, scientific research and nanoscience are advancing quickly due to the nanotechnology revolution, which has a significant impact on every aspect of society. The unique properties of NPs over macro-scale structures and bulk electrodes are the basis for these

technological advancements [19]. Crucially, rational electrode material design for sensing and catalytic applications depends on the knowledge of materials at the nanoscale [20].

Successful materials development often requires a bottom-up strategy that addresses the fundamental understanding of NPs' electrochemical activity. Metallic nanoparticles have attracted significant scientific attention because of their unique optical, catalytic, magnetic, electrical, and biological capabilities, which are attractive for a variety of industrial applications [19]. Because of their unique catalytic, electrical, and surface characteristics, cobalt (Co) nanoparticles have garnered significant interest as potential nanostructured modifiers for electrochemical sensing platforms.

Co-based nanoparticles can improve the electrocatalytic oxidation of target analytes by facilitating electron-transfer processes and offering a large number of electroactive sites [21]. Because of their redox-active $\text{Ce}^{3+}/\text{Ce}^{4+}$ pair, oxygen-vacancy-rich surface, and capacity to enhance the electrochemically active surface area and charge-transfer properties of modified electrodes, cerium compound nanoparticles have become appealing electrode modifiers. An efficient method for enhancing the electrocatalytic performance of modified electrodes is the synergistic interaction between Co nanoparticles and cerium compound nanoparticles [22].

Co–Ce nanoparticles were effectively produced and completely characterized utilizing a variety of analytical techniques in this study. The resulting Co–Ce nanocomplex was then used to modify a SPCE to create a voltammetric sensor for the sensitive measurement of MTZ. The developed modified screen-printed carbon electrodes (SPCE) with Co-Ce nanoparticles (CC-SPCE) showed good analytical properties toward the reduction of MNZ. Additionally, successful identification of MNZ in real samples demonstrated the suitability and reliability of the proposed sensor for quick, easy, and highly sensitive detection of MNZ.

Experimental

Equipment and reagents

PGSTAT 12 N Autolab (Metrohm, the Netherlands) was used for electrochemical measurements and investigations. Metrohm DropSens (Spain) supplied the disposable commercial SPCEs (DRP-110) with a three-electrode configuration (graphite working electrode, graphite counter electrode, and Ag pseudo-reference electrode). To measure the pH of various solutions precisely, a pH meter was used.

Synthesis of ion-pair complex

Initially, 5,5-dmbpy ligand (0.51 mM) was added to a 20 mL H_2O -methanol mixture, and then $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.170 mM) was added. The final product was heated to 60 °C for two hours after being dissolved in 20 mL of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.70 mM) with NaNO_3 (0.50 mM) in the H_2O -methanol mixture. After three days, the $[\text{Co}(\text{dmbpy})_3][\text{Ce}(\text{NO}_3)_6]$ crystals that had developed were collected and rinsed with diethyl ether.

Synthesis of nano-complex

After immersing a high-density ultrasonic probe in a 25 mL methanol solution containing 0.50 mM $[\text{Co}(\text{5,5-dmbipy})_3][\text{Ce}(\text{NO}_3)_6]$, the probe was exposed to radiation for 15 minutes at 100 W. After filtration, the precipitates were air-dried. The analytical data revealed that the nano complex broke down at 270 °C

Preparation of the screen-printed carbon electrodes with Co-Ce nanoparticles

The following is the process for modifying SPCE: First, a Co-Ce-NPs suspension was made by ultrasonication of 1.0 mg of Co-Ce-NPs in 1 mL of water for at least 25 minutes. Subsequently, 3.0 μL

of Co-Ce-NPs solution was applied to the SPGE surface. The Co-Ce-NPs /SPCE sensor was ready after drying at room temperature.

Preparation of samples

Five MNZ tablets (250 mg each) were ground to prepare the pharmaceutical sample. It was put into a 100 mL volumetric flask. After adding enough deionized water to the flask, it was ultrasonicated for at least 20 minutes. The acquired sample was then diluted with phosphate buffer solution (PBS) to a predetermined volume. To determine MNZ quantitatively, differential pulse voltammetry (DPV) was used with the standard addition method.

Preparation of urine samples

Human urine samples were centrifuged for five minutes at 6000 rpm. The centrifuged sample was then diluted 1:5 with PBS. The urine samples were spiked with different MNZ concentrations and analysed quantitatively using the standard addition method.

Results and discussion

Electrochemical behaviour of metronidazole at different electrodes

The electrochemical responses of bare SPCE and CC-SPCE were measured by cyclic voltammetry (CV) in PBS containing MNZ (150.0 μM) to compare and assess the impact of Co-Ce-NPs (Figure 1).

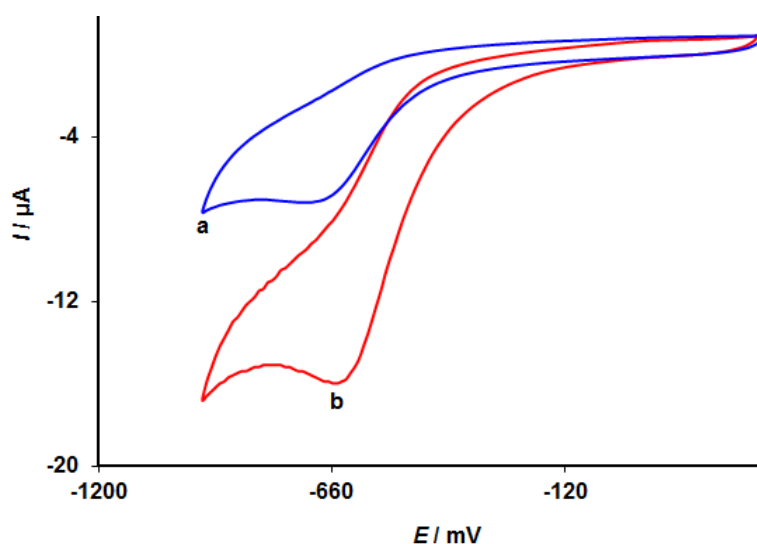


Figure 1. CVs captured of (a) bare SPCE and (b) CC-SPCE for MNZ (150.0 μM) in PBS at 50 mV s^{-1}

As shown, MNZ on bare SPCE produced a current peak (I_{pc}) of around -8 μA (voltammogram a). In contrast to the bare SPCE, the CC-SPCE (voltammogram b) showed a significantly increased I_{pc} (-16 μA) and a shifted reduction peak potential (E_{pc}) toward positive values ($E_{\text{pc}} = -630 \text{ mV}$). Therefore, compared with bare SPCE, CC-SPCE is an effective electrode for low-potential MNZ detection.

Effect of sweep rate on the reduction reaction of metronidazole on screen-printed carbon electrodes with Co-Ce nanoparticles

Figure 2 displays the CV responses of MNZ at various scan rates in order to examine the impact of scan rate on the reduction reaction of MNZ on the CC-SPCE. The MNZ reduction peak current rises with scan rate. Figure 3 shows the linear relationship between reduction peak currents and the square root of the scan rate ($\nu^{1/2}$). The MNZ reduction peak current increases proportionately with increasing $\nu^{1/2}$. This indicates that the MNZ reduction on the CC-SPCE surface is diffusion-controlled.

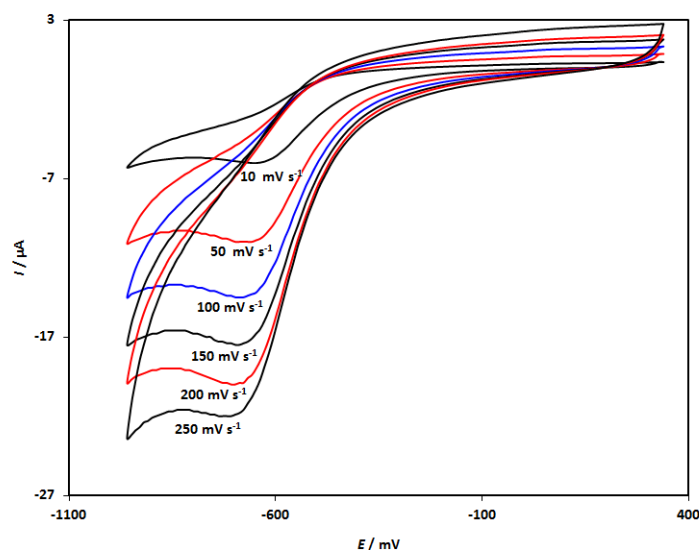


Figure 2. CVs for MNZ (100.0 μM) in PBS on CC-SPCE at variable scan rates

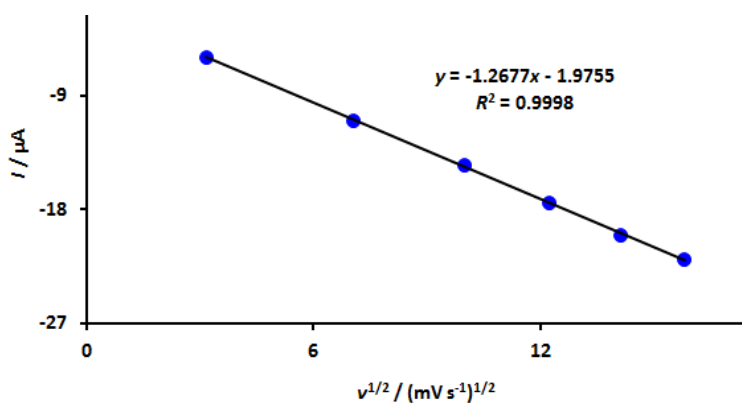


Figure 3. Plot of I_{pc} against $v^{1/2}$ for CVs in Figure 2

Chronoamperometric studies

The working electrode potential was set at -660 mV for different MNZ concentrations to perform chronoamperometric measurements of MNZ at the CC-SPCE (Figure 4).

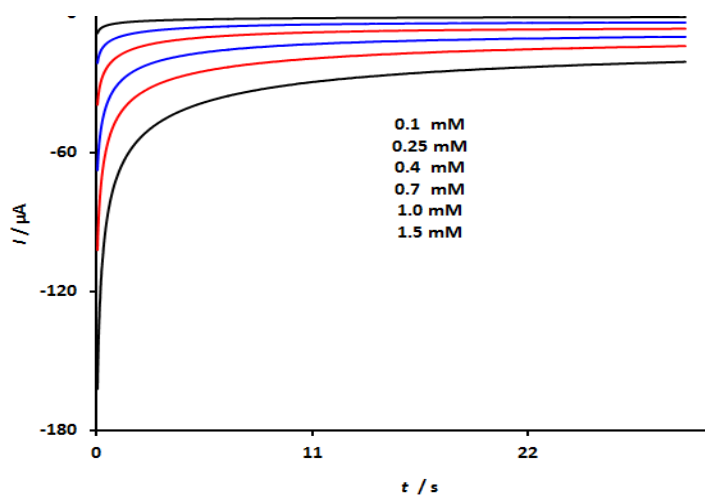


Figure 4. Chronoamperograms captured for the CC-SPCE in the exposure to variable MNZ concentrations

The Cottrell Equation (1) describes the current relation with a diffusion coefficient of $D / \text{cm}^2 \text{s}^{-1}$

$$I = nFAD^{1/2}C\pi^{-1/2}t^{-1/2} \quad (1)$$

Here, $C / \text{mol cm}^{-3}$ is the bulk concentration. Experimental plots of I vs. $t^{1/2}$ were used for different MNZ concentrations (Figure 5A). The slopes were then plotted vs. MNZ concentrations (Figure 5B). D was found to be $1.6 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$

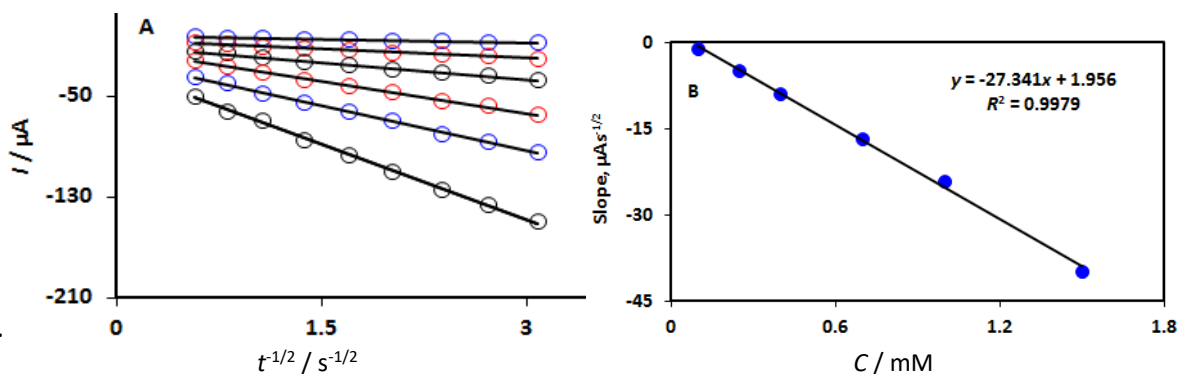


Figure 5. A - plots of I vs. $t^{1/2}$ and B - plot of straight-line slope vs. MNZ level

Calibration plot and limit of detection

MNZ in solution can be determined using the peak current of MNZ reduction at the modified electrode's surface. Accordingly, DPV studies were conducted at various MNZ concentrations (Figure 6). The concentration of MNZ within 0.008 to 450.0 μM was correlated with the oxidation peak currents of MNZ (Figure 7). MNZ was discovered to have a LOD of 0.005 μM .

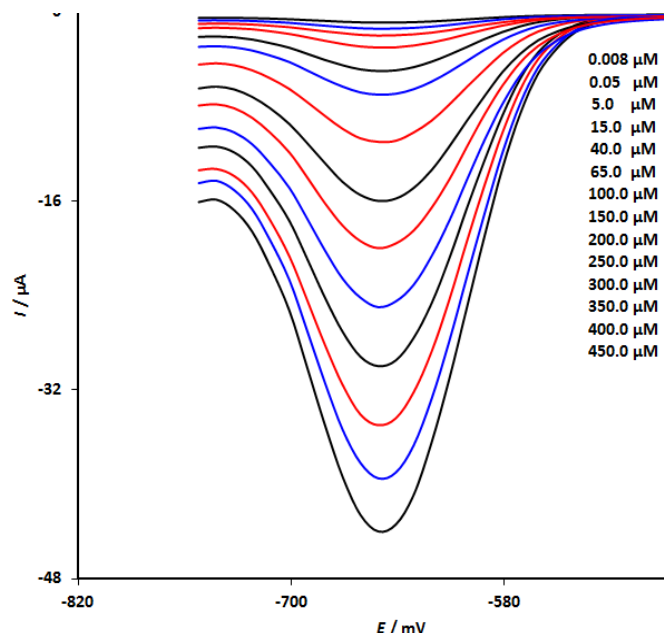


Figure 6. DPVs captured for the CC-SPCE in the exposure to variable MNZ concentrations

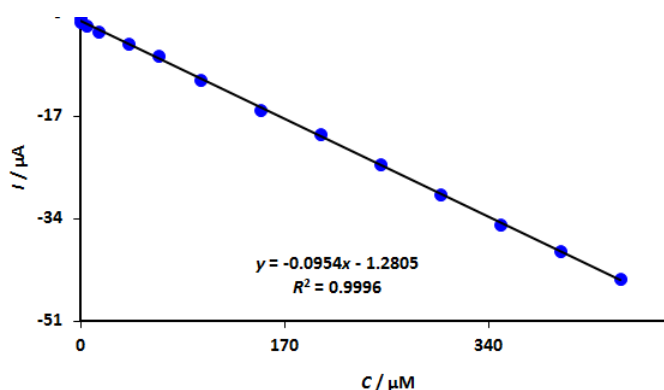


Figure 7. Relationship between I and MNZ levels

Stability and reproducibility studies

DPV measurements were used to assess the stability and repeatability of the CC-SPCE sensor for MNZ determination. The CC-SPCE sensor's stability was evaluated by keeping it at room temperature for a few days and using it every three days to measure 50.0 μM MNZ. The peak current was kept at 96.3 % of the initial value after 15 days of storage. Five CC-SPCEs were made using the same technique to assess repeatability. Under identical conditions, 50.0 μM MNZ was determined using the constructed electrodes. The peak currents' relative standard deviation (RSD) was determined to be 3.7 %.

Interference studies

For 30.0 μM MNZ, the interference effects of certain species were evaluated. The results showed that the DPV response of MNZ was unaffected by a 100-fold excess of common ions (Al^{3+} , Na^+ , Ca^{2+} , K^+ , Cu^{2+} , Fe^{2+} , Mg^{2+} , Cl^- , NH_4^+ and SO_4^{2-}), a 40-fold excess of sucrose, glucose, folic acid and urea, and a 5-fold excess of ascorbic acid, dopamine, and uric acid (less than ± 5 %).

Real sample analysis

The amount of MNZ in commercial tablets and urine samples was measured. To verify the accuracy of the recovery experiments, genuine samples were spiked with MNZ at different concentrations using the conventional addition procedure (Table 1). The range of recovery values was 98.0 to 104.1 %. Additionally, the low RSD indicates good precision.

Table 1. Experimental results from the analysis of in urine and tablets of MNZ samples using CC-SPCE

Sample	MNZ amount, μM		Recovery, %	RSD, %
	Added	Found		
MNZ tablet	0	3.9	-	3.0
	1.0	5.1	104.1	2.7
	3.0	6.8	98.6	2.5
Urine	0	-	-	-
	5.0	4.9	98.0	2.9
	7.0	7.2	102.9	2.1

Conclusions

In this paper, Co-Ce-NPs are added to the SPCE surface to measurement of MNZ. The electroanalytical performance of the redesigned electrodes was thoroughly assessed. To improve sensitivity, the effects of several experimental factors, such as pH, buffer, and scan rate, on sensor behaviour for MNZ detection were investigated and optimized. Compared with bare SPCE, the modified electrodes showed increased electrocatalytic overpotential shifts and peak current values. The DPV method was used to obtain calibration graphs for MNZ under ideal conditions. The outcomes demonstrated the high stability of the Co–Ce–NP-based nanosensors on the SPCE surface in MNZ tests. The modified sensors successfully measured MNZ in an actual sample. The modified electrodes could be effectively employed as electrochemical sensors in environmental or other domains for analyte determination with the aforementioned performance.

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