J. Electrochem. Sci. Eng. **12(1)** (2022) 199-208; http://dx.doi.org/10.5599/jese.1231

Open Access : : ISSN 1847-9286

https://pub.iapchem.org/ojs/index.php/JESE

Original scientific paper

A sensitive Cu(salophen) modified screen-printed electrode for simultaneous determination of dopamine and uric acid

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Received: January 2, 2022; Accepted: February 13, 2022; Published: February 21, 2022

Abstract

This research applied a nanostructured electrochemical sensor with a screen-printed electrode (SPE) for examining the dopamine (DA) electrocatalytic oxidation when uric acid (UA) was present. Cu(salophen) nanostructured modified SPE (Cu(salophen)/SPE) was employed to investigate the electrochemical behavior of DA. At optimal pH (pH 7.0), oxidation of DA at the modified electrode takes place at a potential around 100 mV less positive than at the unmodified SPE. Chronoamperometry was used to determine the diffusion coefficient of DA ($D = 1.96\times10^{-5}$ cm^2 s^{-1}). Differential pulse voltammetry (DPV) showed linear response in the range between 0.2-450.0 µM for DA. The limit of detection (LOD) of DA was computed to be 0.05 µM. Moreover, Cu(salophen)/SPE was employed for determining DA in the presence of UA using DPV. The DPV results showed that at the modified electrode, two well-separated oxidation peaks of DA and UA could be obtained at potentials of 180 and 325 mV, respectively. This separation forms the basis for the co-detection of these two materials on the surface of Cu(salophen)/SPE. This sensor was then employed to determine DA and UA in real specimens.

Keywords

Dopamine; uric acid; screen printed electrode; voltammetry, chemically modified electrodes, real sample analysis

Introduction

Considerable benefits for designing and developing electrochemical sensors are provided by chemically modifying inert substrate electrodes via mediators [1-8]. Redox-active sites facilitate the electron transfer rate decreasing the activation overpotential [9-21]. Another benefit of the chemically modified electrodes is that they have less susceptibility to surface fouling and oxide formation in comparison with inert substrate electrodes [22-33].

Recently, authors have increasingly focused on nanostructured materials, such as nanomaterials, nanoparticles, nanowires, and nanotubes, because of their specific physicochemical features, which may offer a prominent and functional ground for electroanalysis, especially when designing modified electrodes for electrochemical sensing [34-42].

Dopamine (DA) is a catecholamine neurotransmitter in the brain of mammals' central nervous system. DA is a chemical secreted through neural cells for sending signals to other nerve cells. Hence, it functions as a chemical messenger, which contributes significantly to the performance of hormonal, renal, and cardiovascular systems. There is a relationship between unusual levels of DA and neurological abnormalities, including Parkinson's disease and schizophrenia [43].

A key catabolic product of purine nucleoside, guanosine, and adenosine is uric acid (UA, 2,6,8-trihydroxypurin). Direct conversion of purines obtained from the dietary nucleic acids catabolism to UA is also completed. A number of illnesses are caused by abnormal amounts of UA in body fluids. Hence, analytical determination of UA in biological samples is of key importance in preventing in treating these illnesses. Among vast analytical procedures for the determination of UA, electrochemical techniques stand out due to their simplicity and versatility [44].

It is of high importance to simultaneously detect DA and uric acid, because above compounds are essential biomedical compositions with a prominent contribution to the human metabolism. As we know, there are only a few studies conducted on the simultaneous detection of DA and UA via modified SPEs.

Transition metal *(e.g.* copper, cobalt and iron) Schiff base complexes are well recognized for their excellent electrocatalytic properties toward the detection of many important analytes. The electrochemical application of Schiff base complexes of various metals is reviewed for a wide range of applications such as electrocatalyst for novel sensors development and ion-carriers in ion-selective electrodes. The selectivity pattern in the electrochemical responses of such chemically modified electrodes can be influenced by the structural characterization of the modifier species [45]. Copper comples nanostructures are known to show attractive properties in electrode modification by improving the analytical sensitivity and selectivity during last few years [46].

The aim of this research was to investigate the suitability of a Cu(salophen) / SPE as a novel electrode in DA and UA oxidation. Afterward, the analytical response of the modified electrode was evaluated to quantify DA in the presence of UA. Eventually, the novel electrochemical sensor was utilized for detecting DA and UA in real specimens.

Experimental

Chemicals and apparatus

A potentiostat/galvanostat Autolab PGSTAT 302N instrument equipped with a general-purpose electrochemical system (GPES) software has been utilized to measure electrochemical parameters. The screen-printed electrode was purchased from DropSens (DRP-110, Spain). A digital pH-meter (Metrohm 710) was employed for measuring the pH values. DA, UA and each remaining reagent were

of analytical grade. Furthermore, they were purchased from Merck. Finally, the phosphate buffer solution (PBS) were prepared from the orthophosphoric acid and the respective salts in a pH 2.0 - 9.0.

Synthesis of Cu(salophen)

The synthesis procedure of the salophen ligand is the same as described in the literature [47]. Cu(salophen) has been procured via an easy low-temperature synthesis path at atmospheric pressures by reacting salophen ligands and copper chloride under reflux. In general, mixing and sonication of 1 mmol $CuCl_2$ $6H_2O$, 1mmol salophen ligand, and 20 mL methanol were done (two hours, 60 °C). In addition, purification of the resulting green solid has been done by a 2-phase procedure through double solvent extraction with methanol and water. Eventually, drying the solid was done in a vacuum desiccator at a temperature of 80 °C for two hours before another analysis or application.

Electrode preparation

Cu(salophen) was utilized for coating a bare SPE. A stock solution of complex in 1 mL aqueous solution was prepared by adding of 1 mg complex. Then 2 µl of it was casted onto the carbon working electrode. Afterward, the electrode was put aside till the solvent evaporation was accomplished at room temperature.

Preparation of real samples

DA ampules (labeled 200 mg per 5 ml, Caspian Tamin Co.; Iran) were used. The ampule solution was diluted to 10 ml of PBS. Different volumes of the diluted solution have been poured into a 25 mL volumetric flask. The analysis of DA was performed by the standard addition method.

Urine was used for the analytical determination of DA in biological samples. Urine samples were stored in a refrigerator upon the collection. Then, centrifugation of 10 mL of the specimens was done for 15 min at 2,000 rpm. Afterward, a 0.45 µm filter was used for purifying the supernatant. For the next step, a various contents of the solution were transferred into a 25 ml cell. Then, its dilution was completed to the mark with PBS (pH of 7.0). The diluted urine specimens were spiked with various levels of DA and UA. Finally, the recommended process has been used to analyze the amounts of DA and UA employing the standard addition technique.

Electrochemical measurements

All the experiments were performed at room temperature. The oxidation signals of dopamine were obtained using DPV, with a scan rate of 50 mVs^{-1} between -0.05 and +0.4 V in 0.1 M PBS. The data were baseline corrected with GPES software. Repetitive measurements were carried out by repeating the above assay formats.

Results and discussion

Cu(salophen) structure and morphology

Figure 1 shows FT-IR spectrum of the synthesized Cu(salophen). On spectrum the bands of O-H, C=N, C=C, C-O, and C-H vibrations emerge at 3358-3086, 1668-1609, 1503, 1293-1053, and 846, 762, 629 cm^{-1}, respectively. Moreover, Cu(salophen) spectra exhibit new absorption bands in the region of 495 and 440 cm^{-1} that can be attributed to Cu-Cl and Cu-N stretching modes [47].

Scanning electron microscopy (SEM) has been used to examine the product morphology. Figure 2 depicts SEM images of Cu(salophen). According to the image, Cu(salophen) shows plate-like non-agglomerated morphology with plane sizes less than 45 nm.

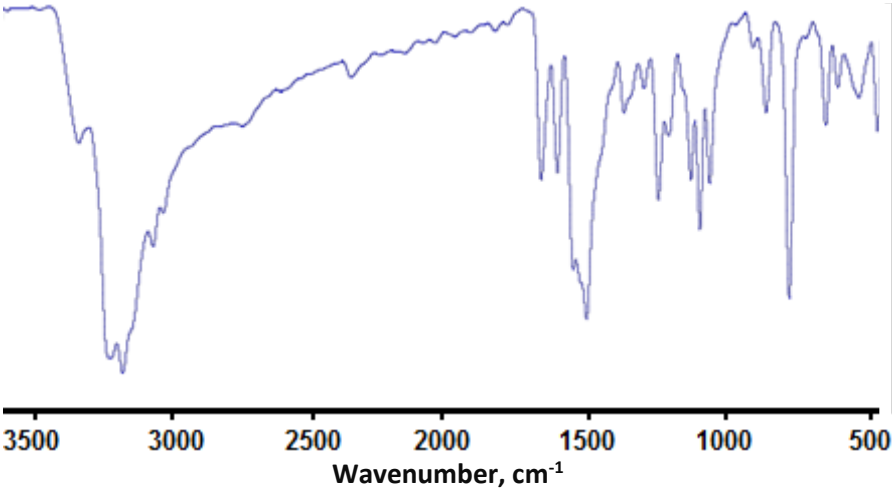

Figure 1. *FT-IR spectra of Cu(salophen)*

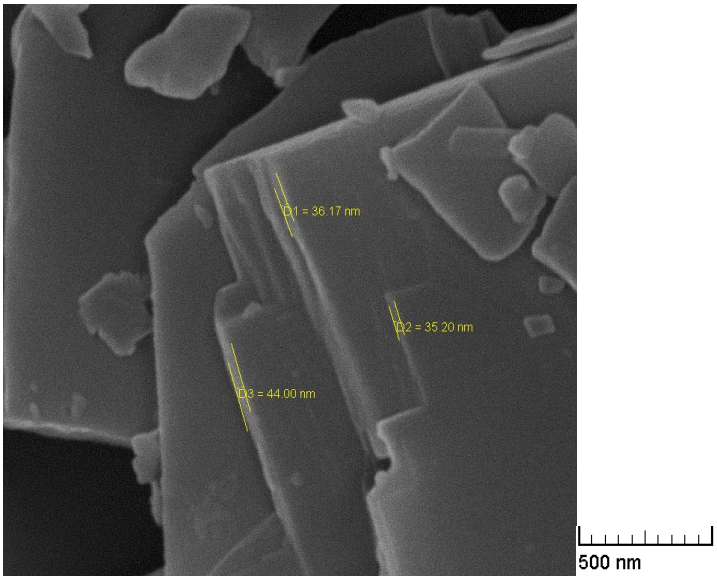

Figure 2. *SEM micrographs of the Cu(salophen)*

For additional confirmation of the compositions of the resulting products, EDX analysis was used (Figure 3A).

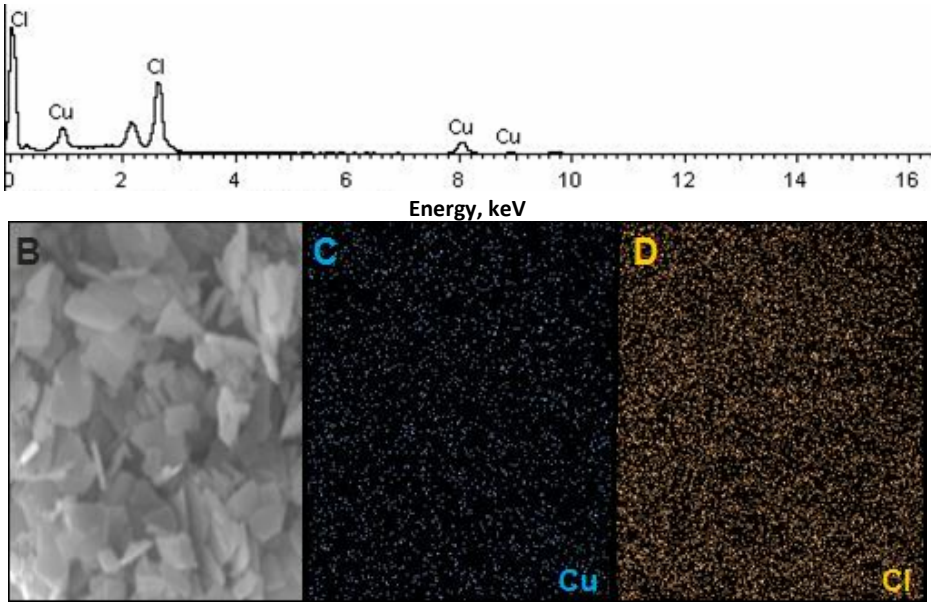

Figure 3. A *- EDX spectra of Cu(salophen);* ***B, C and D*** *- Map images of nanocrystal complex*

Figure 3A shows the predominance of Cl and Cu atoms in the analysed sample. The map images of the complex are shown in Fig. 3B.

Electrochemical behaviour of DA at the surface of different electrodes

Figure 4 gives cyclic voltammetry (CV) response for electrochemical oxidation of 100.0 µM DA at the unmodified SPE (curve a) and Cu(salophen)/SPE (curve b). The effect of the surface is clearly visible on the obtained cyclic voltammograms. It is obvious that modified electrode facilitates the electron transfer and that the obtained cyclic voltammograms approach reversible behaviour. The potential of the anodic peak is approximately 300 mV at the bare SPE surface (curve b) and 200 mV on the surface of Cu(salophen)/SPE (curve a).

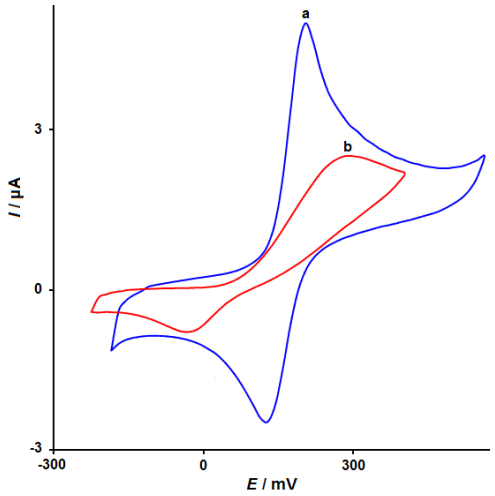

Figure 4. *CVs of Cu(salophen)/ SPE (a) unmodified SPE (b) in 0.1 M PBS (pH 7.0) containing 100.0 µM DA. In all cases the scan rate were 50 mV s^{-1}*

It is widely known that the electrochemical oxidation of DA is sensitive to the pH of the aqueous solution. Thus, optimizing the solution pH is crucial for the electroanalysis of DA. Hence, CV was used to explore the dopamine electrochemical behaviour in 0.1 M PBS at various pH-values (2.0 < pH <9.0) at Cu(salophen)/SPE surface. The results showed that the neutral conditions favour the DA electrochemical oxidation at the Cu(salophen)/SPE surface compared to the basic or acidic media. Therefore, pH 7.0 has been selected as an optimized pH for electrochemical DA oxidation at Cu(salophen)/SPE surface.

Impact of scan rate

We examined the impact of the potential scan rate on the cyclic voltammograms of DA (Fig. 5).

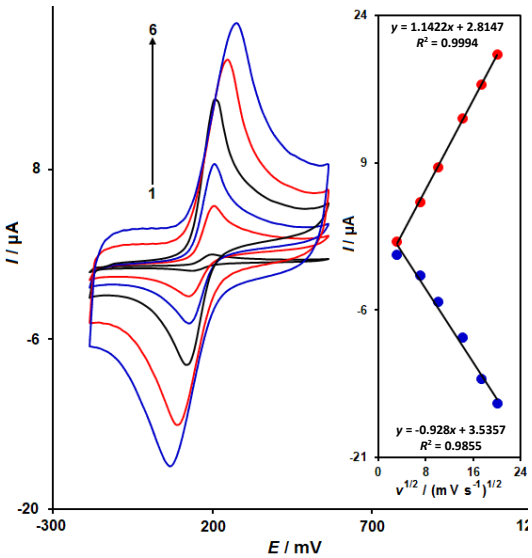

Figure 5. *CVs of Cu(salophen)/SPE in 0.1 M PBS (pH 7.0) containing 100.0 µM DA at various scan rates (10, 50, 100, 200, 300 and 400 mV s^{-1}). Inset: Variation of anodic and cathodic peak current vs. $v^{1/2}$*

With the increase of scan rate, the height of the current peaks also increases. As demonstrated in the inset of Figure 5 the anodic and cathodic peak currents linearly depend on the square root of the potential scan rate ($v^{1/2}$), indicating diffusion control of the reaction [48].

Chronoamperometric analysis

The scan rate dependence allowed us to calculate the diffusion coefficient by a chronoamperometric technique. To determine it, a potential step from 0 V to the potential of 0.25 V was applied (Figure 6). From the slope of the straight lines of I versus $t^{-1/2}$ and using Cotrell equation the diffusion coefficient value for DA was estimated at 1.96×10^{-5} cm^2 s^{-1} [48].

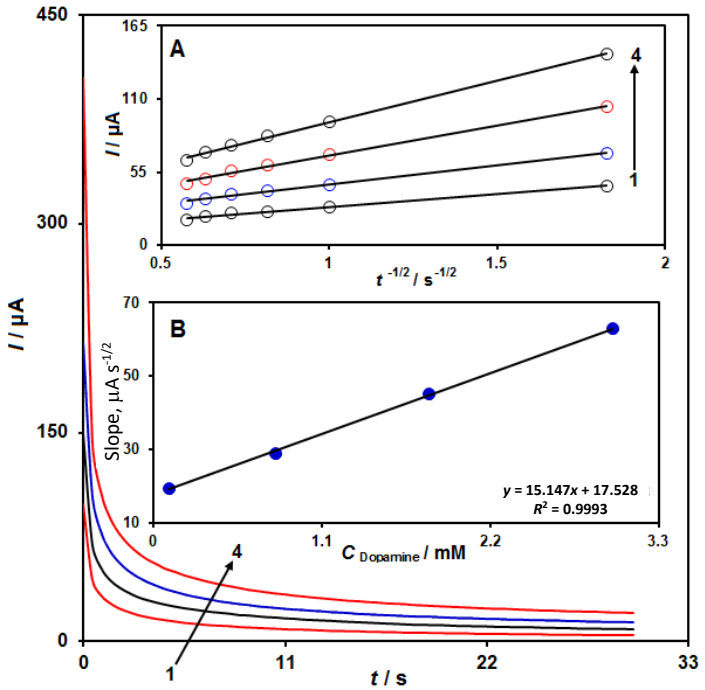

Figure 6. *Chronoamperograms obtained at Cu(salophen)/SPE in 0.1 M PBS (pH 7.0) for different concentrations of DA (0.1, 0.8, 1.8 and 3.0 mM). Insets: (A) Plots of* I vs. t$^{-1/2}$ *obtained from chronoamperograms 1-4. (B) Plot of the slope of the straight lines against DA concentration*

Calibration curve

It should be noted that the peak current of DA using Cu(salophen)/SPE has been utilized for quantitative analysis of DA. Since DPV has benefits in terms of the greater sensitivity and better application features, the modified electrode was employed as working electrode in analysing of DA. Regarding the DPV of DA using the Cu(salophen)/SPE, linear response was obtained in a concentration range from 0.2 to 450.0 µM and 0.9995 correlation coefficient. In addition, the related LOD has been 0.05 µM.

Simultaneous analysis of DA and UA

The analysis was performed to assess the applicability of Cu(salophen)/SPE for simultaneous detection of DA and UA in 0.1 M PBS (pH 7.0). The DPV oxidation peak currents were enhanced linearly with coelevation of DA and UA concentrations, almost with no change in their oxidation peak potentials (Figure 7). Moreover, two distinct separated oxidation signals were found for DA (180 mV) and UA **(**325 mV) based on the difference in their DPVs, which were enough for their simultaneous detection on the modified electrode surface.

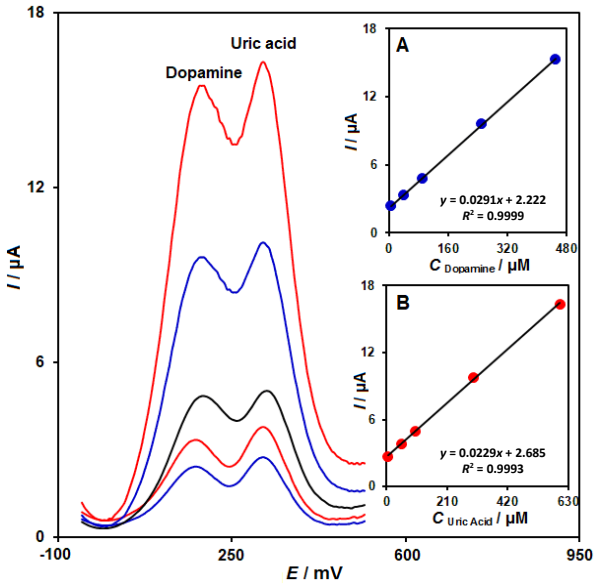

Fig. 7. *DPVs of Cu(salophen)/SPE in 0.1 M PBS (pH 7.0) containing different concentrations of DA and UA from inner to outer: 5.0 + 5.0, 40.0 + 50.0, 90.0 + 100.0, 250.0 + 300.0 and 450.0 + 600.0 µM respectively. Insets (A) plot of* I_p *vs. DA concentration and (B) plot of* I_p *vs. UA concentrations.*

Analyzing real sample

For estimating analytical usability of the recommended technique, it has been utilized for detecting DA and UA in the real samples. Table 1 reports the outputs. Findings showed good recovery and mean relative standard deviation (RSD) of the experimental outputs for DA and UA.

Table 1. *Determination of DA and UA in DA ampoule and urine samples (n=5).*

Sample	*C* / µM				Recovery, %		RSD, %	
	Spiked		Found					
	DA	UA	DA	UA	DA	UA	DA	UA
DA ampoule	0.0	0.0	7.5	-	-	-	2.4	-
	5.5	10.0	12.8	10.2	98.5	102.0	1.8	2.4
	10.5	15.0	18.2	15.1	101.1	100.6	2.6	3.1
	15.5	20.0	22.8	19.7	99.1	98.5	3.1	2.9
	20.5	25.0	27.6	25.1	98.6	100.4	3.2	1.9
Urine	0.0	0.0	-	-	-	-	-	-
	10.0	15.0	10.1	14.9	101.0	99.3	1.8	2.9
	20.0	25.0	19.7	25.7	98.5	102.8	3.1	3.1
	30.0	35.0	29.8	34.8	99.3	99.4	3.3	1.7
	40.0	45.0	40.2	45.2	100.5	100.4	2.6	2.6

Conclusion

A SPE modified with Cu(salophen) has been built in this research to investigate electrochemical behavior of DA. Electrochemical behaviors of DA at Cu(salophen)/SPE surface revealed that catalyzing DA electrooxidation has been done at pH 7.0. The modified electrode substantially removes the coincided voltammetric peaks of DA and UA so that it shows higher selectiveness in DPV measurements of DA and UA into their mixed solution. Ultimately, the modified electrode has been investigated to detect DA and UA in real specimens. The constructed sensor had some advantages such as wide dynamic range, low detection limit, easy of preparation and good selectivity for resolving the oxidation peaks of DA and UA.

Acknowledgment: *The authors acknowledge the financial support provided for this project by the Bam University of Medical Sciences, Bam, Iran and Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran.*

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