



## Research article

# Voltammetric determination of uric acid using multiwall carbon nanotubes coated-poly(4-amino-3-hydroxy naphthalene sulfonic acid) modified glassy carbon electrode

Molla Tefera<sup>a,\*</sup>, Merid Tessema<sup>b</sup>, Shimelis Admassie<sup>b</sup>, Walegion Wubet<sup>a</sup><sup>a</sup> Department of Chemistry, University of Gondar, P. O. Box 196, Gondar, Ethiopia<sup>b</sup> Department of Chemistry, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

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## ABSTRACT

In this study, an electrochemical sensor based multiwalled carbon nanotubes (MWCNTs)-poly (4-amino-3-hydroxy naphthalene sulfonic acid) modified glassy carbon electrode (MWCNTs/poly (AHNSA)/GCE) was developed for the determination of uric acid (UA). The composite electrode was prepared first by electropolymerization of the monomer (AHNSA) on GCE using cyclic voltammetry within the potential range of  $-0.8$  V to  $+2.0$  V vs Ag/AgCl for 15 cycles followed by drop coating of MWCNTs solution on the surface of poly (AHNSA)/GCE. Under optimal conditions, MWCNTs/poly (AHNSA)/GCE showed a linear current response with UA concentrations in the range of  $1 \times 10^{-6}$  to  $1 \times 10^{-4}$  M with  $R^2 = 0.9972$ . The sensor exhibited low detection limit with a value of  $0.024 \mu\text{M}$ . The sensors have been applied to successfully quantify UA in urine samples.

## 1. Introduction

Uric acid (2,6,8-trihydroxypurine, UA) is the primary product of protein and urine metabolism in the human body and normally present in urine and in serum at various levels [1, 2, 3]. It is a natural antioxidant, which can scavenge free radicals, superoxide, hydroxyl radical, and singlet oxygen. In addition, it binds iron so as to inhibit iron dependent ascorbate oxidation. Owing to these properties, UA prevents the destruction of human tissues and cells [4, 5]. Humans have higher UA levels in serum when compared to other mammals due to the lack of uricase enzyme, which catalyses UA to more soluble end product (allantoin). Recently, improvement of dietary life causes over nutrient of high protein on human being, so that the level uric acid is increasing in blood through physiological reaction. Besides, overtaking of alcoholic beverages, under exercise and stress can also influence the levels of uric acid [6]. Extreme level of UA may lead to several diseases, such as gout, hyperuricemia, pneumonia, obesity, diabetes, cholesterol, kidney disease and heart disease [7]. Monitoring UA level inhuman body fluids can be an indicator for diagnosis of many health problems [8]. Due to high clinical and pharmaceutical significance, it is essential to develop new sensors with improved sensitivity for UA determination.

Various analytical techniques including high performance liquid chromatography [9], high performance liquid chromatography-mass

spectrometry [10], spectrophotometric method [11, 12] and chemiluminescence [13] have been attempted for the determination of uric acid. However, most of these methods need very expensive instruments, require advanced expertise and time-consuming. Over the last few decades, because of the electroactive nature of uric acid electroanalytical methods have gained considerable attention for the determination of uric acid [14].

Previous reports showed that UA has been determined electrochemically using various electrodes. Platinum nanosheets/fullerene (C60)/glassy carbon electrode [15], graphene-nickel hydroxide/glassy carbon electrode [16], SnO<sub>2</sub>/graphene nanocomposite/glassy carbon electrode [17], poly (glyoxal-bis(2-hydroxyanil))/glassy carbon electrode [18], graphene oxide/glassy carbon electrode [19], over-oxidized poly (3, 4-ethylene dioxythiophene) nanofibers/pencil graphite electrode [8], multiwalled carbon nanotubes/glassy carbon electrode [20], sodium dodecyl sulfate surfactant modified graphene paste electrode [21] and polymerized luminol film/glassy carbon electrode [22] electrodes have been utilized for UA determination.

The performance enhancement of chemically modified electrodes over bare electrode is due to improvement in kinetics of electron transfer [23]. Owing to remarkable tensile strength, high surface area, flexibility, mechanical, excellent electrical and physicochemical properties, multi-wall carbon nanotubes have been used to modify electrodes [24, 25, 26].

\* Corresponding author.

E-mail addresses: [mollatef2001@gmail.com](mailto:mollatef2001@gmail.com), [molla.tefera@uog.edu.et](mailto:molla.tefera@uog.edu.et) (M. Tefera).

Polymers have also widely used as sensing materials due strong adherence to the electrode surface, large surface area, electronic and ionic conductivity. Even though the electrocatalytic activity of the polymers or carbon nanotubes individually showed good results, some properties such as mechanical stability, sensitivity for different techniques, and electrocatalysis for some compounds are poor [27, 28].

In recent years, studies have been conducted in the preparation of composite electrodes composed of both CNTs and conjugated polymers. Incorporation of CNTs in conjugated polymers resulted in the formation of new composite materials having the properties of each component with a synergistic effect such as high aspect ratio and high surface area, increasing the rate of electron transfer and high accessibility of the analyte to the surface of the electrode. As a consequence, they can enhance electrocatalytic properties, reduce the required overpotential, increase reaction rate, increase sensitivity and stability, and prevent surface fouling of the electrode. A  $\pi$ - $\pi$  and hydrophobic interactions between MWCNTs and polymers resulted in successful dispersion in forming homogeneity for electrochemical sensors [27, 29, 30].

The poly (4-amino-3-hydroxy naphthalene sulfonic acid is the common conductive polymer which was widely applicable for the electrochemical determination of drugs [31, 32, 33, 34]. In this study, a sensor based on MWCNTs/poly (AHNSA)/GCE was prepared by electrodeposition of poly (AHNSA) on to glassy carbon electrode and then drop-coated with MWCNTs. The sensor was utilized for the electrochemical investigation of uric acid in urine samples.

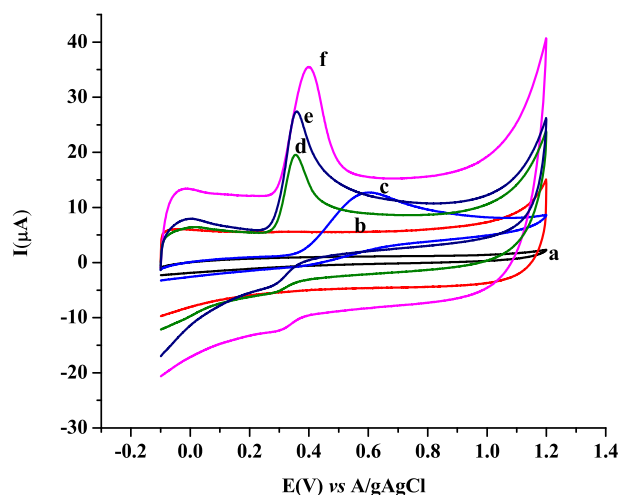
## 2. Experimental

### 2.1. Reagents

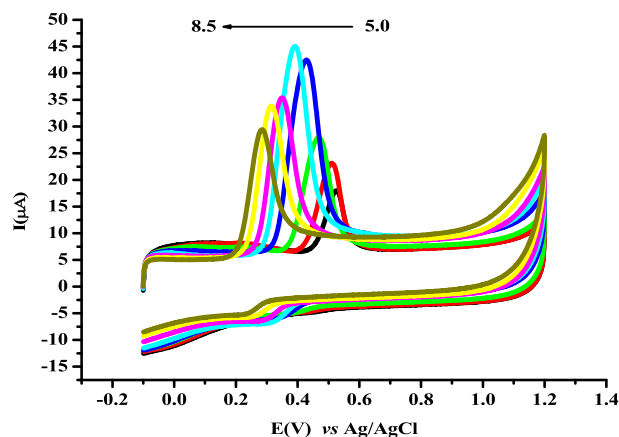
Uric acid (UA) was purchased from Sigma Aldrich (Germany). All other reagents were analytical grade and used without any prior treatment. Phosphate buffer solution (PBS) was prepared by mixing 0.1 M of  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{HPO}_4$  and the pH was adjusted with addition of  $\text{HNO}_3$  and NaOH. All the solutions prepared with double distilled water. The standard solution of UA was dissolved in double distilled water and the working solutions were prepared by diluting the stock solution with PBS.

### 2.2. Apparatus and instrumentation

All measurements were carried out with a CHI760D electrochemical workstation (CH Instruments, USA). A three-electrode system consisting



**Figure 1.** Cyclic voltammograms of unmodified GCE 0.1 M PBS of pH 7 (a) and 1.0 mM UA (c) and MWCNTs/Poly (AHNSA)/GCE in 0.1 M PBS of pH 7 (b), Poly (AHNSA)/GCE (d), MWCNTs/GCE (e) and MWCNTs/Poly (AHNSA)/GCE (f) at scan rate of  $0.1 \text{ V s}^{-1}$ .



**Figure 2.** Cyclic voltammograms for 1.0 mM uric acid in 0.1 M PBS of various pH (pH 5.0–8.5) at MWCNT/poly (AHNSA)/GCE at scan rate of  $0.1 \text{ V s}^{-1}$ .

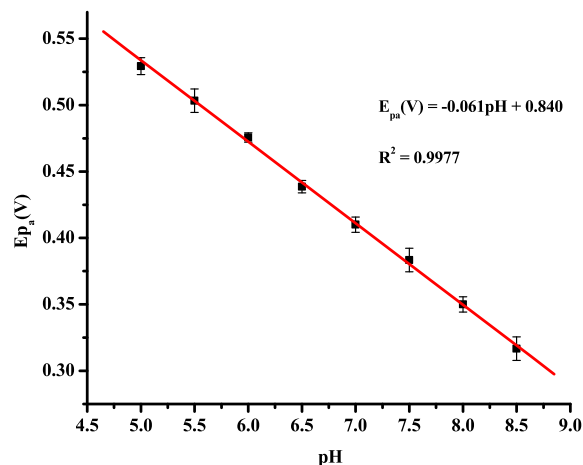
of platinum wire as counter electrode, a silver-silver chloride (3 M KCl) as reference electrode and GCE, poly (AHNSA)/GCE, MWCNTs/GCE and MWCNTs/poly (AHNSA)/GCE as working electrodes were used.

### 2.3. Preparation of modified electrodes

Before modification, GCE (diameter of 3 mm) was polished first with alumina slurry of different sizes (1.0, 0.3 and  $0.05 \mu\text{m}$ ) until a mirror like surface was obtained and then rinsed with doubly distilled water. Finally, it was sonicated with ethanol and doubly distilled water and allowed to dry in air. MWCNTs solution was prepared by mixing 1 mg acidified MWCNTs in 1 mL double distilled water followed by sonication. The MWCNTs/GCE was prepared by drop coated MWCNTs suspension on GCE and allowed it to dry at room temperature. Poly (AHNSA)/GCE was prepared by electropolymerization of 4-amino-3-hydroxynaphthalene sulfonic acid solution by sweeping the potential between  $-0.8 \text{ V}$  and  $+2.0 \text{ V}$  vs Ag/AgCl in 0.1 M  $\text{HNO}_3$  at a scan rate of  $0.1 \text{ V s}^{-1}$  for 15 cycles as described in literature by Amare and Menkir [32]. The MWCNTs/poly (AHNSA)/GCE composite electrode was fabricated by drop coated MWCNTs on poly (AHNSA)/GCE and letting it to dry at room temperature.

### 2.4. Sample preparation

Human urine was obtained from an adult and stored in fridge until analysis. The sample was diluted 100-fold of buffer (pH 7.0) without any further pretreatment. To analyze the accuracy of the sensor, recovery experiments were carried out with addition of various concentrations of



**Figure 3.** Effects of pH on the anodic peak potential for 1.0 mM uric acid in PBS solution.

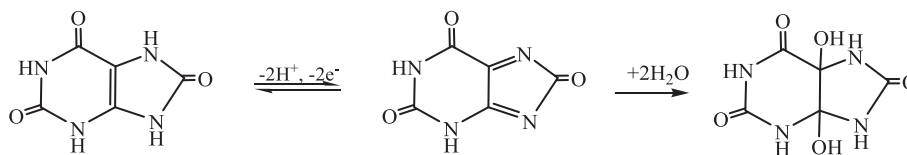


Figure 4. Mechanism of uric acid oxidation.

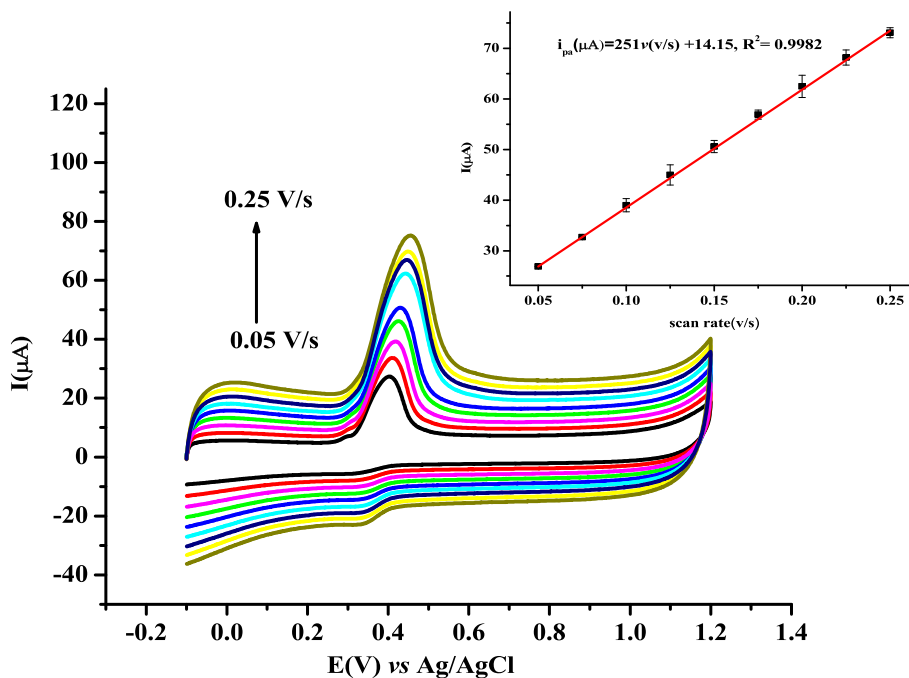


Figure 5. Cyclic voltammograms for 1.0 mM UA in PBS (pH 7.0) at various scan rates (0.05–0.25 V s<sup>-1</sup>). Inset: plot of peak currents vs scan rates.

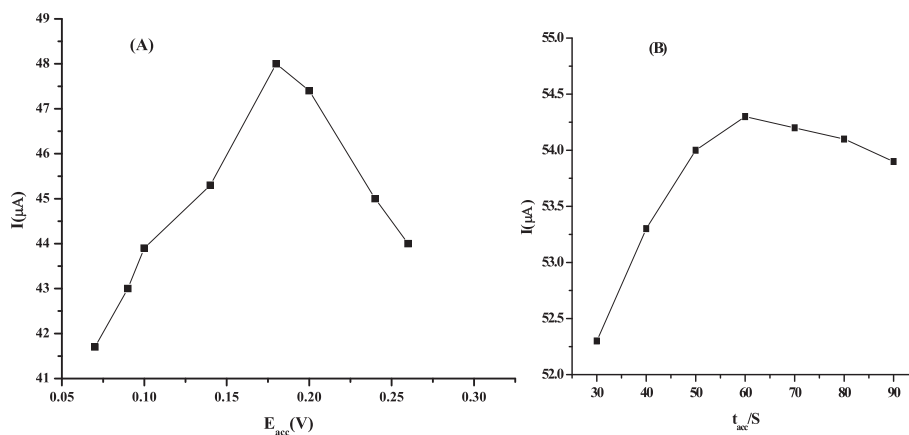


Figure 6. Effect of accumulation potential (A) and accumulation time (B) on the peak current for 1.0 mM UA.

standard solution of UA into urine sample, and then analyzed by square wave voltammetry (SWV).

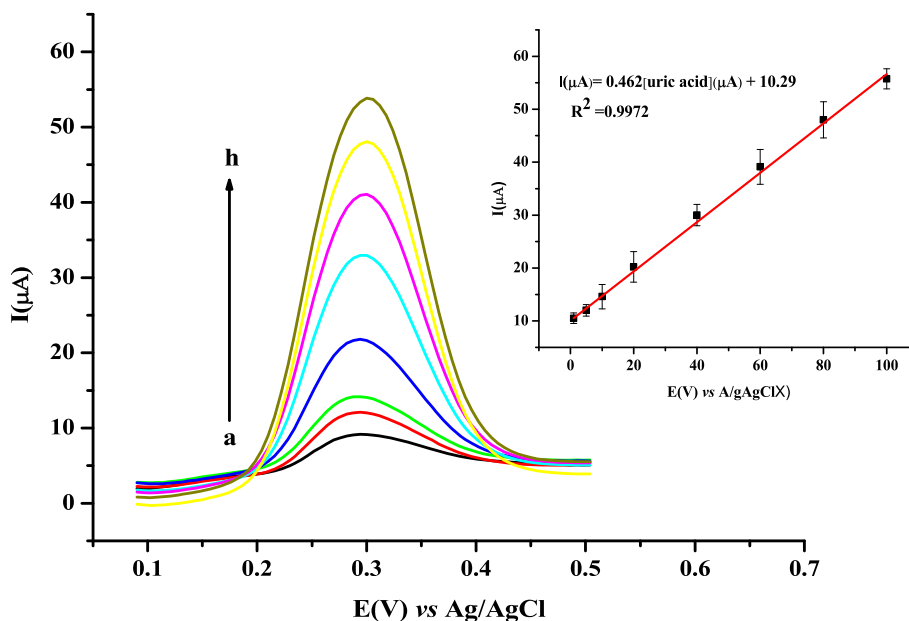
### 3. Results and discussion

#### 3.1. Electrochemical detection of uric acid

The electrochemical nature of UA was evaluated using cyclic voltammetry at the surface of bare GCE and poly (AHNSA), MWCNTs and MWCNTs/poly (AHNSA) modified electrodes at a scan rate of 0.1 V s<sup>-1</sup>. Figure 1 shows a broad oxidation peak without any peak in the reverse scan was observed at a +0.595 V vs Ag/AgCl at the surface of bare GCE

(curve c). After modification with poly (AHNSA) and MWCNTs, the oxidation peak current was shifted to +0.354 V, which was decreased by 0.241 V than at bare GCE with a remarkably increment in its response. A very small reduction peak current appeared in the reverse scan near +0.281 V vs Ag/AgCl at these electrodes is due to reduction of diimine to UA. Thus, UA shows a quasi-reversible electrochemical reaction at the surface of poly (AHNSA)/GCE (curve d) and MWCNTs/GCE (curve e).

The current response for UA at MWCNTs/poly (AHNSA)/GCE (curve f) was higher than at poly (AHNSA)/GCE, MWCNTs/GCE and GCE. In addition, the current response obtained at the composite was found to be three-fold higher than observed at the GCE. The remarkable signal



**Figure 7.** SWV responses for various concentrations of UA (from a to h): 1.0, 5.0, 10, 20, 40, 60, 80 and 100  $\mu\text{M}$  at MWCNT/Poly (AHNSA)/GCE. Inset: plot of peak current vs the concentration of UA.

**Table 1.** Comparison of the proposed sensor with previously reported sensors for the determination of UA.

Electrode	Method	Linear Range ( $\mu\text{M}$ )	D. Limit ( $\mu\text{M}$ )	Ref.
PSRA/MCNTPE	DPV	20–64	1	[1]
Pt NSs/C60/GCE	DPV	9.5–1187	0.63	[15]
SnO <sub>2</sub> /graphene/GCE	SWV	0.1–200	0.28	[17]
Poly (glyoxal-bis(2-hydroxyanil))/GCE	DPV	1.0–200	0.3	[18]
Reduced graphene oxide/GCE	DPV	0.5–60	0.5	[19]
		10–1000	4.7	[21]
Poly-(luminol)/GCE	DPV	0.3–100	2	[22]
NiOH/solar graphene/GCE	DPV	2–15	0.46	[38]
NanoSnO <sub>2</sub> /MWCNTs/Cpe (sim)	DPV	3–200	1.0	[39]
Poly (Aspartic Acid)AuNPs/GCE	LSV	5–100	0.3	[40]
Graphene/GCE	CV	2–120	0.6	[41]
Cetylpyridinium chloride/GCE	DPV	0.50–110	0.13	[42]
MWCNTs/Poly (AHNSA)/GCE	SWV	1–100 $\mu\text{M}$	0.024	This work

PSRA-poly (Solid Red A).

**Table 2.** Effects of interferences on the response of UA.

Interferent	Conc. ( $\mu\text{M}$ )	Change in peak current (%)
AA	1000	+4.3
Glucose	1000	+2.3
Urea	1000	+4.1
Co <sup>2+</sup>	2000	+1.5
Cu <sup>2+</sup>	2000	-3.1

enhancement at the composite modified electrode (MWCNTs/poly (AHNSA)/GCE) shows that MWCNTs and poly (AHNSA) have a synergistic electrocatalytic effect in electrochemical detection of UA. However, the background currents were much larger than at the bare GCE, which mainly results from the increased active area of the GCE as a result of large amount of active oxygen groups in the carbon nanotube, amine and hydroxyl group of poly (AHNSA). Besides, these functional groups interact electrostatically with the oxygen and nitrogen atoms of UA.

Therefore, highest current response of MWCNTs/poly (AHNSA)/GCE towards UA was selected as the best sensing electrode for the determination of UA used in this study.

### 3.2. Effect of pH

The influence of pH of PBS on the electrochemical response for UA at poly (AHNSA)/MWCNTs/GCE was studied over pH range 5.0–8.5 by cyclic voltammetry. The anodic peak current of UA at MWCNTs/poly (AHNSA)/GCE increased gradually with increasing of pH until it attained the maximum at pH 7.0, and then decreased rapidly when the pH increased (Figure 2). This trend attributed to the electrostatic repulsion exerted between the anionic UA ( $\text{pK}_a = 5.4$ ) and negatively charged sulfonic functional and  $\text{COO}^-$  groups on the polymer film and multiwall carbon nanotube [34, 35, 36]. Thus, pH 7.0 was chosen as the optimum value for subsequent experiments, which is in agreement with reported values [35].

Furthermore, influence of pH on the peak potential on the oxidation of UA at MWCNTs/poly (AHNSA)/GCE was studied. The potential

showed negative shift within the range 5.0–8.5 pH, suggesting participation of protons in UA oxidation (Figure 3).

The peak potential ( $E_{pa}$ ) related with the solution pH by Eq. (1).

$$E_{pa}(V) = -0.061\text{pH} + 0.840, R^2 = 0.9977 \quad (1)$$

The slope 0.061 V/pH is close to the 0.059 V/pH (at 25 °C) given by the Nernstian equation, which indicates the involvement of equal numbers of protons and electrons in UA oxidation as described in Figure 4 [37].

### 3.3. Effect of scan rate

The influence of scan rate ( $\nu$ ) on the anodic oxidation peak current ( $i_{pa}$ ) of UA was evaluated on the range of 0.050–0.25 V s<sup>-1</sup> scan rate. Figure 5 shows the influence of scan rate on the  $i_{pa}$  of UA at the MWCNTs/poly (AHNSA)/GCE. The  $i_{pa}$  of UA in PBS (pH 7.0) increased with increase in scan rate over the range of 0.050–0.25 V s<sup>-1</sup>. The  $i_{pa}$  showed linear relationship with the scan rate as shown in Eq. (2).

$$i_{pa}(\mu\text{A}) = 251\nu(\text{V s}^{-1}) + 14.15, R^2 = 0.9982 \quad (2)$$

This indicating that oxidation of UA at the MWCNTs/poly (AHNSA)/GCE is an adsorption-controlled process (inset of Figure 5). Besides, the oxidation peak potentials of UA slightly shifted in a positive direction with an increase in scan rate, demonstrating that the oxidation of UA is quasi-reversible.

### 3.4. Accumulation potential and time

Since the electrochemical oxidation of UA at MWCNTs/poly (AHNSA)/GCE is an adsorption-controlled process, accumulation potential ( $E_{acc}$ ) and time ( $t_{acc}$ ) can influence the magnitude of the peak current. The effect of  $E_{acc}$  on the oxidation peak current of 1.0 mM UA in PBS of pH 7.0 was studied using SWV over the range 0.07 V–0.28 V (Figure 6A). A maximum peak current was observed at 0.18 V vs Ag/AgCl and then it decreased with increasing potential. Therefore, 0.18 V was chosen as the optimal accumulation potential for further measurements.

On the other hand, the effect of  $t_{acc}$  on the oxidation peak current for 1.0 mM UA was also studied, and the results are illustrated in Figure 6B. The peak current increased rapidly with increasing  $t_{acc}$  in the range between 30 and 60 s and remained almost unchanged when the  $t_{acc}$  was further increased due to surface saturation of the electrode. As a consequence, 60 s was selected as the optimum  $t_{acc}$  for further studies.

### 3.5. Calibration curve

Under the optimal conditions, the peak currents of different concentrations of UA ([UA]) were recorded by square wave voltammetry. The oxidation peak currents were linearly related with the concentration of UA in the range of 1.0  $\mu\text{M}$ –100  $\mu\text{M}$  in PBS of pH 7.0 (Figure 7). The linear regression equation of the  $I_{pa}$  vs concentration is given in Eq. (3).

$$I_{pa}(\mu\text{A}) = 0.462[\text{UA}](\mu\text{M}) + 10.29, R^2 = 0.9972. \quad (3)$$

The detection limit calculated to be 0.024  $\mu\text{M}$  based on the three times signal-to-noise ratio (3S/N).

The linear range and detection limit for UA at the MWCNTs/poly (AHNSA)/GCE were compared with reported values (Table 1). The sensor exhibited low detection limits compared with reported values. Besides, the preparation of composite modified electrode and modification were very simple and not time consuming.

### 3.6. Repeatability and stability

The repeatability of the modified electrode was investigated in ten successive measurements of the current response for  $1 \times 10^{-4}$  M UA

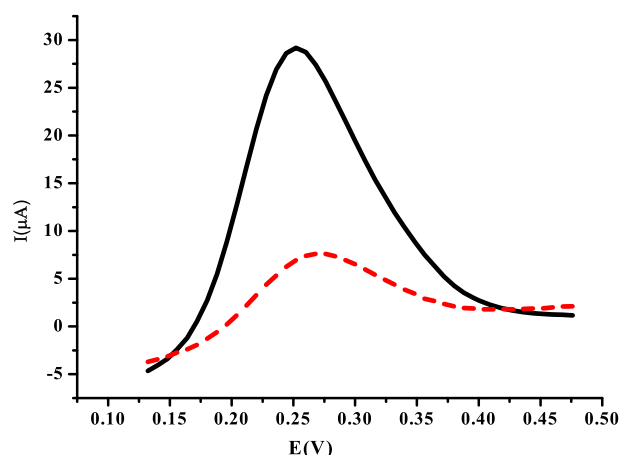


Figure 8. SWVs of 5 mL urine sample diluted with 0.1 M PBS (pH 7.0) (dash line) and after addition of 50  $\mu\text{M}$  UA to the urine sample (solid line).

Table 3. Recovery study of UA in urine sample (n = 3).

Sample	Added ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Recovery ( $\mu\text{M}$ )
Urine	-	6.12	-
	15	21.57 ( $\pm 0.42$ )	102.9 ( $\pm 2.81$ )
	25	31.71 ( $\pm 1.16$ )	102.4 ( $\pm 3.37$ )
	40	42.84 ( $\pm 0.74$ )	92.0 ( $\pm 1.73$ )

under the same experimental conditions. The relative standard deviation (RSD) of the response was found to be 2.3%, which indicates good repeatability of UA determination at MWCNTs/poly (AHNSA)/GCE. The stability of the sensor was also determined. The stability of the MWCNT/Poly (AHNSA)/GCE towards UA oxidation was also evaluated by measuring the response for  $1 \times 10^{-4}$  M UA in 0.1 M PBS (pH 7.0) by keeping the electrode in a buffer solution for a week. The current response decreased only 2.3%, suggesting that MWCNTs/Poly (AHNSA)/GCE has a good stability.

### 3.7. Interference study

To evaluate the selectivity of the MWCNTs/poly (AHNSA)/GCE, the influence of possible interferents which may coexist with UA in real samples on the electrochemical determination of 20  $\mu\text{M}$  UA was examined under the optimum conditions. As shown in Table 2, a 100-fold excess concentrations of  $\text{Co}^{2+}$  and  $\text{Cu}^{2+}$ , and 50-fold concentrations of ascorbic acid, glucose and urea were shown no interference on the response current of UA (signal change below 5%), indicating that MWCNTs/Poly (AHNSA)/GCE possessed excellent selectivity towards UA.

### 3.8. Applications

The applicability of the MWCNTs/poly (AHNSA)/GCE sensor for UA analysis was tested for urine samples obtained from a normal person. After diluting the urine sample with PBS pH 7.0, an oxidation peak was observed at 0.24 V. To confirm the oxidation peak was due to UA, the sample was spiked with known concentrations of standard UA. The standard solution of UA added to the urine sample caused an increase in the oxidation peak current, which confirms that the peak observed for the urine sample only was due to the oxidation of UA (Figure 8).

Standard addition method was applied to validate the method developed for UA determination in urine samples by spiking urine samples with different concentration of UA standard solutions. The percent recoveries were found in the range 92.0%–102.9% (Table 3). These

results indicate that MWCNT/poly (AHNSA)/GCE has good reliability for detecting UA in human urine.

#### 4. Conclusion

MWCNTs/poly (AHNSA)/GCE was applied for the determination of uric acid in human urine. The sensor exhibited higher sensitivity to UA as compared to the MWCNTs/GCE, poly (AHNSA)/GCE and the bare GCE. This is due to the synergistic effect of larger surface area, higher accumulation efficiency of the MWCNTs film and the electrostatic interaction between the hydroxyl, sulphonic and amine groups of the polymer with the UA. The MWCNTs/poly (AHNSA)/GCE also showed remarkable electrochemical advantages such as high repeatability and stability, wide linear range and low detection limit. Moreover, the sensor was applied for UA analysis in human urine sample and a recovery values ranged from 92.0% to 102.9% were obtained.

#### Declarations

##### Author contribution statement

Molla Tefera: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Merid Tessema, Shimelis Admassie: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Walegn Wubet: Analyzed and interpreted the data; Wrote the paper.

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##### Data availability statement

The authors do not have permission to share data.

##### Declaration of interests statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

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