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*Original scientific paper*

# Electrochemical determination of methyldopa in the presence of hydrochlorothiazide using carbon paste electrode modified with $MoS_2$/Ni- metal organic framework hybrid nanosheets

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## Abstract

**Background and purpose:** Methyldopa (MDP) and Hydrochlorothiazide (HCTZ) are advised for the treatment of essential hypertension. Electrochemical techniques are used for their determination in biological samples. **Experimental approach***:* This study uses a carbon paste electrode (CPE) modified with $MoS_2$ nanosheets and a nickel-based metal-organic framework (MSN-MOF/CPE sensor) for simultaneous voltammetric detection of MDP and HCTZ. **Key results***:* Electrochemical analysis of the modified electrode revealed that, in comparison to an unmodified CPE, MDP oxidation takes place at about 100 mV lower potentials under ideal conditions. With a limit of detection (LOD) of 0.01 µM, the sensor shows a sensitive response to MDP in the range of 0.05 to 260.0 µM. Additionally, the MSN-MOF/CPE sensor showed high sensitivity for the simultaneous determination of MDP and HCTZ. The defined and separated oxidation peaks of a mixture of MDP and HCTZ showed significant peak potential differences of 240 mV. **Conclusion:** Ultimately, the suggested method was effectively used to determine MDP and HCTZ in pharmaceutical samples and urine as real samples.

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## Keywords

Hypertension; heart disease; oedema; diabetic insipidus; two-dimensional nanosheets

## Introduction

Pharmaceutical substances are typically used to treat diagnosed diseases and have biological efficacy in the patient's body. After patient administration, analytical measurements are crucial for verifying drug bioavailability, assessing efficacy, and determining the required dosage [1].

For this goal, a variety of analytical techniques have been developed, such as spectrophotometry, spectrofluorometry, chemiluminescence, capillary zone electrophoresis, and chromatography. For pharmaceutical testing, electrochemical techniques have been regarded as a sensitive, economical, and fast method [2]. For the evaluation of a variety of pharmacological variables, electrochemical determination has been shown to be highly sensitive with reduced interference from non-electroactive chemicals compared with other analytical techniques [3].

Since the 1960s, methyldopa (MDP), a compound with functionality, has been used as an antihypertensive medication to treat high blood pressure. It features an amino acid skeleton with a catechol group and a methyl group on the α-carbon of the side chain, making it a structural counterpart of the anti-Parkinsonian drug dopa (dihydroxyphenylalanine) [4]. In the adrenergic nerve terminals, MDP functions by biotransforming into methyl norepinephrine. One of its roles may be to inhibit aromatic amino acid decarboxylase (DOPA decarboxylase), an enzyme involved in the catecholamine biosynthesis pathway that transforms L-DOPA into the dopamine precursor of adrenaline and norepinephrine [5].

Many analytical techniques, including spectrophotometry [6], gas chromatography [7], high-performance liquid chromatography (HPLC) [8], chemiluminescence [9], kinetic methods [10], HNMR [11], and voltammetric determination [12,13], which have been described in the literature for the quantification of catecholamine drugs in dosage forms and biological fluids, can be used to determine MDP for both pharmaceutical and clinical purposes.

However, each technique has drawbacks, including limited selectivity, the need for organic solvents, lengthy analysis times, difficult sample preparation, and high cost. Among them, electrochemical techniques require no sample pretreatment and are quick, easy, and very sensitive [12,13].

Hydrochlorothiazide (HCTZ) is a commonly used diuretic. It is advised for the treatment of essential hypertension, oedema, and diabetic insipidus [14]. Numerous methods have been used to measure HCTZ in bulk and pharmaceutical formulations [15]. Reports also describe electrochemical methods for assessing HCTZ [16]. MDP and HCTZ are used together to treat high blood pressure [16].

Since Ralph N. Adams [17] created the CPE in 1958 and offered it as a replacement for the dropping mercury electrode, this type of electrode (and sensors based on it) has advanced significantly. The development's potential is enhanced by several advantages, such as low background current, low cost, high sensitivity, a wide potential window, and a renewable surface [17]. However, this material has been modified in several ways because the manufacturing process requires only mixing graphite with suitable binders to form a paste [18].

Ultrathin two-dimensional nanosheets have been suggested as promising electrode materials, especially in nanotechnology, because of their extraordinary electrical properties, enormous specific surface area, and excellent mechanical strength [19]. $MoS_2$ is a layered quasi-2D chalcogenide material [20]. It has several uses in lithium batteries, catalysts, supercapacitors, and sensors, due to its outstanding catalytic activity [20].

Metal-organic frameworks (MOFs) are produced by combining metal ions or clusters with organic ligands based on coordination chemistry [20]. MOFs are used in many applications, such as gas separation, catalysis, fluorescence, and magnetism [21]. 2D MOFs have distinctive properties compared with bulk MOF materials, such as high porosity and nanoscale thickness, which promote improved electron transmission and rapid mass movement. In contrast to bulk MOFs, 2D MOFs have many accessible active sites on the layer surface, making it easier for active centres to interact with substrates and enhancing MOF catalytic performance [21]. Because all components operate synergistically, activity may also improve [21,22].

Here, we exploited the complementary qualities of $MoS_2$ and Ni-MOF NSs to develop a relatively simple method for creating a $MoS_2$ nanosheet-modified nickel-based metal-organic framework (MSN-MOF), which we then used to modify the CPE for MDP electrooxidation. The findings demonstrated MSN-MOF/CPE's excellent electrocatalytic activity in MDP oxidation and reduction. The MSN-MOF/CPE sensor offers good sensitivity, a wide dynamic range, and a relatively low limit of detection (LOD) for MDP analysis. Notably, the


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MSN-MOF/CPE allows simultaneous measurement of HCTZ and MDP. Furthermore, in real-sample measurements for MDP and HCTZ analysis, the MSN-MOF/CPE sensor exhibits strong recovery results.

## Experimental

### *Reagents and chemicals*

All chemicals used in this investigation were analytical grade and used as supplied. Phosphate buffered solution (PBS, pH 7.0, 0.1 M) was used in this study. Deionized water used for each test was produced using the Millipore Direct-Q® 8 UV (ultraviolet) apparatus (Millipore, Germany). The Netherlands' Eco Chemie Autolab PGSTAT 12N potentiostat/galvanostat system was used to assess electrochemical characteristics. Next, we used a standard 3-electrode cell at 25±1 °C and constructed a general-purpose electrochemical system (GPES) software to monitor the electrochemical parameters. Additionally, the reference, working, and auxiliary electrodes were an Ag/AgCl/KCl (3.0 M) electrode, MSN-MOF/CPE, and a platinum wire. The pH values were measured using a combination glass electrode with a Metrohm pH-713 Model pH meter.

### *Fabrication of $MoS_2$*

Ammonium heptamolybdate tetrahydrate (3 mmol) and thiourea (2.3 g) were combined in a 50-mL Teflon autoclave with 30 mL of deionized water and heated to 200 ˚C for 24 hours. After thorough rinsing with a 1:1 volume ratio mixture of ethanol and deionized water, the product was vacuum-dried for 6 h at 50 °C.

### *Fabrication of $MoS_2$/Ni- metal-organic frameworks F hybrid nanosheets*

The hybrid MSN-MOF was created by the *in situ* growth of Ni-MOF on the $MoS_2$ surface. Following a 30-minute ultrasonication of $MoS_2$ (0.32 g) in dimethylformamide (DMF) (15 mL), $Ni(NO_3)_2{\cdot}6H_2O$ (0.87 g, 3 mmol) dispersed in deionized water (10 mL) was added under another 30-minute ultrasonication. After that, a DMF solution (10 mL) containing terephthalic acid (0.17 g, 1 mmol) and 0.4 M KOH (5 mL) was gradually added to the obtained solution. The resulting solution was subjected to a solvothermal reaction in a 50 mL Teflon-lined stainless steel autoclave at 120 °C for 24 hours after an additional two hours of sonication. The product was then allowed to cool to ambient temperature. The mixture was centrifuged to obtain the precipitate. The precipitate was then completely washed with deionized water/DMF (1:1 volume ratio) and dried at 65 °C for 24 hours under vacuum. Additionally, NiMOF NSs were prepared as a control using the same procedure as the $MoS_2$/NiMOF hybrid NSs, except that $MoS_2$ was added before solvothermal treatment.

### *Preparation of modified electrode*

Using a mortar and pestle, thoroughly mixed graphite powder, liquid paraffin, and MoS2/Ni-MOF nanohybrid sheets to prepare the modified electrode. The final paste was packed into a 15 cm long tube with an inner diameter of 3.4 mm. To create the electrical contact, a copper wire was then inserted into the electrode. Additionally, the same method was used to prepare unmodified CPE without incorporating $MoS_2$/Ni-MOF nanohybrid sheets into the mixture. These were used for comparison.

### *Procedure of real samples preparation*

Five 250 mg MDP tablets and five 50 mg HCTZ tablets were ground individually, and tablet solutions were prepared by ultrasonically dissolving 250 or 50 mg of MDP and HCTZ powder in 100 mL of water, respectively. Next, various quantities of the diluted solution were placed in a 10 mL volumetric flask and diluted with PBS to the appropriate level. Using the standard addition method, the suggested method was used to verify MDP and HCTZ quantities.

Immediately following collection, urine samples were kept in the refrigerator. Each sample (10 mL) was centrifuged at 2000 rpm for 20 minutes. After removing the supernatant, the filtrate was filtered through a 0.45 µm membrane. A 10 mL volumetric flask was then filled with multiple volumes of the filtrate and diluted with PBS to the appropriate level. Lastly, various concentrations of MDP and HCTZ were added to the diluted urine.

*Results and discussions*

*Evaluation of the electrochemical activity of the carbon paste electrode modified with $MoS_2$ nanosheets and a nickel-based metal-organic framework towards methyldopa*

pH is an important factor affecting the sensor's sensitivity. Therefore, the effect of solution pH on MSN-MOF/CPE sensor efficiency was investigated in the pH range of 4.0 to 9.0. A pH of 7.0 showed greater sensitivity in identifying MDP. As a result, pH 7.0 was chosen for upcoming analytical tests.

100.0 µM MDP was evaluated using cyclic voltammetry (CV) at unmodified CPE (curve a) and MSN-MOF/CPE (curve b) (Figure 1). It is evident from comparing the obtained voltammograms that the unmodified CPE (voltammogram a) exhibits a faint signal for MDP oxidation. It was confirmed by the voltammetric reaction of MDP at the MSN-MOF/CPE surface (voltammogram b) that the MSN-MOF/CPE significantly outperformed unmodified CPE in terms of higher current intensity and lower potential.

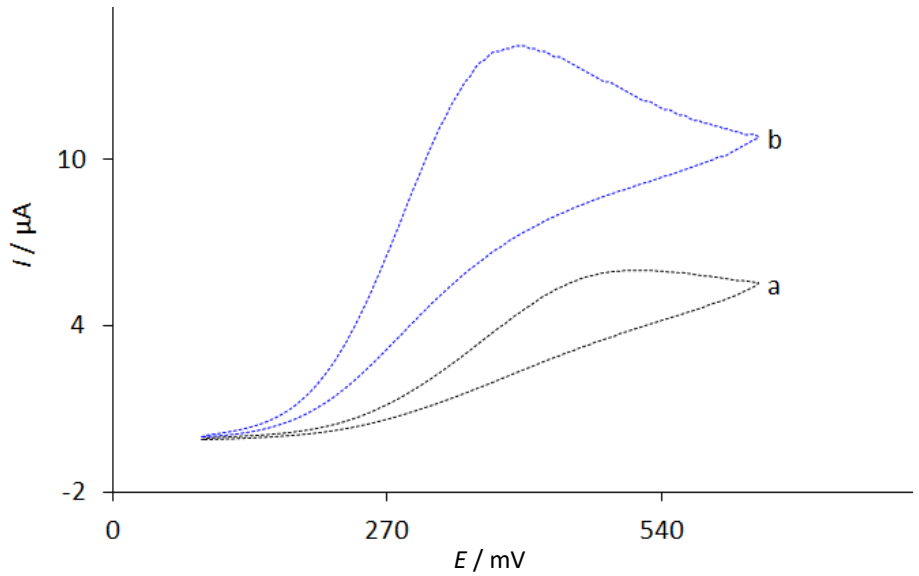



**Figure 1.** CV responses of 100.0 µM MDP: a - at unmodified CPE and b - at MSN-MOF/CPE

*Effect of scan rate on the electrochemical behaviour of methyldopa at the carbon paste electrode modified with $MoS_2$ nanosheets and a nickel-based metal-organic framework*

Using CV at different scan rates, the electrochemical behaviour of MDP produced in PBS (pH 7.0) at the MSN-MOF/CPE was examined. Figure 2 displays the corresponding CV responses at different scan rates. The anodic peak potentials shifted to more positive values at higher scan rates, indicating kinetic constraints, as shown by the cyclic voltammograms. Furthermore, the voltammograms' peak currents showed a linear relationship with the square root of the scan rate (Figure 3). These results show that MDP's electrochemical reaction mechanism on the MSN-MOF/CPE sensing platform is mostly diffusion-controlled.


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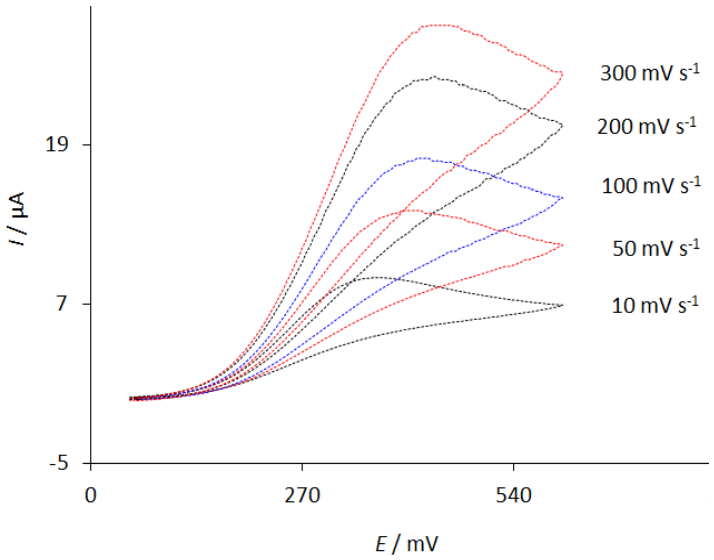



**Figure 2.** CV curves of 100.0 µM by MSN-MOF/CPE at various scan rates

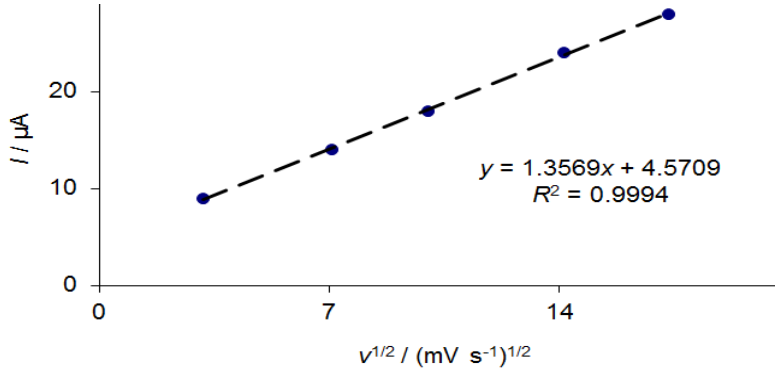



**Figure 3.** The linear relationship between *I* and $v^{1/2}$

*Chronoamperometric studies*

The oxidation process was investigated using the MSN-MOF/CPE sensor employing a chronoamperometry approach to determine the diffusion coefficient of MDP (Figure 4).

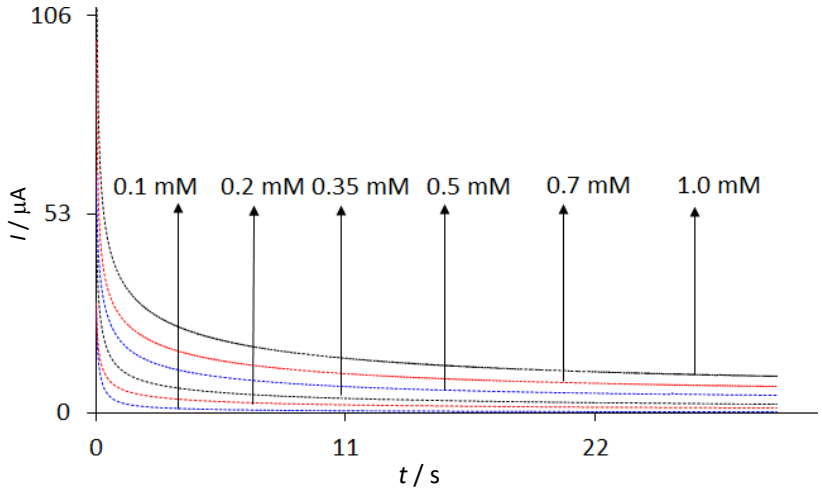



**Figure 4.** Chronoamperograms at MSN-MOF/CPE for various concentrations of MDP

The Cottrell equation [23] can describe the relation between *I* and $t^{-1/2}$ (Figure 5A). The slopes of the linear plots were then plotted against MDP concentration (0.05 to 260.0 µM) (Figure 5B). The diffusion coefficient (*D*) of MDP was determined to be $5.1\times10^{-6}$ $cm^2$ $s^{-1}$ by applying the Cottrell equation.

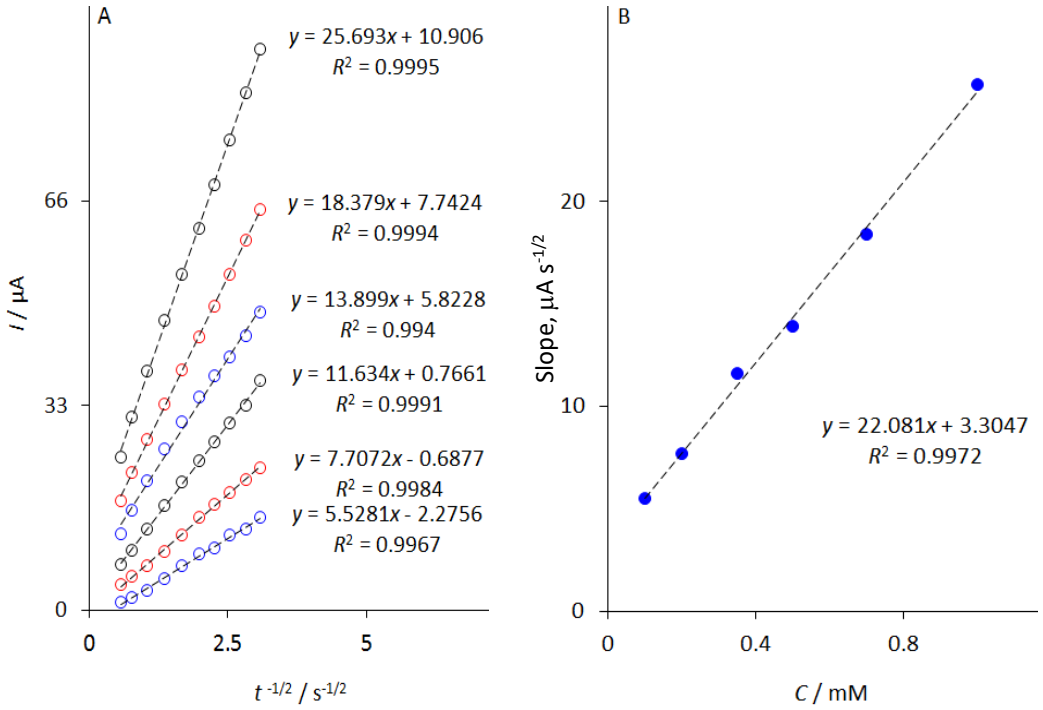



**Figure 5.** A - *p*lots of *I vs.* $t^{-1/2}$; B - plot of the slope against the MDP concentration

*Quantitative measurements of methyldopa at carbon paste electrode modified with $MoS_2$ nanosheets and a nickel-based metal-organic framework sensor using differential pulse voltammetry*

The MDP concentration was measured using differential pulse voltammetry (DPV). Figure 6 displays the DPVs for the oxidation of MDP obtained at the MSN-MOF/CPE. The DPV oxidation peak currents for MDP increased progressively with its concentration over a range of 0.05 to 260.0 µM (Figure 7). The LOD was determined using the formula LOD = $3S_{Blank}/m$, where $m$ is the calibration curve's slope, and $S_{Blank}$ is the standard deviation of the response from the blank solution ($n$ = 12). Based on this equation, the LOD for MDP at the suggested sensor was 0.01 µM.

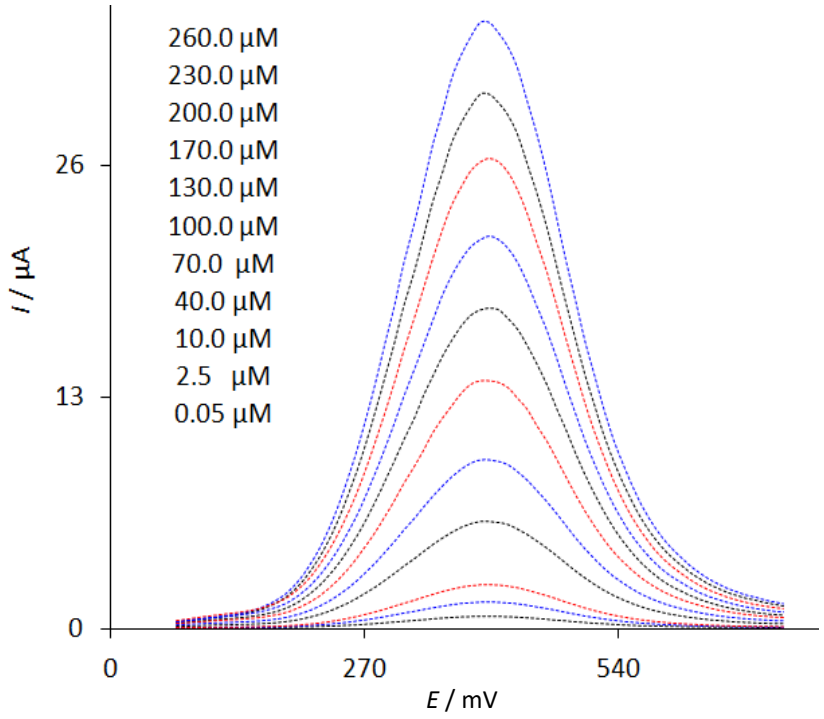



**Figure 6.** DPV responses of MSN-MOF/CPE 0 to different MDP concentrations


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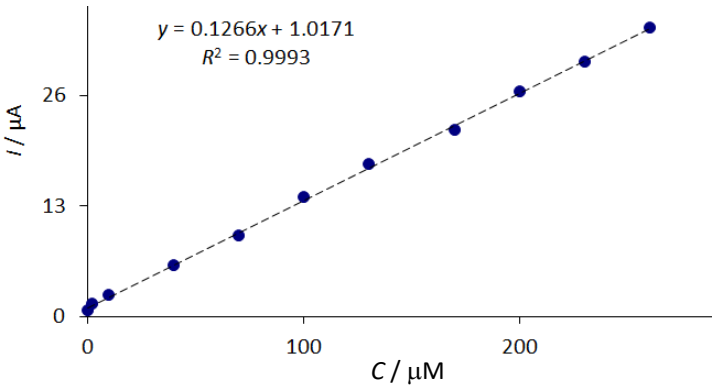



**Figure 7.** Linear dependence of *I* on MDP concentrations

*The simultaneous determination of methyldopa and hydrochlorothiazide at carbon paste electrode modified with $MoS_2$ nanosheets and a nickel-based metal organic framework sensor*

This investigation primarily focused on the simultaneous determination of MDP and HCTZ. Figure 8 shows the DPVs for the MDP and HCTZ combination on MSN-MOF/CPE after the concentrations of *MDP* and HCTZ were changed synchronously. With a peak difference of 240 mV, the current responses for the oxidations of MDP (400 mV) and HCTZ (640 mV) grew linearly. The MSN-MOF/CPE sensitivity to MDP oxidation in the presence of HCTZ was approximately 0.1259 µA/µM, very close to the value obtained in the absence of HCTZ (0.1266 µA/µM) (Figure 9).

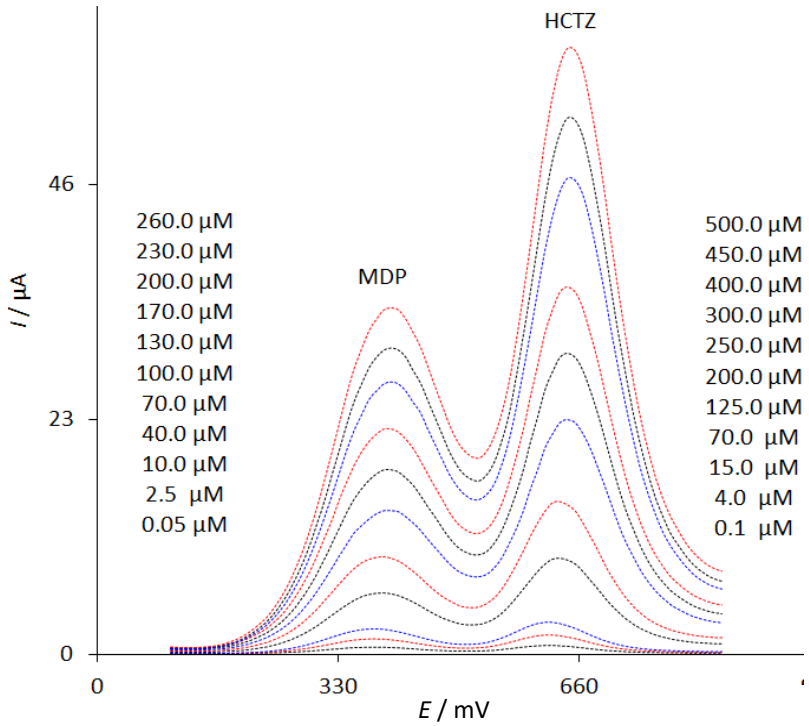



**Figure 8.** DPVs accepted *MSN-MOF/CPE* containing diverse concentrations of MDP and HCTZ

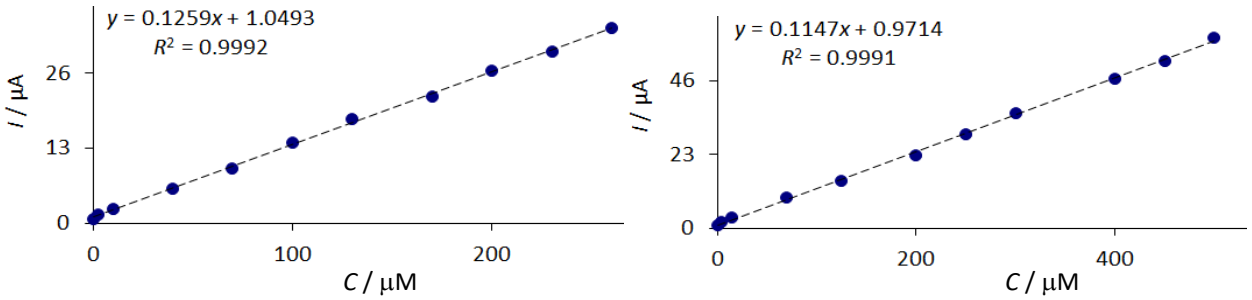



**Figure 9.** A) Plot of *I* versus MDP concentrations. B) Plot of *I* versus HCTZ concentrations

*Real sample analysis*

Determination of MDP and HCTZ in pharmaceutical preparations

The approach was used to determine MDP and HCTZ in MDP tablets and HCTZ tablets to assess its analytical applicability. Table 1 lists the findings.

**Table 1.** Determination of MDP and HCTZ in real pharmaceutical preparations using the MSN-MOF/CPE. (*n* = 5)

| Sample | Amount, µM | | | | Recovery, % | | RSD, % | |
|---|---|---|---|---|---|---|---|---|
| | Spiked | | Found | | | | | |
| | MDP | HCTZ | MDP | HCTZ | MDP | HCTZ | MDP | HCTZ |
| MDP tablet | 0 | 0 | 4.9 | - | - | - | 3.6 | - |
| | 1.0 | 5.0 | 5.8 | 5.2 | 98.3 | 104.0 | 2.2 | 3.3 |
| | 3.0 | 7.0 | 8.0 | 6.8 | 101.3 | 97.1 | 3.0 | 2.9 |
| HCTZ tablet | 0 | 0 | - | 3.8 | - | - | - | 2.3 |
| | 5.5 | 2.0 | 5.6 | 5.7 | 101.8 | 98.3 | 3.2 | 2.0 |
| | 7.5 | 3.0 | 7.3 | 8.8 | 97.3 | 101.1 | 2.1 | 3.1 |

By comparing the results with those stated on the medicinal preparations' labels, the reliability of the proposed modified electrode was also assessed (Table 2).

**Table 2** Comparison of content of MDP and HCTZ with declared values in the labels of the sample (*n* = 5).

| Content in tablet, mg $ml^{-1}$ | Declared value | Found value | RSD, % |
|---|---|---|---|
| MDP | 250.0 | 249.5 | 2.9 |
| HCTZ | 50.0 | 49.9 | 2.5 |

Determination of MDP and HCTZ in urine sample

The suggested method was also used to determine MDP and HCTZ in a urine sample to assess its analytical applicability. Table 3 presents the findings.

**Table 3.** Determination of MDP and HCTZ in urine sample using the MSN-MOF/CPE (*n* = 5)

| Amount, µM | | | | Recovery, % | | RSD, % | |
|---|---|---|---|---|---|---|---|
| Spiked | | Found | | | | | |
| MDP | HCTZ | MDP | HCTZ | MDP | HCTZ | MDP | HCTZ |
| 0 | 0 | - | - | - | - | - | - |
| 5.5 | 5.0 | 5.6 | 4.9 | 101.8 | 98.0 | 3.3 | 2.4 |
| 7.5 | 7.0 | 7.3 | 7.2 | 97.3 | 102.9 | 2.3 | 3.5 |

## Conclusion

Using the solvothermal approach, the $MoS_2$/Ni-MOF nanohybrid sheets were effectively generated, characterized, and used to modify CPE to create an electrochemical sensing platform for voltammetric detection of MDP in the presence of HCTZ. For determining MDP, the CV responses of MSN-MOF/CPE and unmodified CPE showed a notable decrease in overpotentials and an increase in oxidation peak currents. The synergistic effect of the constituents in the resulting nanocomposite is linked to the enhanced electrocatalytic capacity of MSN-MOF/CPE toward MDP oxidation.

A low LOD of 0.01 µM was found when quantitative measurements of MDP based on the DPV method were analysed using MSN-MOF/CPE. The sensitivity was 0.1266 µA $\mu M^{-1}$. Additionally, the MSN-MOF/CPE, used for their simultaneous determination, successfully separates the oxidation peaks of MDP and HCTZ in a binary combination. Furthermore, MDP and HCTZ in urine samples and MDP and HCTZ tablets were measured using the as-developed MSN-MOF/CPE sensing platform with satisfactory results, suggesting that this platform may be useful for electrochemical sensing applications.


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