



# Electrocatalytic response of poly(cobalt tetraaminophthalocyanine)/ multi-walled carbon nanotubes-Nafion modified electrode toward sulfadiazine in urine\*

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**Abstract:** A highly sensitive amperometric sulfadiazine sensor fabricated by electrochemical deposition of poly(cobalt tetraaminophthalocyanine) (poly(Co<sup>II</sup>TAPc)) on the surface of a multi-walled carbon nanotubes-Nafion (MWCNTs-Nafion) modified electrode is described. This electrode showed a very attractive performance by combining the advantages of Co<sup>II</sup>TAPc, MWCNTs, and Nafion. Compared with the bare glassy carbon electrode (GCE) and the MWCNTs-Nafion modified electrode, the electrocatalytic activity of poly(Co<sup>II</sup>TAPc)-coated MWCNTs-Nafion GCE generated greatly improved electrochemical detections toward sulfadiazine including low oxidation potential, high current responses, and good anti-fouling performance. The oxidation peak currents of sulfadiazine obtained on the new modified electrode increased linearly while increasing the concentration of sulfadiazine from 0.5 to 43.5 μmol/L with the detection limit of 0.17 μmol/L.

**Key words:** Sulfadiazine, Electrochemical detection, Cobalt tetraaminophthalocyanine, Multi-walled carbon nanotubes  
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## 1 Introduction

Sulfonamides are broad-spectrum and low-cost synthetic antibiotics used in treatment of diseases such as urinary tract infections, pneumocystis pneumonia, chronic bronchitis, meningococcal meningitis, and toxoplasmosis (Catalano-Pons *et al.*, 2004; Joseph and Kumar, 2010). Over-dosage of sulfonamides can cause allergic reactions, antibiotic resistance, and carcinogenic effects (Hasebe and Osteryoung, 1975). Because of poor water solubility, sul-

fonamides can also lead to urinary tract lithiasis such as renal calculus and vesical calculus (Catalano-Pons *et al.*, 2004). Therefore, quick systematic monitoring of sulfonamides in body fluids is an important analytical task.

Several methods have been developed for measurement of sulfonamide concentration including high performance liquid chromatography coupled with fluorimetric detection (HPLC-Fluo) (Pang *et al.*, 2003; Maudens *et al.*, 2004) or ultra-violet detection (HPLC-UV) (Malintan and Mohd, 2006), gas chromatography (GC) (Chiavarino *et al.*, 1998), mass spectrometry (MS) (Hartig *et al.*, 1999; Göbel *et al.*, 2004; Conley *et al.*, 2008), enzyme-linked immunosorbent assay (ELISA) method (Assil *et al.*, 1992; Diaz-Cruz *et al.*, 2003; Shelver *et al.*, 2008), and time-resolved fluoroimmunoassay (TRFIA) (Zhang *et al.*, 2010). Although these methods have advantages

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of high sensitivity and accuracy, they have drawbacks such as the high cost of the instruments, tedious sample preparation, and the necessity of skilled laboratory personnel. Consequently, it is of great importance to develop a sensitive, selective, cheap, and convenient analytical method for monitoring the level of sulfonamides and their metabolites in body fluids such as blood and urine.

Electrochemical methods have several advantages over other methods such as simplicity, low cost, and facility of use (Tsai and Chiu, 2007). Mediators such as rhodium particles deposited polyvinyl pyridine film (Shaidarova *et al.*, 2004), palladium particles deposited polyaniline film (Shaidarova *et al.*, 2006), cobalt phthalocyanine complex (Wang *et al.*, 1988), and ruthenium oxide-ruthenium cyanide composites film (Shaidarova *et al.*, 2008; 2011) were successfully used as the electrode modifier for enhancing electron-transfer kinetics and lowering the overvoltage. Modified electrodes such as carbon nanotubes (CNTs)-Nafion modified electrodes (Issac and Kumar, 2009), boron-doped electrodes (Preecharapapun *et al.*, 2006; Souza *et al.*, 2008), metalloporphyrin modified electrodes (Joseph and Kumar, 2010), bismuth film electrodes (Campestrini *et al.*, 2010), and amperometric DNA-peroxidase sensors (Evtugyn *et al.*, 2005) were designed for sulfonamide antibiotics determination. The results demonstrated that electrochemical methods have advantages of high sensitivity, good stability, and promising selectivity. Hence, electrochemical determination methods will be a new avenue for sulfonamide antibiotics detection.

Electrochemical detection based on CNTs-modified electrodes is an attractive research area. As electrode materials, CNTs exhibit fast electron transfer kinetics, high surface area, low over-potential, and promising surface anti-fouling capability (Kachoosangi *et al.*, 2009; Zhou *et al.*, 2011). Surface-bound mediators, such as gold nanoparticles (Li and Lin, 2007), metalloporphyrins (Chen *et al.*, 2001), and metallophthalocyanines (Wang *et al.*, 1988), were covalently or non-covalently stabilized onto CNTs fibers to acquire fantastic synergistic effect in electrocatalytic reactions. As versatile electrocatalysts, cobalt phthalocyanine (Co<sup>II</sup>Pc) complexes were shown to decrease the electro-oxidation potential by several hundred millivolts toward a wide variety of oxidizable species (Wang *et al.*, 1988). However, non-

covalently adsorbed Co<sup>II</sup>Pc complexes are not stable at higher pH conditions due to the decomposition of Co<sup>II</sup>Pc (Tse *et al.*, 1995). It was noticed that potential scanning around the cobalt tetraaminophthalocyanine (Co<sup>II</sup>TAPc) oxidation potential in dimethylformamide or dimethylsulfoxide solution could cause electrochemical oxidative polymerization of Co<sup>II</sup>TAPc and deposition of an electroactive poly(Co<sup>II</sup>TAPc) onto the surface of the electrode (Li and Guarr, 1989).

In the present study, we aimed at developing a voltammetric sensor fabricated by using poly(Co<sup>II</sup>TAPc) coated multi-walled carbon nanotubes (MWCNTs) modified glassy carbon electrode (GCE) for trace sulfadiazine determination in urinary samples.

## 2 Materials and methods

### 2.1 Chemicals and apparatus

Sulfadiazine (analytical grade) and Nafion (5% (w/w) in low aliphatic alcohols and water) were purchased from Sigma-Aldrich (Shanghai, China). MWCNTs crude material (diameter 10–30 nm, length 5–15  $\mu\text{m}$ , purity >95%) was obtained from Shenzhen Nanotech Port, China. Other pure analytical quality chemicals such as *N,N'*-dimethylformamide (DMF), sulfuric acid (98% (w/w)), nitric acid (70% (w/w)), and hydrogen peroxide (30% (w/w)) were obtained from standard suppliers and used as received. All solutions were prepared with Milli-Q water from an Academic Millipore system (Millipore Inc., MA, USA).

Electrochemical measurements were performed by using a CHI 660A electrochemical workstation (CH Instruments Inc., Austin, USA). The electrochemical cell was assembled with a conventional three-electrode system: GCE (3.0 mm diameter), MWCNTs-Nafion or poly(Co<sup>II</sup>TAPc)/MWCNTs-Nafion modified GCE as working electrodes, an AgCl/Ag electrode (saturated potassium chloride) as the reference electrode, and a platinum wire as the auxiliary electrode. Indium-tin oxide (ITO) glass was used as the substrate for morphologic and elementary characterization. All experiments were carried out under ambient conditions.

### 2.2 Fabrication of modified electrode

Crude MWCNTs were pretreated according to the method of Liu *et al.* (1998) prior to use. In brief,

100 mg of MWCNTs crude material was refluxed in 2.6 mol/L of nitric acid for 48 h at 80 °C. Then, the purified MWCNTs were rinsed to neutral with double-distilled water. After drying in a vacuum drying oven at 60 °C, the nitric acid purified MWCNTs were dispersed in a mixture of concentrated sulfuric and nitric acids (3:1 in volume) and then sonicated for 6 h. Then, the pretreated MWCNTs were rinsed to neutral with double-distilled water and dried at 60 °C in a vacuum drying oven. A suspension of MWCNTs was prepared by dispersing 2 mg of pretreated MWCNTs into 2 ml of DMF solution containing 0.1% (w/w) of Nafion through ultrasonication and a black suspension was obtained.

Prior to electrode modification, a GCE was polished to a mirror-like surface with 0.05 µm alumina slurry, thoroughly rinsed with double-distilled water, and then sonicated in anhydrous ethanol and double-distilled water (each for 3 min). Then, 2 µl of above-mentioned black suspension was cast onto the surface of the cleaned GCE and dried in ambient temperature to fabricate MWCNTs-Nafion modified GCE. The poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified GCE was prepared by successive voltammetric scanning in 0.2 mol/L phosphate buffer solution (pH 7.8) containing 110 mg/L of  $\text{Co}^{\text{II}}\text{TAPc}$  with MWCNTs-Nafion modified GCE as the working electrode. MWCNTs-Nafion modified ITO electrode and poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified ITO electrode were prepared for morphological and elementary characterization by using cleaned ITO glass as electrode substrate. The morphologies of electrode surfaces and energy-dispersive X-ray spectra were studied by field-emission scanning electron microscope (FESEM, Hitachi S-4800, Japan) equipped with an energy-dispersive X-ray spectroscopy (EDS, Hitachi, Japan).

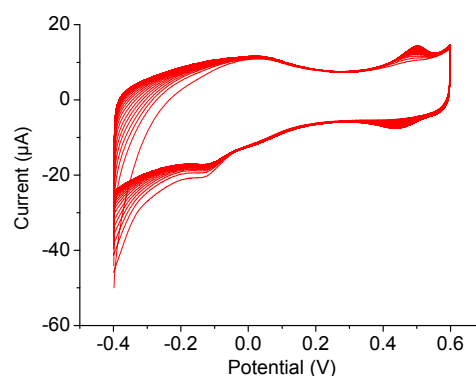
### 2.3 Sample preparation

Drug-free human urine samples used in this study were obtained from a healthy donor and stored in a refrigerator immediately after collection. After centrifugation for 5 min at 1500 r/min, the urine supernatant was diluted five times with sulfuric acid to reach pH 0.7. Then, the solution was applied for analysis without any further pretreatment. The standard addition method was used to calculate the recovery of this method.

## 3 Results and discussion

### 3.1 Electrochemical deposition of $\text{Co}^{\text{II}}\text{TAPc}$

$\text{Co}^{\text{II}}\text{TAPc}$  was electrochemically deposited onto MWCNTs-Nafion modified electrode by cyclic voltammetric scanning in a phosphate buffer solution (pH 7.8) containing 110 mg/L of  $\text{Co}^{\text{II}}\text{TAPc}$ . As depicted in Fig. 1, the currents of redox couple around 0.45 V steadily increased with voltammetric cycling. After scanning for 32 cycles, a stable redox response was obtained. This signifies the electro-deposition of  $\text{Co}^{\text{II}}\text{TAPc}$  onto the surface of MWCNTs-Nafion modified electrode. The redox peaks occurring between 0.4 and 0.6 V are assigned to  $\text{Co}^{\text{II}}\text{TAPc}$  film oxidation and reduction, respectively.

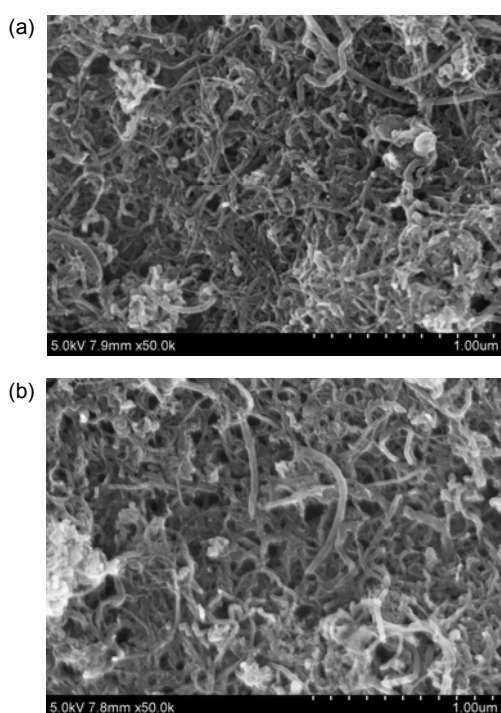


**Fig. 1 Electrochemical deposition of  $\text{Co}^{\text{II}}\text{TAPc}$  onto MWCNTs-Nafion modified electrode**

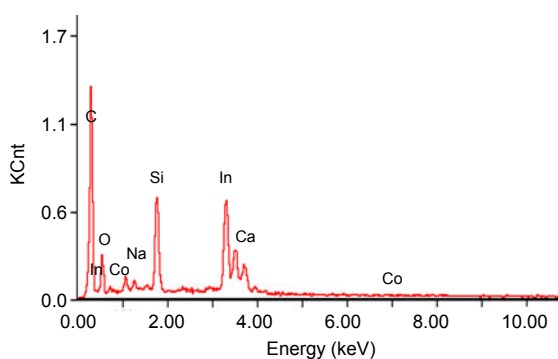
The scanning electron microscopic (SEM) characterization of the electrode modified with different complexes was carried out. Fig. 2 shows the morphologies of MWCNTs-Nafion (Fig. 2a) and poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion (Fig. 2b) electrode surfaces. It is discernible that poly( $\text{Co}^{\text{II}}\text{TAPc}$ ) film deposition took place as the morphology of these images was different. In Fig. 2a, MWCNTs (about 20–30 µm in diameter) can be seen clearly and distribute homogeneously on the surface of the electrode. However, in Fig. 2b, it can be observed that small particles are adsorbed on the tubes and the edges of tubes become indistinct. This phenomenon could be attributed to the aggregation of poly( $\text{Co}^{\text{II}}\text{TAPc}$ ) molecules onto the surface of MWCNTs-Nafion.

The EDS surface analysis was also performed to provide information on materials present on the electrodes' surfaces. In Fig. 3, the dominating carbon (C)

peaks were expected, as the CNTs immobilized on the electrode surface. Strong peaks due to silicon (Si), indium (In), sodium (Na), and calcium (Ca) are observed and these attribute to the ITO substrate. Weak peaks (Co) at about 0.8 and 6.9 keV are also observed in the EDS spectrum. The presence of the Co (L and K) orbital peaks from the modified electrode shows the presence and the successful deposition of poly( $\text{Co}^{\text{II}}\text{TAPc}$ ) onto MWCNTs-Nafion surface to form poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified electrode.



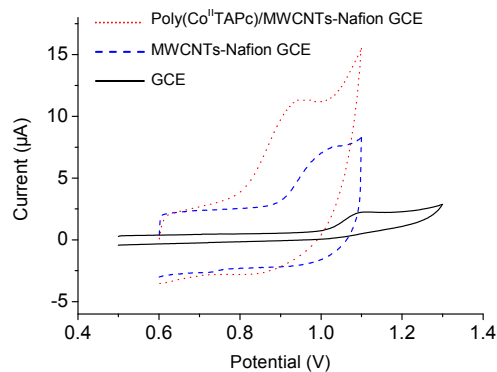
**Fig. 2** SEM images of MWCNTs-Nafion film (a) and poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion film (b) on ITO substrates



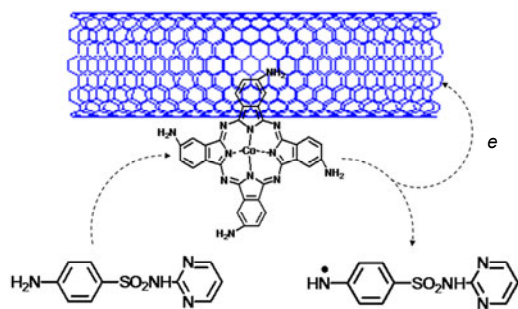
**Fig. 3** EDS spectrum for a poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified electrode  
KCnt: 1000 counts

### 3.2 Electrocatalytic oxidation of sulfadiazine at poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified electrode

In order to test the potential electrocatalytic oxidation of the modified electrodes, cyclic voltammograms of poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified GCE, MWCNTs-Nafion modified GCE, and GCE were obtained in 0.1 mol/L sulfuric acid solution with the presence of 0.09 mmol/L of sulfadiazine. As shown in Fig. 4, a weak anodic peak at about 1.1 V is observed (solid line) which is a typical electrochemical response of sulfadiazine corresponding to the oxidation of a phenylamino group (Msagati and Ngila, 2002). As expected, when MWCNTs-Nafion modified GCE was used as the working electrode, the anodic peak of sulfadiazine was greatly enhanced (dashed line) with the anodic potential shifting negatively to 1.0 V. Surprisingly, when poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified GCE was used as the working electrode, the anodic peak was enhanced much greater (dot line) than that at MWCNTs-Nafion modified GCE. The anodic potential shifted to 0.9 V (dotted line), which was lower than that obtained on GCE or MWCNTs-Nafion modified GCE. The enhanced current responses and low anodic potential observed on poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified GCE probably attribute to the synergistic effect of MWCNTs and poly( $\text{Co}^{\text{II}}\text{TAPc}$ ) coated on GCE, as depicted in Fig. 5. The enhanced sensibility of poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified GCE suggests the possibility of sensitive determination of sulfadiazine at trace levels.



**Fig. 4** Cyclic voltammograms of 0.09 mmol/L of sulfadiazine in sulfuric acid solution (0.1 mol/L) at GCE, MWCNTs-Nafion modified GCE, and poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified GCE



**Fig. 5 Mechanism of sulfadiazine oxidation on poly(Co<sup>II</sup>TAPc)/MWCNTs-Nafion surface**

### 3.3 Effect of pH

The pH value of the support electrolyte solution is regarded as a most important impact factor in sensor performance and its detection of the analyte. The influence of pH on electrocatalytic oxidation of sulfadiazine was studied in the presence of 0.09 mmol/L sulfadiazine. Several kinds of solutions, such as 0.1 mol/L sulfuric acid, 0.1 mol/L hydrochloric acid, 0.2 mol/L sodium acetate buffer, and 0.2 mol/L phosphate buffer, at pH values ranging from 0.7 to 7.0 were used as the supporting electrolytes, respectively. The results showed that the pH value of the buffer medium has an important effect on the electrochemical oxidation of sulfadiazine. The anodic peak currents decreased as pH increased from 0.7 to 5.8. When the pH value of the electrolyte was higher than 7.0, the anodic peak disappeared. Hence, sulfuric acid (0.1 mol/L, pH 0.7) is the optimum electrolyte for the sensor.

### 3.4 Analytical properties

Since amperometry under stirred conditions has a much higher current sensitivity than cyclic voltammetry, it was chosen for sensitive determination of

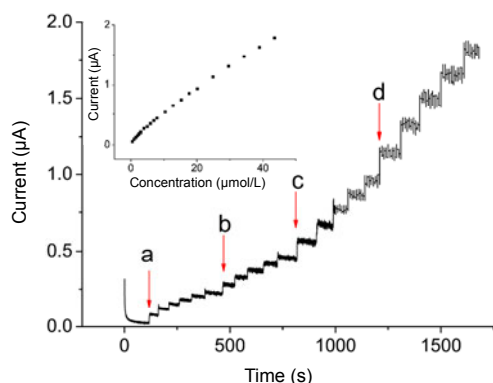
trace sulfadiazine. Under the optimal detection conditions (electrolyte of 0.1 mol/L sulfuric acid, pH 0.7; amperometry with applied potential of 0.9 V vs. AgCl/Ag (saturated KCl)), the response current change of sensor was plotted against sulfadiazine concentration by amperometric detection (Fig. 6, inset). When an aliquot of sulfadiazine solution was added into 10.0 ml of electrolytes, the oxidation currents increased steeply to reach a stable value in less than 3 s (Fig. 6). The current response increased linearly with sulfadiazine concentration in the range of 0.5 to 43.5  $\mu\text{mol/L}$ . The corresponding regression equation was  $I=0.09144+0.04042C$ , where  $I$  was the current change ( $\mu\text{A}$ ),  $C$  was the sulfadiazine concentration ( $\mu\text{mol/L}$ ), and the coefficient  $R$  was 0.998. The detection limit of 0.17  $\mu\text{mol/L}$  was calculated according to  $3S_b/m$  (Hasebe and Osteryoung, 1975), where  $m$  was the slope of the linear range of the respective calibration plot, and  $S_b$  was estimated as the standard deviation of the signals obtained in a blank solution. In Table 1, references on determination of sulfadiazine were listed and compared with this work. Although the detection limit of this work is inferior to HPLC methods, it is the lowest in all electrochemical methods listed in Table 1.

The repeatability of five measurements for solutions of 10  $\mu\text{mol/L}$  sulfadiazine was excellent with a relative standard deviation (RSD) of 3.6%. The proposed poly(Co<sup>II</sup>TAPc)/MWCNTs-Nafion modified GCE was stored in air under ambient conditions and its sensitivity was checked daily. The current response of poly(Co<sup>II</sup>TAPc)/MWCNTs-Nafion modified GCE maintained 93% of its initial response after one week, indicating good stability of the new electrode.

**Table 1 Comparison of different methods for determination of sulfadiazine**

Study	Method	Linear range ( $\mu\text{mol/L}$ )	Detection limit ( $\mu\text{mol/L}$ )
Rao <i>et al.</i> , 2000	HPLC (AD)	0.05–50	0.05
Preechaworapun <i>et al.</i> , 2006	HPLC (AD)	0.2–400	0.044
Pingarron Carrazon <i>et al.</i> , 1987	DPV (GCE)	15–60	5.4
Souza <i>et al.</i> , 2008	SWV (BDDE)	8.01–119	2.19
Diaz <i>et al.</i> , 1996	DPP (HgE)	2.0–32	4.9
Msagati and Ngila, 2002	SWV (P3MT/GCE)	20–3200	4.0
Braga <i>et al.</i> , 2010	SWV (GCE)	62.7–340	10.9
Campestrini <i>et al.</i> , 2010	DPV (BiFE)	3.2–20	2.1
This work	Amperometry	0.5–43.5	0.17

HPLC: high performance liquid chromatography; AD: amperometric detector; DPV: differential pulse voltammetry; GCE: glassy carbon electrode; SWV: square wave voltammetry; BDDE: boron doped diamond electrode; DPP: differential pulse polarography; HgE: mercury electrode; P3MT: poly(3-methylthiophene); BiFE: bismuth film electrode



**Fig. 6** Amperometric current-time curve response obtained for successive addition of 5 (a), 10 (b), 25 (c) and 50 (d)  $\mu\text{l}$  of 1.0 mmol/L sulfadiazine in each step

Inset shows the plot of current change versus sulfadiazine concentration

According to the interference in real samples, ascorbic acid (AA) widely exists in biological samples. The oxidation potential of AA is about 0.35 V in 0.1 mol/L sulfuric acid, which means AA will be oxidized simultaneously with sulfadiazine at applied potential of 0.9 V during amperometry monitoring. Hence, determination of sulfadiazine with AA coexisting was carried out by background subtraction. The obtained current is 0.27  $\mu\text{A}$  for a solution containing 10  $\mu\text{mol/L}$  AA and 10  $\mu\text{mol/L}$  sulfadiazine when 0.6 V was applied. The observed current was due to the oxidation of AA. However, when 0.9 V was applied to the same solution, the current was 0.78  $\mu\text{A}$  due to the oxidation of AA and sulfadiazine. Subtracting the current obtained for AA (0.27  $\mu\text{A}$ ) from the total current gave the current of 0.51  $\mu\text{A}$  for sulfadiazine alone. This current was consistent with the current obtained for the individual addition of 10  $\mu\text{mol/L}$  sulfadiazine in Fig. 6. Potential interference from dopamine and uric acid can also be calibrated by background subtraction due to their low anodic potential (Xiao *et al.*, 2007).

### 3.5 Analytical application

The practical application of the modified electrode was demonstrated by measuring the concentration of sulfadiazine in real urine samples. The standard addition method was used for the determination of sulfadiazine in urine samples supplied by a healthy donor. The anodic currents of sulfadiazine in real samples were calculated by subtracting background currents (obtained at 0.6 V) from total currents (ob-

tained at 0.9 V). Based on the anodic currents of amperometric measurements and using the calibration plots which are shown in Fig. 6 (inset), the sulfadiazine concentration in spiked urine samples was obtained. The results are given in Table 2. The proposed method showed a better recovery of spiked sulfadiazine in urine samples suggesting that this method could be used for practical applications.

**Table 2** Determination and recovery results of sulfadiazine in urine samples at a modified electrode

Sample	$c_s$ ( $\mu\text{mol/L}$ )		RSD (%) $n=5$	Recovery (%)
	Added	Found		
1	<LOD			
2	5.0	4.4	5.8	88.0
3	20.0	18.6	3.4	92.8

$c_s$ : sulfadiazine concentration; RSD: relative standard deviation; LOD: limit of detection

## 4 Conclusions

In this work, we have demonstrated a highly sensitive electrochemical determination of sulfadiazine using a new poly( $\text{Co}^{\text{II}}\text{TAPc}$ )/MWCNTs-Nafion modified GCE. The attachment of poly( $\text{Co}^{\text{II}}\text{TAPc}$ ) on MWCNTs was confirmed by SEM and EDS analyses. The synergistic effects of poly( $\text{Co}^{\text{II}}\text{TAPc}$ ) and MWCNTs efficiently catalyzed the electrochemical oxidation of sulfadiazine on electrode surface. The modified electrode showed a fast response, high precision and sensitivity, and could detect sulfadiazine concentrations as low as 0.17  $\mu\text{mol/L}$  in acidic buffer solution. The practical application of the modified electrode was demonstrated by measuring the concentration of sulfadiazine in urine samples.

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