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Original scientific paper

$NiMoO_4$ nanosheets modified carbon paste electrode for simultaneous determination of epinine and dobutamine

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Abstract

In this work, a carbon paste electrode was modified with ionic liquids and $NiMoO_4$ nanosheets (NMs/IL/CPE) for voltammetric epinine detection. The NMs/IL/CPE showed good electrocatalytic activity for epinine oxidation compared to unmodified CPE. The limit of detection is 0.05 µM, and under ideal circumstances, the oxidation peak currents of epinine show a linear relationship with epinine concentration from 0.1 to 300.0 µM. Additionally, when dobutamine was present, the NMs/IL/CPE showed good activity toward epinine determination. Its appropriateness for the simultaneous detection of these two medicines using differential pulse voltammetry is shown by the separation of the oxidation peak potentials of 200 mV. Lastly, epinine and dobutamine analysis in real specimens confirmed the proposed sensor's applicability with outstanding findings (recovery 96.0 to 103.5 %, and relative standard deviation <3.6 %).

Keywords

Electrochemical sensor; deoxyepinephrine; catecholamine; cardiovascular disease; dobutamine

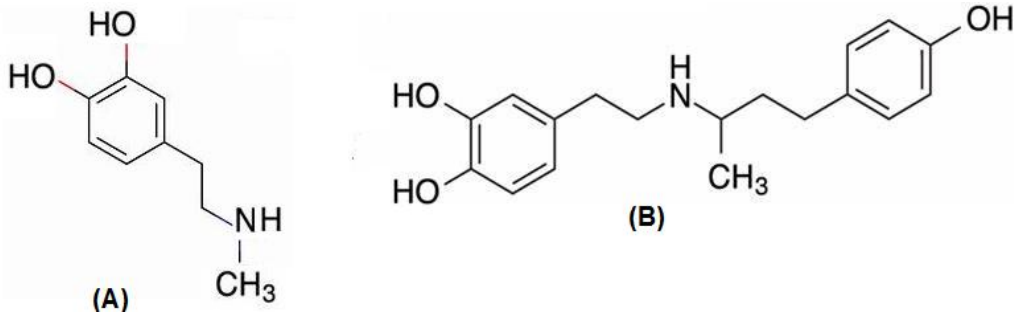
Introduction

The 1950s saw the development of carbon paste electrodes (CPE), a straightforward but effective electrode material that was the product of research into a liquid carbon electrode intended to replace mercury in polarography [1]. Because of its ease of preparation and surface renewal [2], CPE offered significant potential applications in anodic voltammetry. Over the next seven decades,

carbon paste electrodes' aforementioned characteristics fuelled the development of carbon paste electrochemical sensors, which are now among the most researched fields and used in the study of drug cells, microbes, and environmental processes. This was due to the fact that the cost of production of carbon paste is lower than that of other electrode materials, it is easier to make in the process, and it does not have a great environmental impact as other methods [3]. This composition is mainly made up of a conductive material, usually graphite, which is cheap and has a high level of conductivity, and a binder material, such as hydrophobic and viscous mineral oil, that is usually used to maintain the needed cohesion of the paste in the liquid medium. The use of ionic binders has become more attractive with the introduction of ionic liquids (ILs) in the 2000s, which brought about the development of carbon ionic liquid electrodes (CILEs) [4]. ILs can replace ordinary binders [5] as an additional component or a special medium for another modifying agent [6]. The CILEs were used in the electroanalytical determination of biologically active compounds, e.g. phenolic derivatives [7], biological markers [8], glucose [9] and proteins [10], thus proving the catalytic nature of the ILs and the process.

Electrochemistry has traditionally offered analytical methods with instrumental simplicity, modest cost, and mobility. These strategies have introduced the most promising approaches for certain applications [11]. However, for ordinary electrodes, these techniques often have limited sensitivity. However, electrode modification can overcome the limited sensitivity and poor selectivity of electrochemical techniques [12,13].

Some natural products, including *Acacia* and the *Peyote cactus*, contain deoxyepinephrine, also known as epinine, a catecholamine derivative having pharmacological action [14]. Because of the increased risk of hypertension in the human body, deoxyepinephrine, an active form of ibopamine, is not prescribed with 1-(3-mercapto-2-methyl-propionyl)-pyrrolidine-2-carboxylic acid (Scheme 1). Deoxyepinephrine is recommended to increase blood pressure and as an appropriate alternative to epinephrine [14]. While there are numerous advantages to using deoxyepinephrine, too much of it can lead to a number of health issues. As a result, deoxyepinephrine detection in biological samples and the human body is crucial [15]. For the quick and accurate identification of epinine, amperometric and voltammetric sensors are excellent options [16-18].



Scheme 1: *Molecular structure of A -epinine and B - dobutamine*

These sensors have been enhanced in the last year to detect pharmaceuticals or biological molecules simultaneously by utilising nanomaterials and other electroconductive mediators [19]. In recent years, there has been increased interest in coupling two or three mediators to create biosensors or electrochemical sensors [20]. In real samples, the modified amperometric and voltammetric-based sensors show good activity for determining various biological, pharmacological, or environmental substances [21]. A novel method for analysing drugs, environmental contaminants, and biological materials at the trace level was developed through the use of nano-based structural sensors [21].

A dangerous condition that seriously damages the heart is cardiovascular disease. According to the National Health and Nutrition Examination Survey, a significant portion of patients in the United States have cardiovascular problems, primarily those 65 years of age or older [22]. In general, it encompasses circumstances involving constricted or blocked blood arteries that may result in angina, heart attacks, or strokes [23]. Dobutamine (DB), a member of the catecholamine family, is mainly used to treat cardiac problems such as coronary heart disease and cardiogenic shock [24]. DB is used primarily for short-term therapy of heart failure brought on by cardiac disease or for patients who have just had surgery. Because of its relative selectivity, DB is regarded as a sympathomimetic medication when taken with beta-adrenoceptors [4]. DB promotes blood flow, alleviates coronary heart disease symptoms, and raises heart rate or cardiac output in congestive heart failure [25]. Since DB has a low bioavailability when taken orally, intravenous infusion is typically used to deliver it. Additionally, it is crucial to monitor the blood's DB level since it is linked to a number of neurological conditions and mental illnesses, including Parkinson's disease and schizophrenia [26]. The literature cites several papers that use various methodologies to determine DB. The simplicity, high sensitivity, quick response, and reduced detection limits of electrochemical methods have made them popular and preferred over other approaches in recent years [27,28].

A few serious diseases (hypertension, neuroblastoma, and pheochromocytoma) may arise from alterations in the body's catecholamine metabolism. It would be possible to record the incidence of certain diseases at an early stage and stop further disease progression if the lower or larger content of the body was properly detected. Furthermore, the amounts of catecholamines in bodily fluids may indicate how they function physiologically in the body [29,30]. To evaluate the levels of dobutamine and epinine in biological fluids, it is crucial to create an analytical method that is extremely sensitive, exact, selective, and economical.

$NiMoO_4$ nanosheets were suggested for epinine detection in this work. Differential pulse voltammetry (DPV), chronoamperometry, and cyclic voltammetry (CV) were used to examine the electrochemical behaviour of epinine and the effectiveness of the $NiMoO_4$ nanosheets/ionic liquids modified carbon paste (NMs/IL/CPE). The high conductivity of ILs, the huge specific surface area and the potent catalytic activity of $NiMoO_4$ nanosheets can be linked to the produced sensor's superior performance in epinine detection. Subsequent research showed that the NMs/IL/CPE sensor can efficiently detect both epinine and dobutamine in combination with different signals and exhibit outstanding electrocatalytic capabilities towards epinine. Dobutamine and epinine levels in actual specimens were accurately determined using the NMs/IL/CPE sensor. To our knowledge, no research has been published in the literature that describes using $NiMoO_4$ nanosheets to alter CPE as a sensing platform for voltammetric epinine measurement in the presence of dobutamine.

Experimental

Materials and reagents

The Sigma-Aldrich and Merck businesses provided the epinine, dobutamine, and other ingredients without further purification. Orthophosphoric acid was used to create the buffer solutions (0.1 M), and aqueous NaOH was used to modify their pH. Deionized water was used to prepare all aqueous solutions.

Instrumentation

To perform the electrochemical experiments, an Autolab potentiostat-galvanostat was used. Three electrodes were used: platinum wire for the auxiliary electrode, Ag/AgCl KCl (3.0 M) for the reference electrode, and bare CPE and NMs/IL/CPE for the working electrode.

Synthesis of $NiMoO_4$ nanosheets

In 10 mL of deionised water, 0.25 g of $Ni(CH_3COO)_2 \times 4H_2O$, 0.2 g of $((NH_4)_6Mo_7O_2 \times 4H_2O$ and 0.24 g of urea were dissolved while being constantly stirred for one hour. The resultant solution was then moved to a 25 mL stainless steel autoclave coated with Teflon, heated for 10 h at a rate of 5 °C min^{-1}, and allowed to naturally cool to ambient temperature. The resulting precipitate was then vacuum-dried for 12 hours at 60 °C after being thoroughly cleaned with ethanol and deionised water [31]. Figure 1 shows an example of a standard SEM.

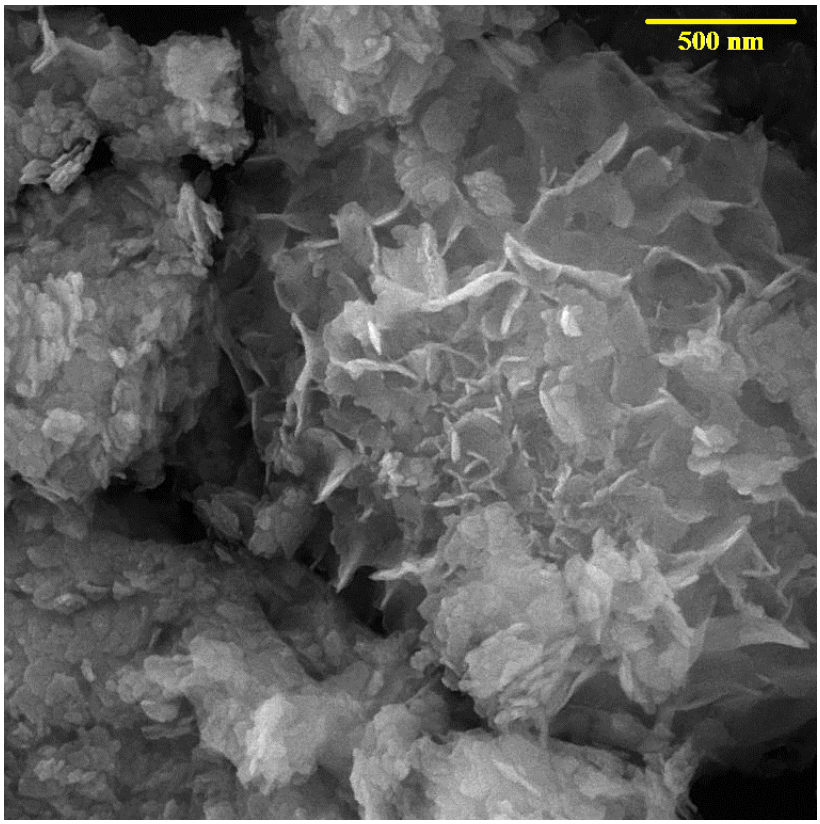


Figure 1. *SEM image of $NiMoO_4$ nanosheets*

Preparing the modified electrode

The NMs/IL/CPE sensor was prepared by thoroughly hand-mixing graphite powder (0.48 g), $NiMoO_4$ nanosheets (0.02 g), and IL (n-hexyl-3-methylimidazolium hexafluoro phosphate) with a suitable amount of paraffin oil in a mortar using a pestle. A portion of this mixture was then firmly packed into a tubular glass holder, with a copper wire positioned at the back of the paste. The external surface of the carbon paste was polished using soft paper. To refresh the electrode surface, the electrode surface was polished.

Preparing the real samples

A 250 mg/ampoule dobutamine ampule was purchased, and 0.1 M phosphate buffer solution (PBS) solution (pH 7.0) was used to dilute it. It was then immediately used to quantify the

dobutamine levels. The electrochemical cell was then used to assess dobutamine using the traditional addition technique after a suitable volume of the final solution was transferred.

After collecting the urine samples, we stored them in a refrigerator and centrifuged 30 mL of the samples for 10 minutes at 3000 rpm. Next, we used a 0.45 µm filter to filter the supernatant. A 50 mL volumetric flask was then filled with 20 mL of the solution, which was diluted with PBS at a pH of 7.0. In order to minimise further matrix effects, the diluted urine samples were spiked with varying concentrations of dobutamine and epinine in the next step. The dobutamine and epinine contents were then examined using this novel methodology in conjunction with the conventional addition method.

Results and discussion

Electrochemical behaviour of epinine on the bare CPE surface and NMs/IL/CPE surface

Varying the pH of the buffer solution, samples of 100.0 µM epinine were examined with cyclic voltammetry (CV) on the NMs/IL/CPE in an aqueous solution of 0.1 M PBS at the pH range of 5.0 to 9.0. Cyclic voltammograms showed one irreversible anodic charge transfer process (Figure 2). Anodic current peak, I_{pa}, reached its maximum value at pH 7.0, so pH 7.0 was the optimum pH and chosen medium for further investigations.

The anodic peak current of epinine at the unmodified CPE was estimated to be 4.92 µA, whereas at NMs/IL/CPE, it was 16.0 µA. I_{pa} of epinine increased considerably at NMs/IL/CPE over bare CPE (Figure 2).

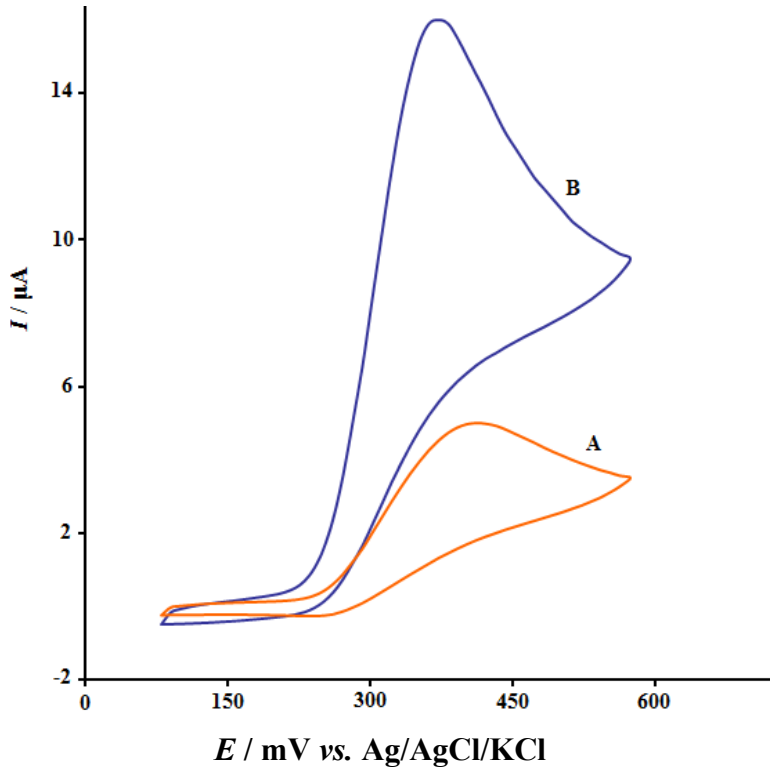


Figure 2. *CVs of unmodified CPE (A) and NMs/IL/CPE (B) in PBS containing epinine (100.0 µM)*

Evaluating the effect of scan rate

Epinine's I_{pa} at different scan rates was determined with CV. The CVs of 100.0 µM of epinine in PBS with various scan speeds are shown in Figure 3. The oxidation peak current was seen to increase proportionally to the scan rate. I_{pa} = 2.1286 $\nu^{1/2}$ + 0.7761 (R^2 = 9966) is a linear equation, which signifies that diffusion is the rate-determining step for epinine oxidation at the NMs/IL/CPE surface.

The correlation coefficient determined between I_{pa} and the square root of the scan rate ($\nu^{1/2}$) is about 0.9966 in the range of 10-100 mV s^{-1}.

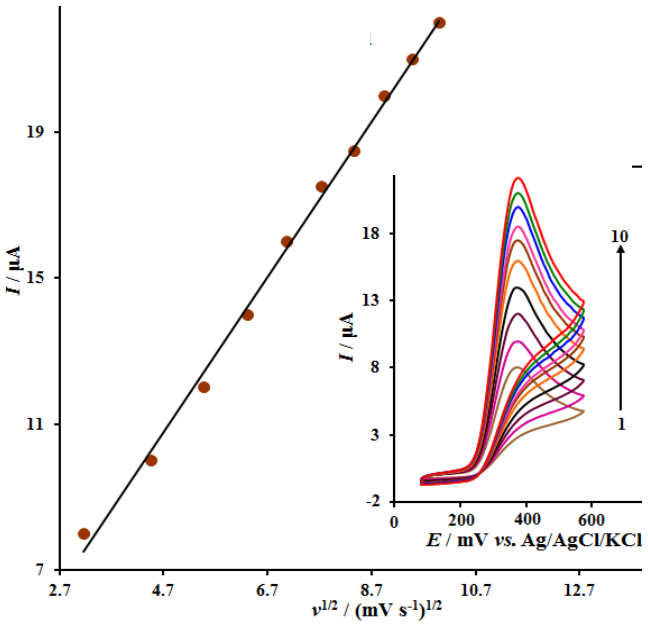


Figure 3. *CV responses and the linear response of the I_{pa} with $\nu^{1/2}$ of the NMs/IL/CPE in the measurement of 100.0 µM epinine in PBS by varying the scan rates. Inset: CVs recorded at (1 to 10): 10, 20,30, 40, 50, 60, 70, 80, 90 and 100 mV s^{-1}*

Chronoamperometric investigations

Chronoamperometry was used to determine the diffusion coefficient (*D*) of epinine. The measurements were performed at a constant potential of 420 mV at various epinine concentrations in PBS (Figure 4).

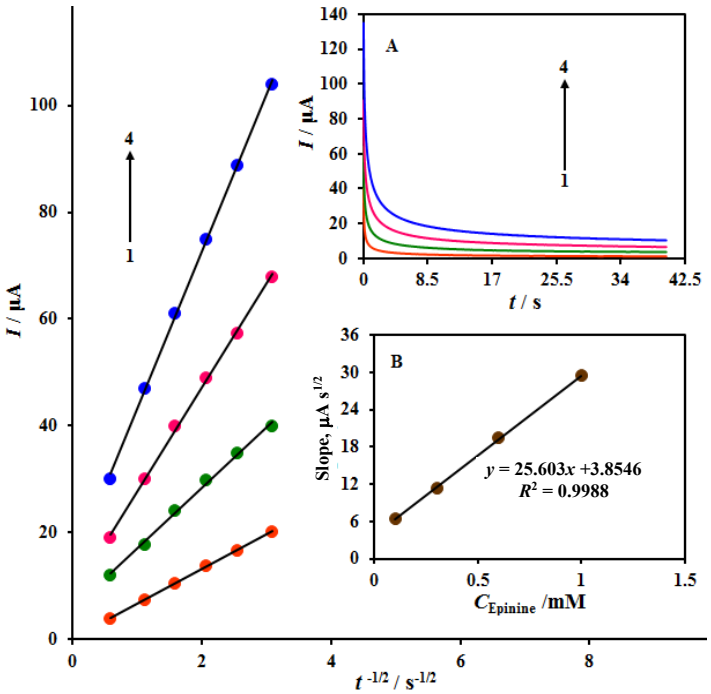


Figure 4. *The chronoamperomtric curves obtained on NMs/IL/CPE in PBS for different concentrations of epinine. The chronoamperograms 1 to 4 represent 0.1, 0.3, 0.1 and 1.0 mM of epinine. Inset* A: I vs. t *graphs composed of chronoamperograms. Inset B: the slope of the lines versus the epinine concentration plot*

The Cottrell equation (1) defines the current response of an electrochemical reaction for an electroactive species. Figure 4 shows *I vs.* $t^{-1/2}$ plots using the Cottrell equation under diffusion control. Figure 4B shows the relationship between the concentration of epinine and the slopes of the straight lines that were formed. The average value of *D* for epinine was found to be 6.8×10^{-6} cm² s^{-1} based on these experiments.

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

Calibration curve

The differential pulse voltammetry (DPV) produced the calibration curve of NMs/IL/CPE in PBS with varying epinine concentrations (Figure 5). The DPVs of epinine at various doses (0.1 to 300.0 µM) were recorded under ideal circumstances. It was discovered that when epinine concentrations increased, the I_{pa} of epinine progressively increased. The anodic current response of epinine increases linearly with the concentration in the range of 0.1 to 300.0 µM (I_{pa} = 0.1425$C_{Epinine}$ + 2.1411 (R^2 = 0.9995)). A limit of detection (LOD) of 0.05 µM was achieved.

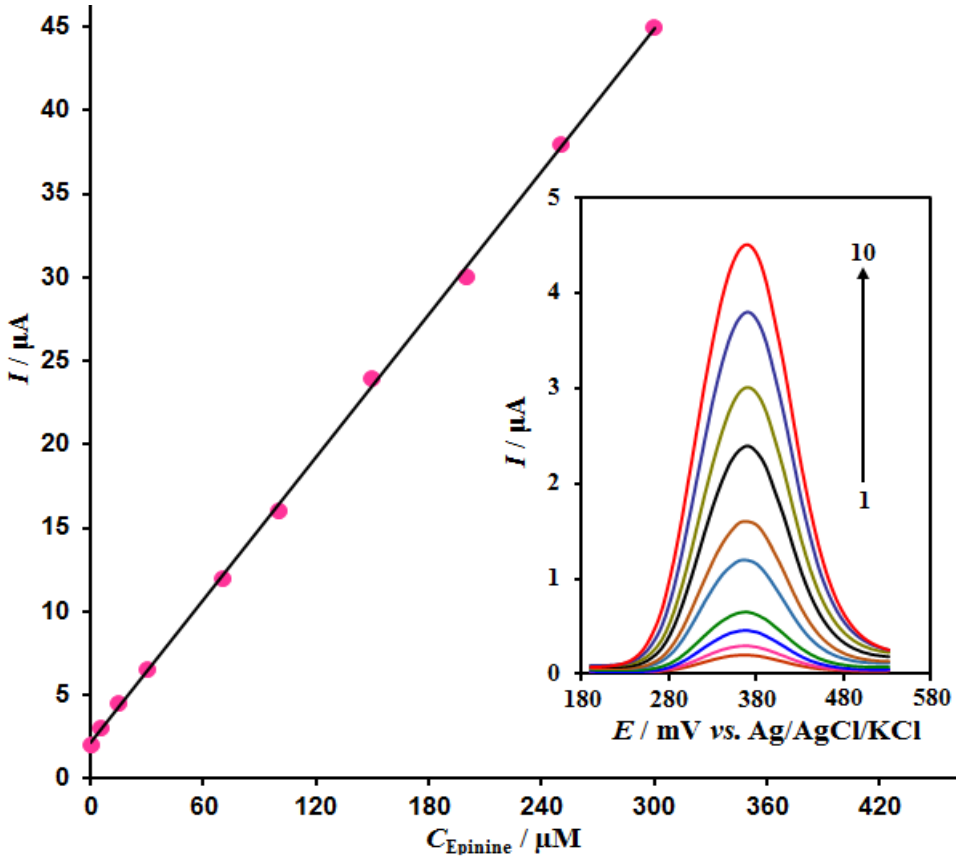


Figure 5. *DPV responses and calibration graph for epinine at NMs/IL/CPE in PBS containing diverse concentrations of epinine. Inset: voltammograms 1 to 10 correspond to 0.1, 5.0, 15, 30.0, 70.0, 100.0, 150.0, 200.0, 250.0 and 300.0 µM of epinine*

Simultaneous voltammetric determination epinine and dobutamine at NMs/IL/CPE

Because of its high sensitivity, the DPV method was used to analyse dobutamine and epinine simultaneously. The DPV responses of NMs/IL/CPE to the oxidation of dobutamine and epinine in PBS while concurrently varying the dobutamine and epinine concentrations were displayed in Figure 6. At 370 mV and 570 mV, respectively, the voltammetric responses showed clear oxidation peaks corresponding to epinine and dobutamine oxidation. This suggests that the NMs/IL/CPE may be used to analyse two substances simultaneously. As epinine and dobutamine concentrations

increased, the anodic peak currents showed a linear pattern, with epinine concentrations between 0.1 and 300.0 µM and dobutamine concentrations between 1.0 and 600.0 µM. Notably, the created sensor's sensitivity to epinine in the presence and absence of dobutamine was almost the same, demonstrating the independence of the epinine oxidation processes at the NMs/IL/CPE. This finding suggests that it is possible to analyse the two substances simultaneously and without interference.

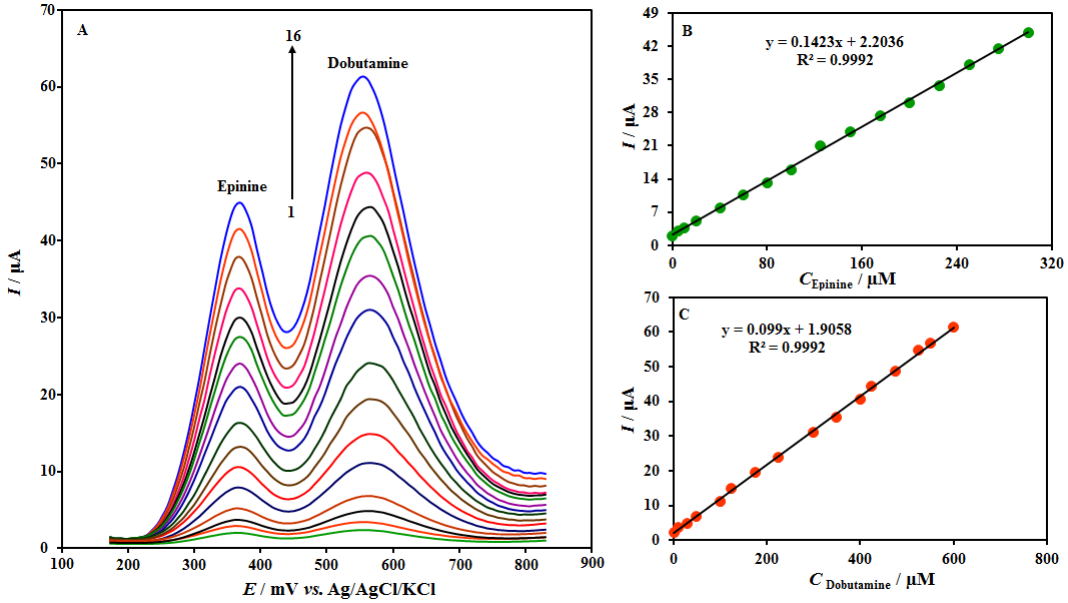


Figure 6. *A -DPV responses of NMs/IL/CPE (voltammograms 1 to 16 correspond to 0.1+ 1.0, 5.0 + 10.0, 10.0 + 30.0, 20.0 + 50.0, 40.0 + 100.0, 60.0 + 125.0, 80.0 + 175.0, 100.0 + 225.0, 125.0 + 300.0, 150.0 + 350.0, 175.0 + 400.0, 200.0 + 425.0, 225.0 + 475.0, 250.0 + 525.0, 275.0 + 550.0 and 300.0 + 600.0 µM epinine and dobutamine, respectively) and calibration graphs for B- epinine and C -dobutamine in PBS containing diverse concentrations of epinine and dobutamine*

Repeatability and selectivity studies

The test to determine the reproducibility of the method was done by measuring the current-voltage responses at NMs/IL/CPE in PBS against 30.0 µM epinine 15 times. Excellent repeatability was demonstrated by the modified CPE's ability to withstand 97.0 % of its initial response in the same particular sets of measurements.

Potential interfering compounds were introduced to 60.0 µM epinine in order to assess the selectivity of the NMs/IL/CPE. The experimental results demonstrated that some organic species, such as vitamin B_9, vitamin B_6, tryptophan, alanine, citric acid, and phenylalanine, as well as commonly occurring inorganic ions, such as K^+, Cl^-, Ca^{2+}, Na+, Mg^{2+} and NO_2^-, did not interact. However, the detection of epinine was interfered with by methyldopa, dopamine, levodopa, carbidopa, isoproterenol, and epinephrine at comparable amounts.

Real sample analysis

To evaluate the NMs/IL/CPE sensor's applicability and efficacy in identifying dobutamine and epinine in urine and dobutamine injection samples, the prepared specimens were analysed using the standard addition method. The results of the study of the actual specimens are shown in Table 1. The suggested electrode's great accuracy and precision were demonstrated by the injection samples' recovery values, which fell between 96.0 and 103.5 %, and their relative standard deviation (RSD), which was less than 3.6 %. As a result, the NMs/IL/CPE is a useful instrument for pharmaceutical specimen examination.

Table 1. *Determination of epinine and dobutamine in real samples using the NMs/IL/CPE. (n=5).*

Sample	Amount, µM				Recovery,%		RSD, %	
	Spiked		Found					
	Epinine	Dobutamine	Epinine	Dobutamine	Epinine	Dobutamine	Epinine	Dobutamine
Dobutamine	0	0	-	2.9	-	-	-	3.0
	5.0	3.0	4.9	6.0	98.0	101.7	3.4	2.0
	7.5	5.0	7.7	7.6	102.8	96.2	2.7	2.4
Urine	0	0	-	-	-	-	-	-
	5.5	6.5	5.6	6.4	101.8	98.5	2.9	1.8
	7.5	8.5	7.2	8.8	96.0	103.5	2.1	3.6

Conclusion

This paper demonstrates how $NiMoO_4$ nanosheets may be created using a straightforward process. Epinine was determined voltammetrically using NMs/IL/CPE. $NiMoO_4$ nanosheets and ILs work together, giving the NMs/IL/CPE exceptional epinine detection capabilities. With a low limit of detection of 0.05 µM, the developed sensor displayed linear dynamic ranges in the range of 0.1 to 300.0 µM of epinine concentration. Additionally, with significant potential differences, the NMs/IL/CPE sensor showed exceptional electrocatalytic activity for the simultaneous determination of dobutamine and epinine. Therefore, due to their considerable peak separations, epinine and dobutamine may be analysed simultaneously using DPV. To sum up, the suggested sensor is a reliable instrument for precisely identifying dobutamine and epinine in actual specimens, producing positive outcomes.

Conflict of interest: The authors have no conflict of interest.

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