



Original scientific paper

## Highly sensitive determination of methyldopa and hydrochlorothiazide using CoMoO<sub>4</sub> nanosheets-modified screen-printed electrode

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### Abstract

A novel screen-printed electrode (SPE) modified by CoMoO<sub>4</sub> nanosheets (CoMoO<sub>4</sub> NSs) was designed to analyse hydrochlorothiazide. Cyclic voltammetry, chronoamperometry and differential pulse voltammetry using the developed electrode were performed to evaluate its electrochemical behaviour. Further, the anodic peak currents for hydrochlorothiazide at CoMoO<sub>4</sub> NSs modified SPE were almost 4 times higher than for the untreated SPE. Between the concentration range of 0.1 to 400.0 μM, the modified electrode's response was directly proportional to the concentration, and it could detect hydrochlorothiazide at a very low level of 0.05 μM (S/N = 3). Hydrochlorothiazide was also measured using the modified electrode when methyldopa was present. The sensor offers various advantages for the analysis of hydrochlorothiazide detection, including moderate cost, ease of preparation and considerable sensitivity.

### Keywords

Voltammetry, drug analysis, real sample analysis, blood pressure drugs

### Introduction

As a member of the thiazide family, hydrochlorothiazide is a diuretic agent that prevents kidney failure. Hydrochlorothiazide is known to lessen the effects of renal tubular acidosis and nephrogenic diabetes insipidus and is used to treat conditions like hypertension, renal oedema, and hypercalcaemia [1].

Hydrochlorothiazide is absorbed through the gastrointestinal tract and is mainly excreted through renal excretion. About 65 to 72 % of hydrochlorothiazide leaves the body through urine [2]. Athletes are known to use thiazide diuretics to excrete illegal substances or for fast and acute loss of weight. This is why the use of diuretics has been banned by the WADA [3]. Consequently, efficient and dependable techniques for the detection and swift online analysis of hydrochlorothiazide are of paramount significance.

Conventional methods used for the analysis of these banned substances are gas and mass spectrometry [4], high-performance liquid chromatography (HPLC) [5], and capillary electrophoresis [6]. These techniques offer high sensitivity yet are very complex, time-intensive, costly and require highly skilled operators. Consequently, developing fast and easy alternatives for detecting diuretics like hydrochlorothiazide is an important research topic. Being a known class of alternative techniques, electrochemical methods offer ease of use and relatively low-cost instrumentation capability for sensitive real-time analyses [7,8]. Electrochemical techniques have widespread applications in the determination of pharmaceuticals [9,10].

High blood pressure is treated with methyldopa (2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid). Methyldopa is categorised as an antihypertensive medication. It dilates the blood vessels to enhance blood circulation throughout the body. Prevalent and untreated, hypertension can adversely affect the kidneys, heart, blood vessels, brain, and other bodily organs. Damage to these organs can lead to heart disease, stroke, vision impairment, heart failure, myocardial infarction, and other complications.

Prevalent and untreated, hypertension can adversely affect the heart, kidneys, brain, blood vessels, and other body organs if left untreated. Renal failure, heart disease, eyesight loss, stroke, heart attack, heart failure, and other issues can result from damage to these organs. As a result, methyldopa detection and quantification are crucial aspects of clinical and pharmacological methods [11].

Methyldopa and hydrochlorothiazide are used together to treat hypertension. Therefore, it is imperative to develop a straightforward, precise, quick, and affordable approach to simultaneously detect these medications [12].

Chemically modified electrodes are very often used in electrochemistry [9,10]. A key quality of modified electrodes is their catalytic abilities, which significantly lower the overpotentials of various species on their surfaces as opposed to unmodified electrodes. Modified electrodes also offer enhanced selectivity [9,10].

Using nanoparticles to chemically modify electrodes can improve their fundamental characteristics without necessarily altering the electrode material's composition [13]. Thus, the smart world of low-dimensional systems and the present developments in building functional nanostructured arrays may be important factors in the development of novel nanotechnology [14-16]. Because of their ability to improve electron transfer, nanostructured materials are essential to the operation and integration of nanoscale sensors [17-19]. Because of their unique capacity to enhance electron transfer between the electrode and the active site, CoMoO<sub>4</sub> nanosheets were used to produce the transducer surface of the hydrochlorothiazide sensor. In this study, the hydrothermal method, a potent technique for the synthesis of large, high-quality crystals, was used to produce CoMoO<sub>4</sub> nanosheets.

Screen-printed electrodes (SPEs) have proven to be versatile devices, capable of being fabricated in diverse geometries and composed of various materials [20,21]. This research uses screen-printed electrodes (SPE) to analyse hydrochlorothiazide. Hydrochlorothiazide quantification was conducted using the voltammetric methods. The resulting electrode offers better selectivity, sensitivity and reproducibility. The analytical performance of the CoMoO<sub>4</sub> NSs-SPE for the assessment of hydrochlorothiazide and methyldopa in actual samples was examined.

## Experimental

### *Materials and instruments*

The electrochemical experiments used the designed electrode and the potentiostat-galvanostat (PGSTAT 12N, Eco-Chemie, the Netherlands) with general-purpose electrochemical software. The SPEs (DropSens, DRP-110, Spain) comprised an unmodified graphite working electrode, an Ag pseudo reference electrode, and a graphite counter electrode. The pH was measured with a Metrohm 710 pH meter.

Merck (Darmstadt, Germany) provided all compounds with methodical evaluation purity, including hydrochlorothiazide and methyldopa. To create PBS (the orthophosphate buffer solutions) within a pH range of 2 to 9,  $\text{H}_3\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$ , and  $\text{Na}_2\text{HPO}_4$ , and  $\text{Na}_3\text{PO}_4$  were used.

### *Preparation of $\text{CoMoO}_4$ nanosheets*

The synthesis of  $\text{CoMoO}_4$  nanosheets was conducted using the following procedure: Initially, 0.25 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and 0.2 g of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  were dissolved in 30 mL of ethanol and 25 mL of distilled water, followed by stirring for 15 minutes. The acquired solution was subsequently placed into an autoclave. The hydrothermal reaction was conducted by positioning the autoclave in an oven maintained at 180 °C for ten hours.

The obtained sample was cooled to ambient temperature and then repeatedly cleaned with ethanol and distilled water before being dried for 15 hours at 70 °C. Later, the material was calcified at 400 °C for 120 minutes.

### *Electrode modification*

5  $\mu\text{l}$  of dispersion of 1 mg  $\text{CoMoO}_4$  NSs in 1 mL of water, homogenised by 45 minutes of ultrasonication, was cast on the SPE surface and let to dry at room temperature in order to modify SPEs.

### *Real samples*

500 milligrams of the powder was sonicated and diluted in 25 millilitres of  $\text{H}_2\text{O}$  after ten hydrochlorothiazide tablets (each labelled 50 mg) were ground. Following that, varying volumes of this solution were diluted with 25 millilitres of PBS (pH 7.0). The analyses were carried out using the standard addition technique.

Five 250 mg tablets of methyldopa were being ground. Then, the solution was made by ultrasonically dissolving 250 milligrams of the powder in a volume of 25 mL of  $\text{H}_2\text{O}$ . Then, the solution was subsequently adjusted with PBS (pH 7) to the suitable concentration and transferred in varying volumes into a 25 mL volumetric flask.

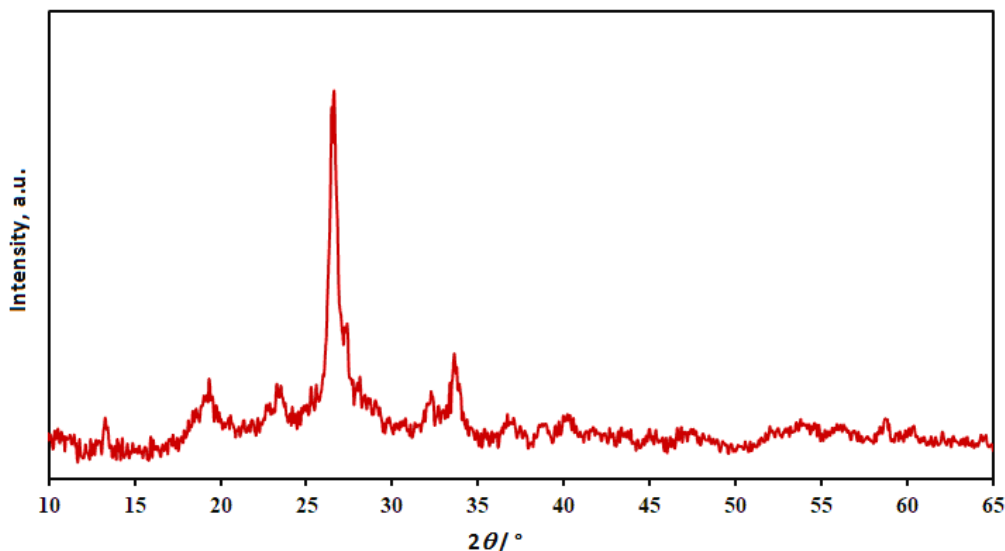
A fridge was used to keep the urine samples. 0.01 mL of the samples were spun at 2000 rpm for fifteen minutes before being filtered through a 0.45  $\mu\text{m}$  filter in preparation for analysis. Next, enough PBS (pH 7.0) was added to a volumetric flask to dilute the resultant sample to 25 mL. The obtained samples were combined with hydrochlorothiazide and methyldopa at different concentrations.

## Results and discussions

### *X-ray diffraction*

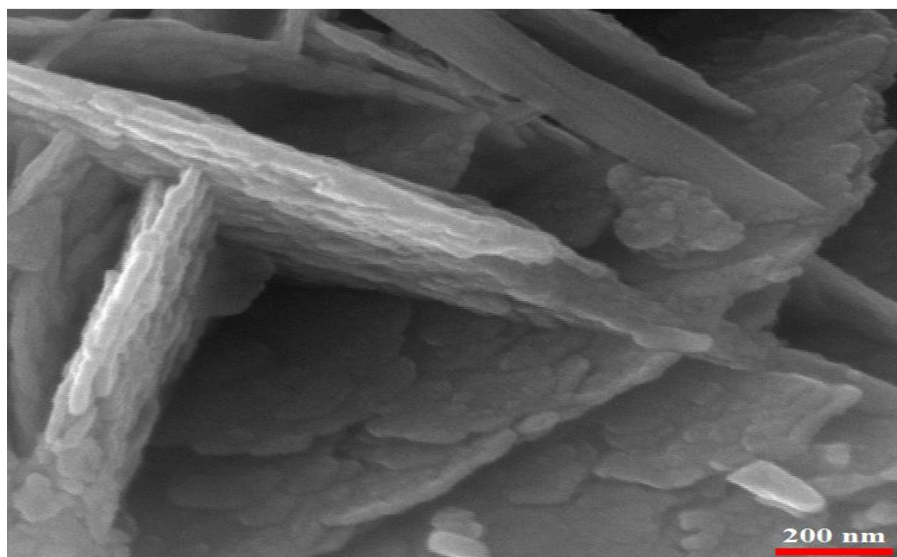
The XRD study was used to examine the crystalline structure of the nanostructure of  $\text{CoMoO}_4$ , as shown in Figure 1. From the XRD pattern, the peaks at  $2\theta = 13.1, 19.3, 23.4, 35.6, 26.5, 27.3, 27.9, 28.9, 29.9, 30.8, 32.2, 33.6, 36.8, 39.0, 40.3, 41.6, 42.8, 43.4, 43.8, 45.1, 45.4, 46.3, 46.9, 47.5, 48.3, 52.1, 52.6, 53.9, 54.9, 56.0, 58.9, 59.7, 60.4, 61.1, 63.8,$  and  $64.5^\circ$  were identified and assigned to

(001), (-201), (021), (201), (002), (-112), (-202), (-311), (310), (-131), (022), (-222), (400), (040), (003), (222), (132), (-422), (-223), (113), (-511), (-403), (241), (042), (-133), (-204), (-441), (-531), (-440), (-532), (024), (-352), (-424), (061), (260), and (243) planes of  $\text{CoMoO}_4$ , respectively (JCPDS = 21-0868).



**Figure 1.** XRD pattern of the  $\text{CoMoO}_4$  nanosheets

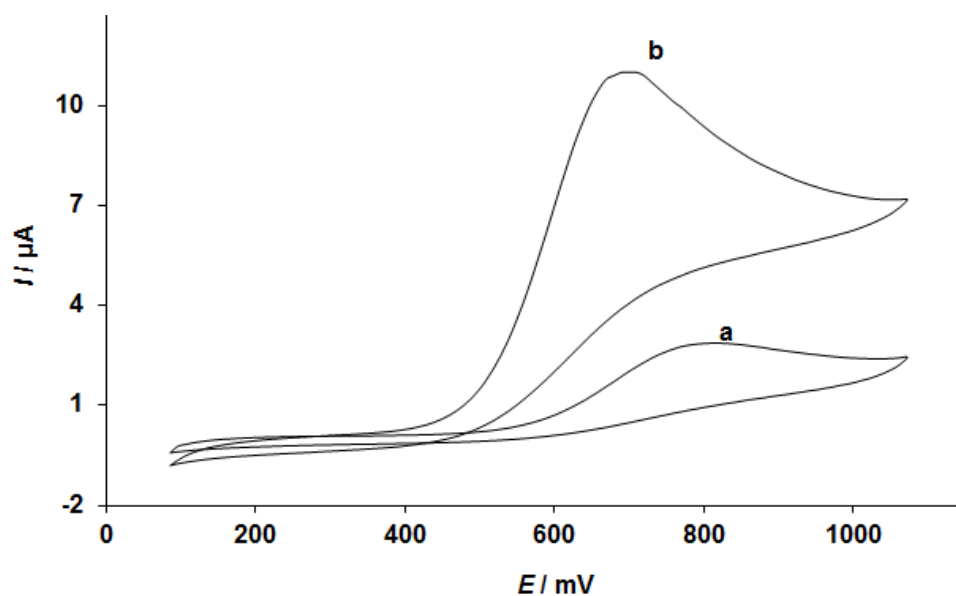
Figure 2 displays the results of the FE-SEM topography study of the  $\text{CoMoO}_4$  nanosheets



**Figure 2.** FE-SEM image for the  $\text{CoMoO}_4$  nanosheets

#### *The behaviour of the $\text{CoMoO}_4$ NSs-SPE*

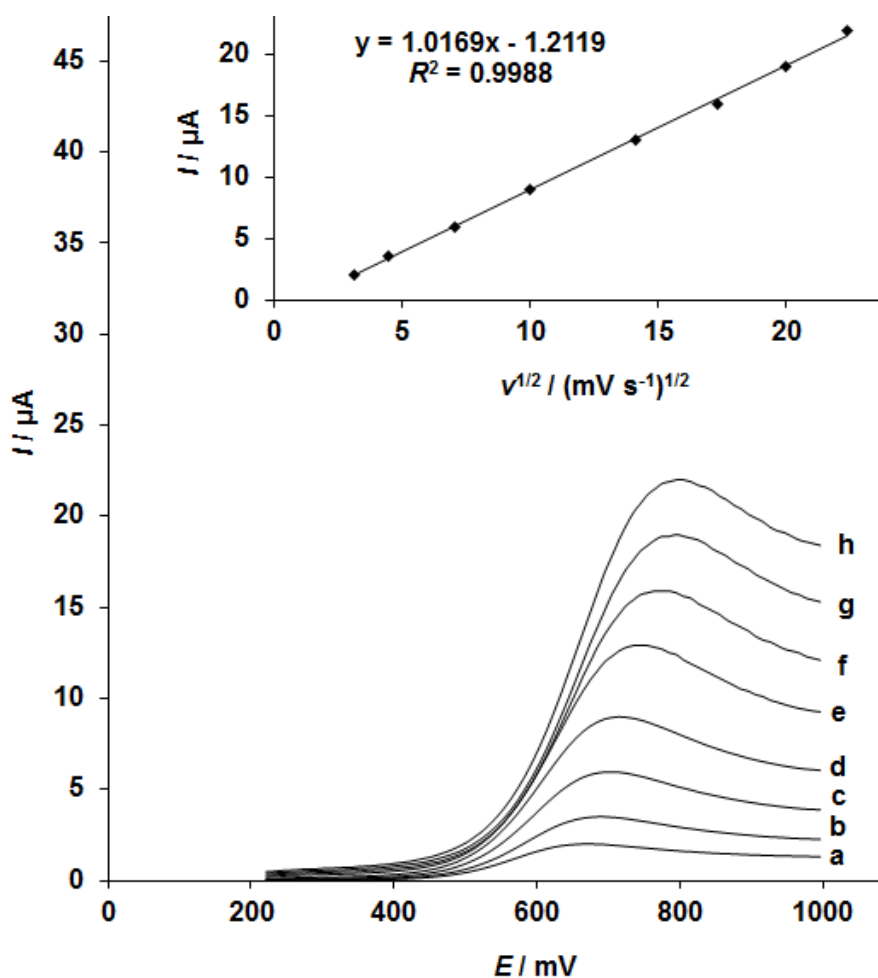
The electrochemical behaviour of hydrochlorothiazide was studied in various pH values in the range of 2 to 9 to determine the optimal pH. The findings demonstrated that the optimal results in terms of oxidation peak height were achieved at pH 7, and consequently, all further experiments were conducted at this pH. The cyclic voltammograms (CVs) of a 0.2 mM hydrochlorothiazide solution utilising the modified and bare SPE can be seen in Figure 3. This figure shows that the peak oxidation current for the  $\text{CoMoO}_4$  NSs-SPE appears at approximately 700 mV, which is about 100 mV more negative than the current peak obtained at bare SPE.



**Figure 3.** Cyclic voltammograms of (a) bare SPE, (b) CoMoO<sub>4</sub> NSs-screen printed electrode in 0.1 M PBS using 200.0 μM hydrochlorothiazide present at the scan rate of 0.05 V s<sup>-1</sup>

#### The effect of the scan rate

The oxidation peak currents increase with the scan rate (Figure 4). A linear dependence of the potential scan rate ( $v^{1/2}$ ) on the peak current ( $I_p$ ) was discovered, revealing a diffusion-controlled process.



**Figure 4.** Cyclic voltammograms of CoMoO<sub>4</sub> NSs-SPE at several scan rates in 0.1 M PBS (pH 7.0) with 0.1 mM hydrochlorothiazide; a-h denote 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 V s<sup>-1</sup>, respectively. The inset exhibits the variability of anodic peak current as a function of  $v^{1/2}$

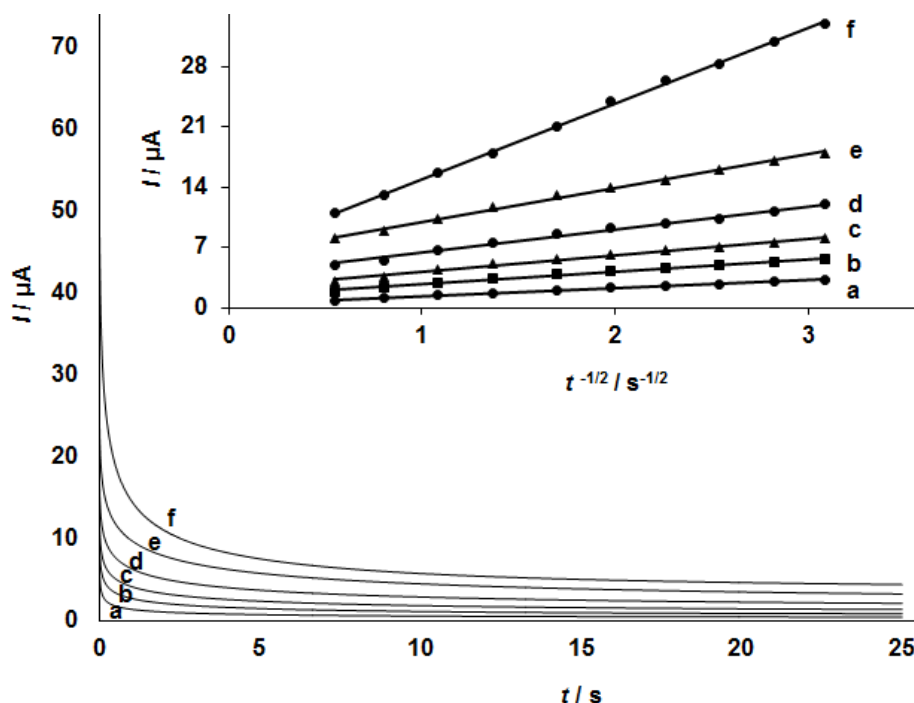
### Chronoamperometric analysis

Chronoamperometric analyses of hydrochlorothiazide samples were conducted using a CoMoO<sub>4</sub> NSs-SPE at 0.75 V, and the results were obtained for various hydrochlorothiazide concentrations in PBS, as seen in Figure 5. The dependence of current on time for a non-steady state diffusion-controlled process is described by the Cottrell equation (1):

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

where  $C_b$  and  $D$  are the bulk concentration and diffusion coefficient, respectively.

Figure 5 inset shows a plot of  $I$  vs.  $t^{-1/2}$  based on best-fit experimental data for different concentrations of hydrochlorothiazide. Based on these data, the mean value of  $D$  was  $2.6 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ , calculated from Cottrell's equation and resultant slope.



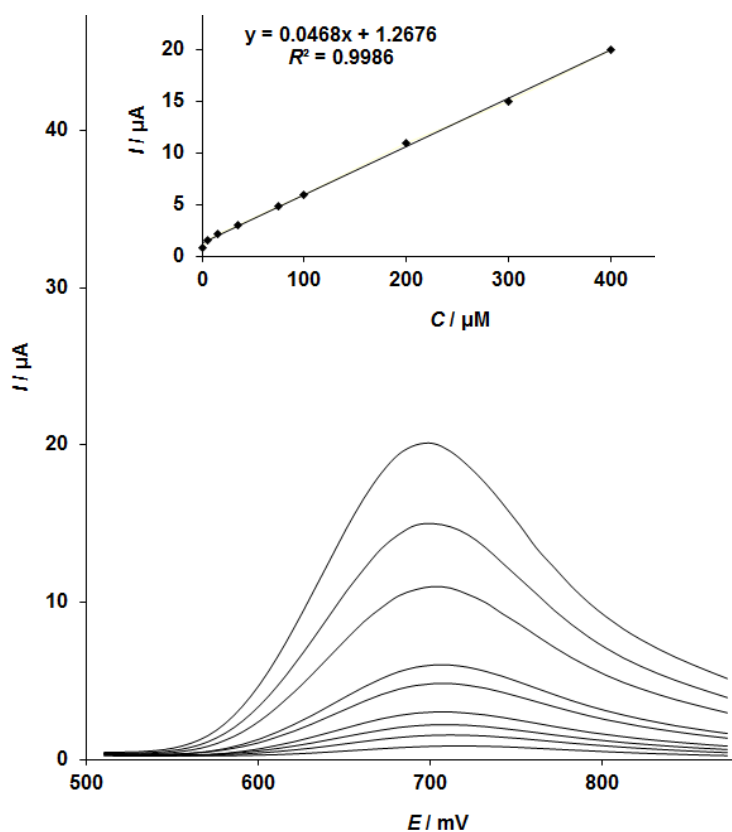
**Figure 5.** Chronoamperograms for varying hydrochlorothiazide concentrations were acquired at CoMoO<sub>4</sub> NSs-SPE in 0.1 M PBS with pH 7.0. The values 0.1, 0.15, 0.2, 0.3, 0.45 and 1.0 mM of hydrochlorothiazide are represented by the letters a to f. Inset: plot  $I$  vs.  $t^{-1/2}$

### Calibration plot

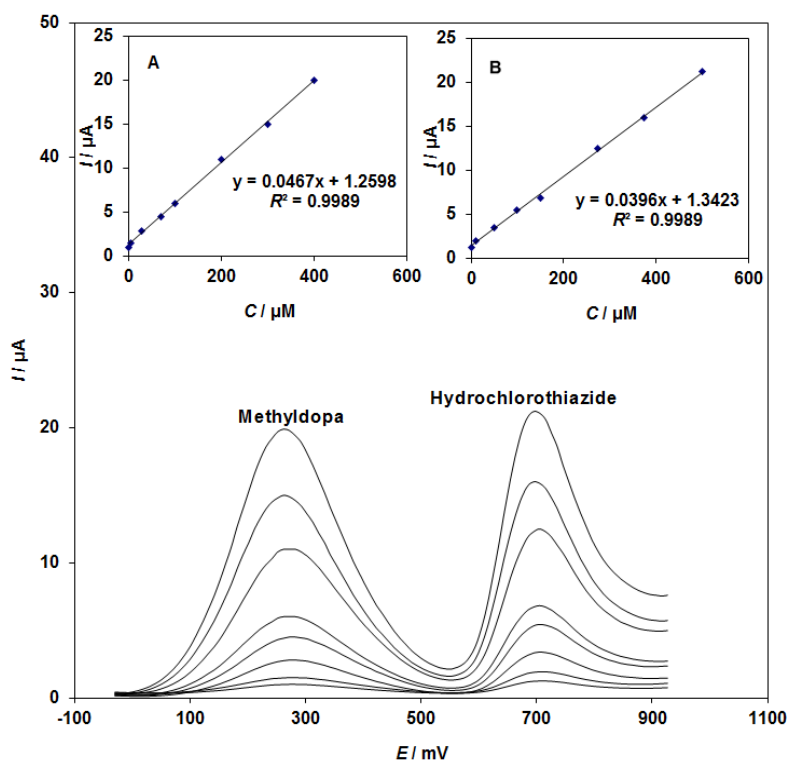
A quantitative evaluation of hydrochlorothiazide was conducted by differential pulse voltammetry (DPV) using the CoMoO<sub>4</sub> NSs-SPE, with the electrode serving as the working electrode. Various standard hydrochlorothiazide solutions in 0.1 M PBS were analysed, as shown in Figure 6. The peak currents exhibited a linear relationship with hydrochlorothiazide concentration within the 0.1 to 400  $\mu\text{M}$  concentration range, yielding a correlation coefficient 0.9986 and a detection limit for hydrochlorothiazide of 0.05  $\mu\text{M}$  ( $S/N = 3$ ).

### Simultaneous determination of hydrochlorothiazide and methyldopa

The mixed solution of hydrochlorothiazide and methyldopa at various concentrations was investigated at CoMoO<sub>4</sub> NSs-SPE utilising DPV. To determine two substances, the concentrations of methyldopa and hydrochlorothiazide were changed concurrently, and the DPVs were recorded (Figure 7). Methyldopa and hydrochlorothiazide oxidation are represented by well-defined anodic peak potentials of 270 and 700 mV, respectively. This suggests that simultaneous detection of both chemicals is possible at the CoMoO<sub>4</sub> NSs-SPE, as seen in Figure 7.



**Figure 6.** DPVs of  $\text{CoMoO}_4$  NSs-SPE in 0.1 M pH 7.0 for varying concentrations of hydrochlorothiazide. The hydrochlorothiazide concentrations were (from inner to outer) 0.1, 5.0, 15.0, 35.0, 75.0, 100.0, 200.0, 300.0 and 400.0  $\mu\text{M}$ . Electrocatalytic peak current against the hydrochlorothiazide concentration is shown in the inset



**Figure 7.** Shows the DPVs of the  $\text{CoMoO}_4$  NSs-SPE in PBS (pH 7.0) with varying methyl dopa + hydrochlorothiazide concentrations of 1.0+1.0, 5.0+10.0, 30.0+50.0, 70.0+100.0, 100.0+150.0, 200.0+275.0, 300.0+375.0 and 400.0+500.0  $\mu\text{M}$  in that order. Inset A - Plots of  $I_p$  vs. methyl dopa concentration; inset B -  $I_p$  versus hydrochlorothiazide concentration

### Analysis of real samples

The developed electrode was found to be capable of analysing hydrochlorothiazide and methyldopa contents of solutions of hydrochlorothiazide and methyldopa tablets and urine samples. Table 1 demonstrates the analyses performed through the standard addition process. The data clearly indicate that the hydrochlorothiazide and methyldopa recoveries were satisfactory. Further, the results were reproducible as reflected by the mean standard deviation of the relative results.

**Table 1.** CoMoO<sub>4</sub> NSs-SPE estimation of methyldopa (MD) and hydrochlorothiazide (HCT) in real samples (n=5)

Sample	Amount, $\mu\text{M}$				Recovery, %		RSD, %	
	Spiked		Found		MD	HCT	MD	HCT
	MD	HCT	MD	HCT				
Methyldopa tablet	0.0	0.0	3.4	-	-	-	3.2	-
	2.0	5.0	5.6	4.9	103.7	98.0	2.8	2.1
	4.0	7.0	7.3	7.2	98.6	102.9	1.8	3.2
Hydrochlorothiazide tablet	0.0	0.0	-	4.9	-	-	-	2.6
	5.5	1.0	5.4	6.0	98.2	101.7	3.2	2.7
	7.5	3.0	7.8	7.7	104.0	97.5	2.7	2.9
Urine	0.0	0.0	-	-	-	-	-	-
	5.0	4.0	4.9	4.1	98.0	102.5	1.8	2.3
	7.0	6.0	7.3	5.8	104.3	96.7	2.7	2.2

### Conclusion

In summary, CoMoO<sub>4</sub> NSs-SPE has significantly enhanced electrocatalytic ability for hydrochlorothiazide compared to bare SPE. It was found to offer excellent performance above a rather wide-ranging concentration range of 0.1 to 400  $\mu\text{M}$  and had a low detection limit of 0.05  $\mu\text{M}$ . It was used to determine hydrochlorothiazide in the presence of methyldopa.

**Conflict of interest:** The author has no conflict of interest.

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