

Fabrication of a sensitive electrochemical sensor based on Ag nanoparticles and alizarin yellow polymer: Application to the detection of an environmental pollutant thiourea

Iraj Jodan^{*}, Kitirote Wantala^{**}, Nader Amini^{*}, Behzad Shahmoradi^{*,†}, Mona Ghaslani^{*},
Seung-Mok Lee^{***,†}, Jixiang Yang^{****}, and Shivaraju Harikaranahalli Puttaiah^{*****}

^{*}Department of Environmental Health Engineering, Environmental Health Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
^{**}Department of Chemical Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen 40002, Thailand
^{***}Department of Environmental Engineering, Catholic Kwandong University, Gangneung 210701, Korea
^{****}Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing, China
^{*****}Department of Water and Health, JSS Academy of Higher Education and Research, Mysuru-570015, India
(Received 2 March 2020 • Revised 2 April 2020 • Accepted 27 April 2020)

Abstract–The harmful and destructive effects of excessive consumption of thiourea in various industries and agriculture have caused health and environmental concerns. Hence, attention has been paid to the need for analysis and detection thiourea at very low concentrations. The aim of this study was to determine the efficiency of electrochemical sensor based on glass carbon electrode modified with Ag nanoparticles and alizarin yellow to detect thiourea as a pollutant. Glass carbon electrode was first modified by alizarin yellow polymer and Ag nanoparticles through electropolymerization and chronoamperometry methods, respectively. The characteristics of the modified electrode were determined through FESEM and EDAX analysis and thiourea concentration was measured through cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. The results showed that the linear range, limit of detection, and sensitivity were 10 to 940 μM , 3.3 μM and 0.181 $\mu\text{A}/\mu\text{M}$, respectively. The stability and reproducibility of the sensor response as well as the interference effect of some anion species were investigated on the performance of the sensor in determining thiourea. The results indicate the optimum stability and reproducibility and no interference effect of the studied species on efficiency of the sensor in determining thiourea.

Keywords: Electrochemical Sensor, Ag Nanoparticles, Alizarin Yellow Polymer, Thiourea

INTRODUCTION

Thiourea (TU) is a white crystalline solid of formula $\text{CH}_4\text{N}_2\text{S}$ whose solubility is about 137 mg/l in water at 20 °C. Other names for this substance are thiocarbamide and sulfourea [1]. It is produced in two forms, natural and synthetic. It has been detected in laburnum shrubs (*Laburnum anagyroides*) and is a natural metabolite of *Verticillium alboatrum* and *Bortryllus cinerea*. It is industrially produced by the reaction between technical-grade calcium cyanamide (CaCN_2) and hydrogen sulfide (H_2S) or one of its precursors in aqueous solutions, for example, ammonium sulfide ($(\text{NH}_4)_2\text{S}$) or calcium hydrogen sulfide ($\text{Ca}(\text{HS})_2$). This compound is structurally similar to the urea molecule, except that it contains sulfur atoms instead of oxygen. Fig. 1 shows the structure of the urea and TU [2]. TU has different applications in different industries, for example, in agriculture as fungicides, herbicides, anti-rodent poison [2], reducing the content of soil salinizing bacteria [3], and causing the ripening of unripe fruits and breaking the sleep of seeds and glands [4]. This material is widely used in photography as fixing and clean-

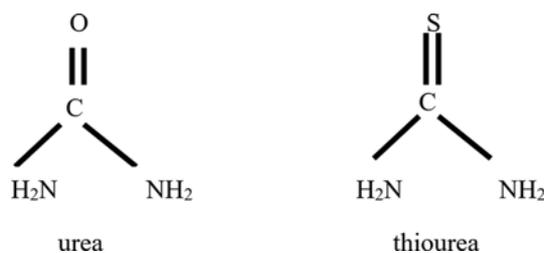


Fig. 1. Structures of the urea and TU.

ing agent of films. It is widely used in the production of metals such as copper, preparation of textiles, paint industrials, pharmaceuticals and rubber and paper manufacturing. TU is also used in cold storage to store citrus [5].

Health and environmental concerns about the deleterious effects of TU necessitate the analysis and monitoring of TU as a pollutant at low concentrations. So far, various methods have been proposed for the determination of TU, including iodine titration [6], bromosoxin imide titration [7], haloamine titration [8], Raman spectroscopy [9], spectrophotometry [10], polarography [11], voltammetry [12], HPLC [13], chemiluminescence [14], and mass spectroscopy [15]. These methods have some disadvantages such as high cost,

[†]To whom correspondence should be addressed.
E-mail: bshahmorady@gmail.com, leesm@cku.ac.kr
Copyright by The Korean Institute of Chemical Engineers.

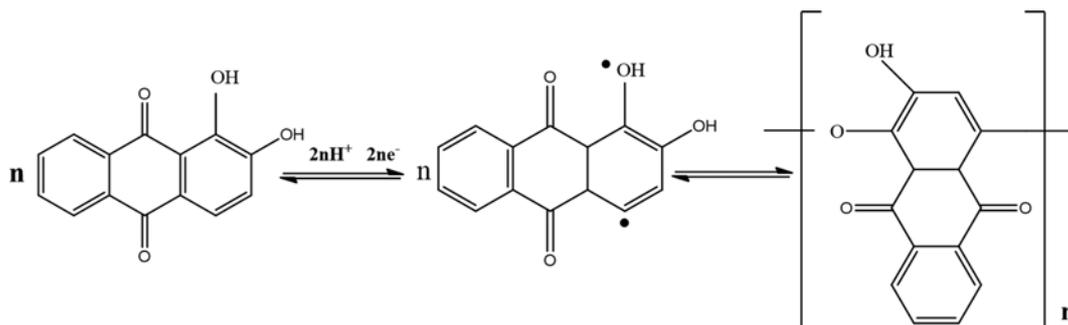


Fig. 2. The mechanism of electropolymerization of alizarin yellow.

low specificity, high detection limit, low sensitivity, and high use of chemical solvents of environmental pollutants. Electrochemical methods could be used to overcome these drawbacks [16].

Recently, the synthesis of self-assembled composite for electrode surface modification and manufacturing of electrochemical sensors has assumed great importance [17-20]. Electrode modification involves a process in which the surface of the electrode is changed to perform or not perform an electrode process. This change is the result of a physical process such as increasing or changing the surface structure or a chemical process of placing a compound on the electrode surface. Chemically modified electrodes (CME) are the product of targeted modification of a conductor surface, resulting in the ability to perform specific tasks that the unmodified electrode is unable to perform [21]. In electrochemical studies, there are many limitations at the electrode surface, for instance, the formation of sediment and the adsorption of undesirable substances that reduce the rate of the progress of electrochemical reactions at the electrode surface; high overvoltage required for the electrode reactions; and the low sensitivity of the measurement as well as the low selectivity of the electrode to the desired electro-active compounds.

Therefore, electrode surface modification is performed to eliminate or reduce such problems. In practice, modifying the electrode surface reduces the overvoltage and increases the kinetics of electrode processes [22]. Silver nanoparticles (Ag NPs) have been used to modify electrochemical sensors due to their excellent properties such as good compatibility and low toxicity and favorable catalytic activity [23]. Alizarin yellow polymer is an organic dye with a structure of salicylic acid used to modify electrochemical sensors. Advantages of conductive polymers are stability, reproducibility, homogeneity in chemical deposition and strong adhesion to the electrode surface, which have highlighted their role and application in electrochemical sensor modification [23]. Many studies have been performed on the detection and determination of TU using modified electrochemical sensors. Safavi et al. fabricated nanosensors for the detection and determination of TU using a modified electrode with silver nanoparticles/graphene/ionic liquid [24]. Lee et al. measured TU using modified electrodes with copper oxide nanoparticles [25]. And Nematollahi et al. designed an electrode by modifying the surface of the platinum electrode with alumina for measuring TU [26].

In the present study, for the first time, modified electrode with alizarin yellow polymer and silver nanoparticles (Ag NPs) was used to measure TU. Some of the advantages of the used method in this

study can be very low detection limit, high sensitivity, low cost, easy preparation method, high linear range and most importantly, non-use of environmental solvents and pollutants to detect and determine TU (Thiourea).

EXPERIMENTAL

1. Materials

Thiourea, AgNO_3 , and alizarin yellow polymer (AYP) (Fig. 2) were purchased from Merck, Germany (with a purity of 99.9%). Buffer solutions (0.1 M) were prepared from di-sodium hydrogen phosphate and sodium dihydrogen phosphate. pH adjustment was performed with 0.2 N hydrogen chloride or sodium hydroxide.

2. Apparatus

Electrochemical measurements were performed in an electrochemical cell using an autolab device of Vortex model (Netherlands). The electrochemical cell had three working, reference, and auxiliary electrodes. Glass carbon electrode modified with AYP and Ag NPs was used as the working electrode, platinum electrode was employed as the auxiliary electrode, and glass electrode was used as the reference electrode. Scanning electron microscopy was used to confirm the deposition of AYP and Ag NPs on the electrode surface. A pH meter was used to prepare buffer solutions with pH 1

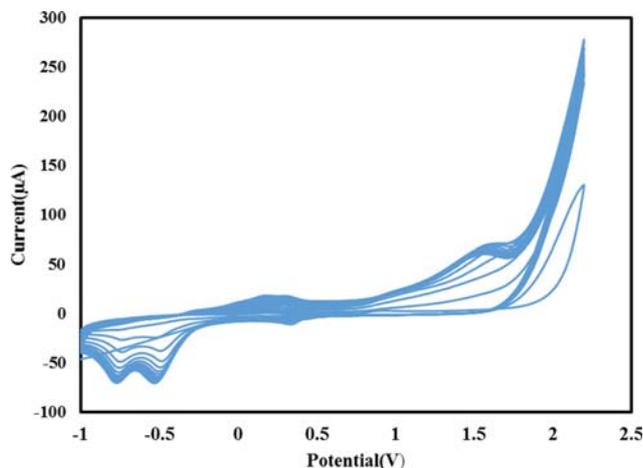


Fig. 3. Cyclic voltammograms for electropolymerization of AYP (0.001 M) in 0.1 M NaOH buffer solution on unmodified GCE surface at scan rate 100 mV/s.

to 13, and an ultrasound device was employed to remove pollutants from the electrode surface after polishing.

3. Preparation of the Modified Electrode

Electrode modification was performed in three steps: At first, the glassy carbon electrode was polished using an alumina paste (Al_2O_3). The electrode was then placed in an ultrasound apparatus for 5 min using a small 20 ml beaker containing water and alcohol in equal proportion to remove possible pollutants from the electrode surface after polishing. After polishing the electrode, the AYP was placed

on the electrode surface by electropolymerization [27] to facilitate electron transfer at the electrode surface. Next, Ag NPs were placed on the electrode surface using chronoamperometry [28] to increase the conductivity of the electrode surface. For this purpose, electrode inside a solution containing 0.001 M AgNO_3 was placed and Ag^+ was reduced by applying 0.2 V in 150 s and deposited on the electrode surface as Ag. After these steps were completed, the electrode was modified and used in subsequent voltammetry studies. The presence of these two agents on the electrode surface facilitates

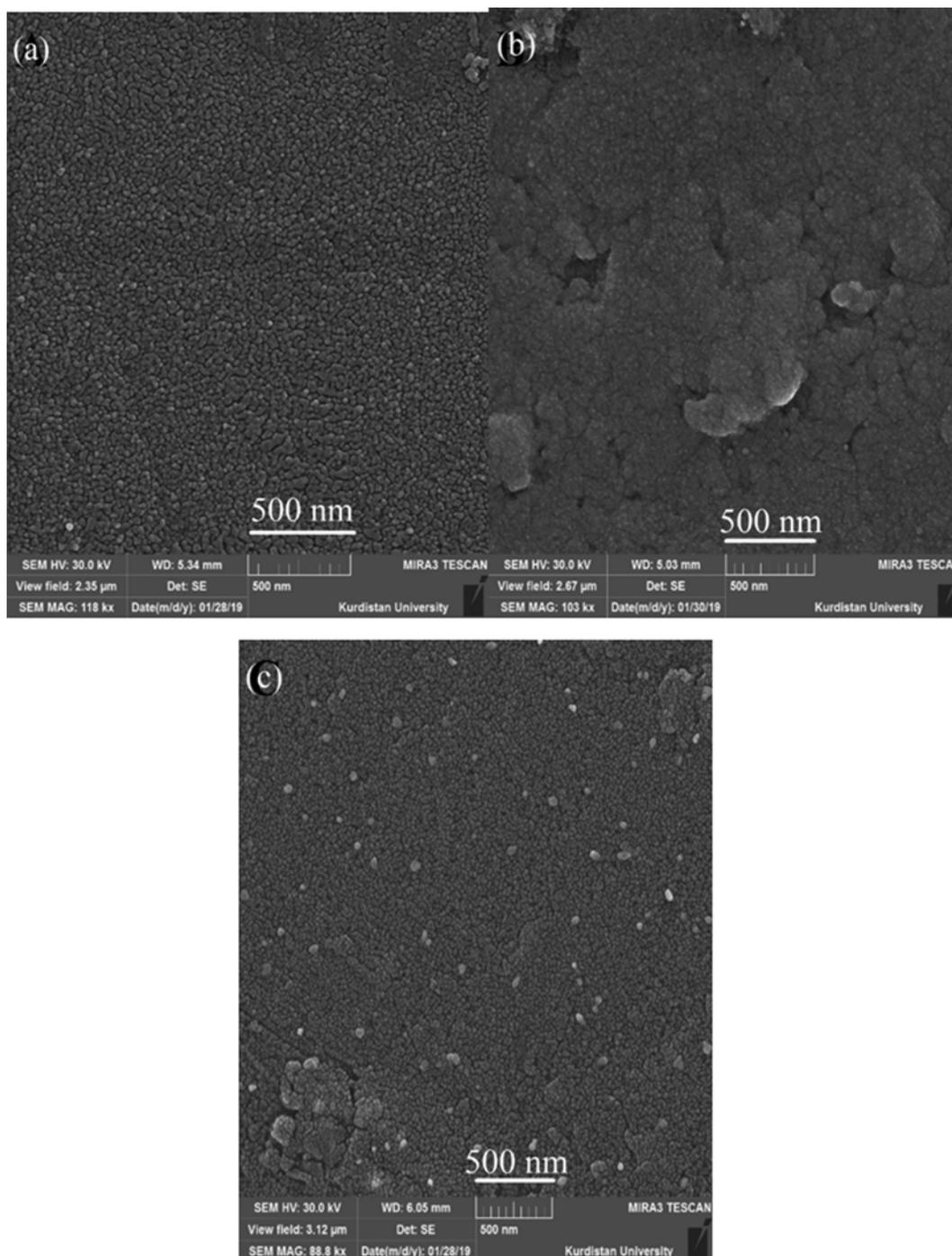


Fig. 4. SEM images of the electrode surface in three modes: (a) Bare GCE, (b) AYP/GCE, (c) Ag NPs/Poly/AYP/GCE.

electron transfer and, in a way, it can be said the conductive AYP and Ag NPs enhance the effect of each other and have increased the conductivity of the electrode.

RESULTS AND DISCUSSION

1. AYP Electropolymerization on Glass Carbon Electrode Surface

Electrochemical polymerization of alizarin yellow (1 mM) on glass carbon electrode (GCE) surface was performed in 0.1 M NaOH buffer solution, in the potential range of -1 to 2.2 V, with scanning rate of 100 mV/s for 15 cycles. Fig. 3 shows the consecutive cyclic voltammograms of electropolymerization of alizarin yellow on the glass carbon electrode surface. One oxidation peak was observed at the potential of 1.6 V and two peaks obtained at the potentials of -0.52 and -0.76 V based on the mechanism proposed below. Over the 15 consecutive cycles, the intensity of peaks current created had increasing trend, indicating the formation of a conductive polymer from the alizarin yellow solution and placing on the electrode surface characteristics of the modified electrode.

2. Surface Characterization of Modified Electrodes

SEM and EDAX analyzes were used for both bare and modified electrodes to determine the morphology and confirm the structure modification. Fig. 4 shows the SEM images obtained from the surface of bare GCE (a), AYP/GCE (b), and Ag NPs/AYP/GCE (c).

It can be easily understood by comparing the images in Fig. 4, that after the electropolymerization step, AYP was uniformly placed on the surface of the GCE (Fig. 4(a), (b)), and after performing the chronoamperometric process, Ag NPs were deposited on the electrode surface and confirmed the formation of the composite of AgNPs/AYP on the electrode surface. The Ag NPs were arranged in an irregular shape with an average diameter of 30 nm on the electrode surface. Fig. 5 shows the energy dispersive X-ray spectroscopy of AgNPs/AYP/GCE surface that verifies the presence of Ag NPs on the electrode surface.

3. Electrocatalytic Oxidation of TU on Ag NPs/AYP/GCE

Cyclic voltammetry (CV) was used for investigating the electro-

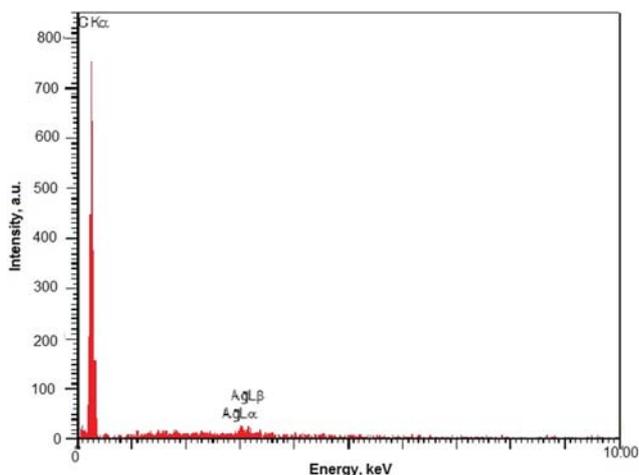


Fig. 5. Energy dispersive X-ray spectroscopy of Ag NPs/AYP/GCE on the surface of GCE.

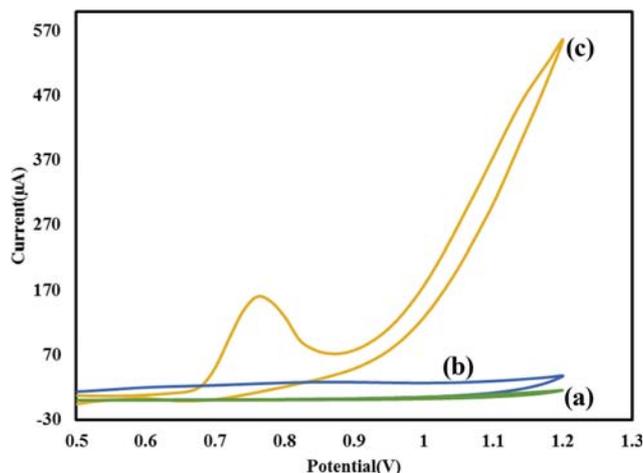


Fig. 6. Cyclic voltammograms of: (a) Bare GCE; (b) AYP/GCE; (c) Ag NPs/AYP/GCE in the presence of thiourea.

oxidation of TU at the surface of various electrodes. Fig. 6 shows the cyclic voltammograms for the comparison of three different electrodes in the presence of TU. As can be seen, there is no redox response of TU in the potential range 0.5 to 1.2 V for the bare electrode (Fig. 6(a)). However, at the AYP/GCE (Fig. 6(b)), the oxidation current slowly increased due to catalytic oxidation of TU. At the Ag NPs/AYP/GCE (Fig. 6(c)), the oxidation current greatly increased due to catalytic oxidation of TU. Therefore, Ag NPs/AYP is suitable as mediator to shuttle electron between TU and working electrode and facilitates electrochemical regeneration following electron exchange with TU.

The effect of pH was investigated to determine the optimum pH (the pH with the highest oxidation current). Cyclic voltammogram was prepared using buffer solutions of pH 1 to 13 and 0.1 M NaOH buffer solution containing 0.001 M TU at a potential range of 0 to 1.2 V, with a scanning rate of 40 mV/s. Fig. 7 shows the cyclic voltammogram of 0.001 M TU solution at different pHs. TU oxidation current peaks at pHs 1 to 13 and 0.1 M NaOH solution were 8.1 , 10.5 , 10.5 , 10.5 , 10.5 , 11.9 , 24.4 , 27.7 , 29.8 , 30.3 , 32 , 32.3 , 63.9 and 97 μ A, respectively.

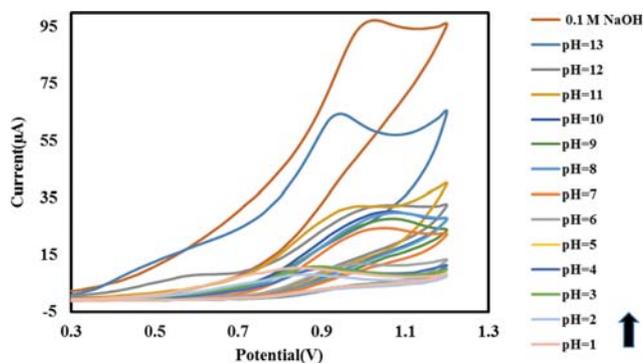


Fig. 7. Cyclic voltammograms in phosphate buffer solutions at pHs 1 to 13 and 0.1 M NaOH solution containing 0.001 M thiourea at Ag NPs/AYP/GCE surface at potential range 0 to 1.2 V with a scanning rate of 40 mV/s.

The results show that as the pH of buffer solution increased, the current intensity also increased; hence, the largest current peak that is related to oxidation of TU in a buffer solution of 0.1 M NaOH, was formed. Safavi et al. observed that the highest oxidation of TU was in 0.1 M NaOH solution [20]. Manea et al. also found similar results in their study; they used 0.1 M NaOH solution as the optimum pH for TU oxidation [29]. Therefore, the electrode response was pH dependent and the modified electrode could be used as a sensor at alkaline pHs. In this research, the 0.1 M NaOH solution was used as the optimum buffer for oxidation of TU at the modified electrode surface. In addition, the effect of the potential scan rate on the response of the AgNPs/AYP/GCE at the presence of 800 μM TU was studied (Fig. 8(a)). At low scanning rates, weak anodic peaks appeared, but the height of the anodic fuselage gradually increased with the increase of the potential scanning rate, and

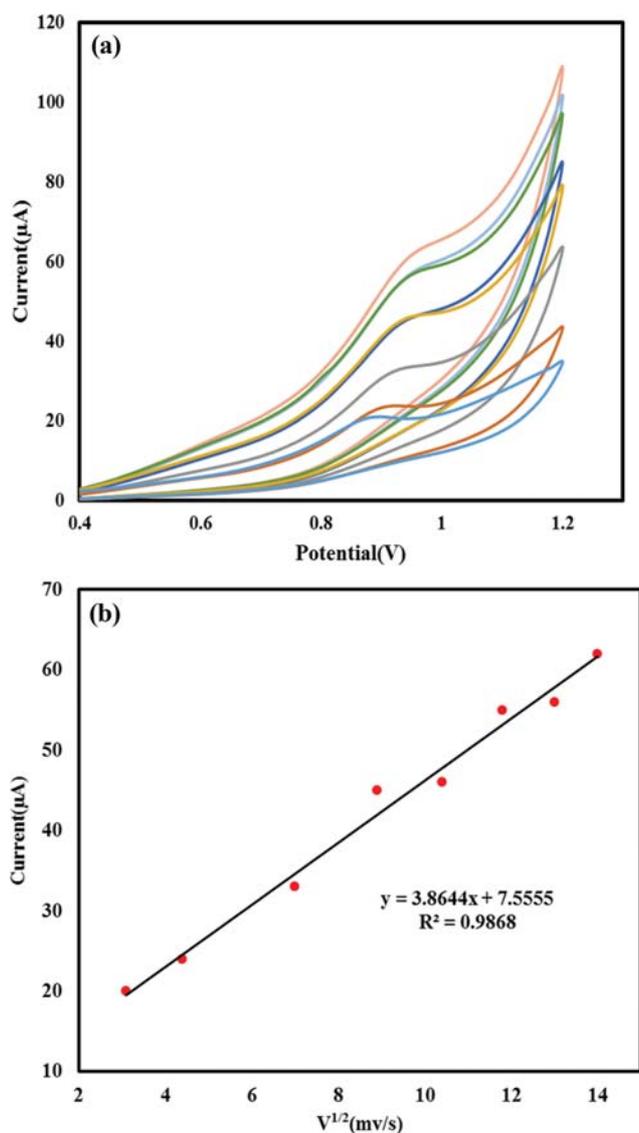


Fig. 8. (a) Cyclic voltammograms in a 0.1 M NaOH solution containing 0.001 M thiourea, at the Ag NPs/AYP/GCE surface, in the potential range of 0 to +1.2 V and different scanning rates; and (b) plot of peak current of thiourea vs. scan rate^{1/2}.

the potential of the anodic fuselage shifted to more positive values with increasing scanning rate. The curves of oxidation currents versus square root of scan rate are linear, indicating that the system is controlled by diffusion function (Fig. 8(b)). Safavi et al. also achieved similar results in their study and observed that with the increase of the scanning rate of potential, the peak of TU oxidation current increased [24]. Spataru et al reported similar results, i.e., the current peak of TU oxidation increases with increasing the scanning rate of potential [30].

For finding the TU linear ranging, the effect of increasing different concentrations of TU on the cyclic voltammogram of modified Ag NPs/AYP/GCE was investigated. According to Fig. 9(a), with increases of the TU concentration, the anodic peak current of the catalytic wave increased as linearly at 0.8 V. The plot of the electrocatalytic current versus TU concentration was obtained; Fig. 9(b) shows a linear response from 190 to 1,110 μM with a good linear

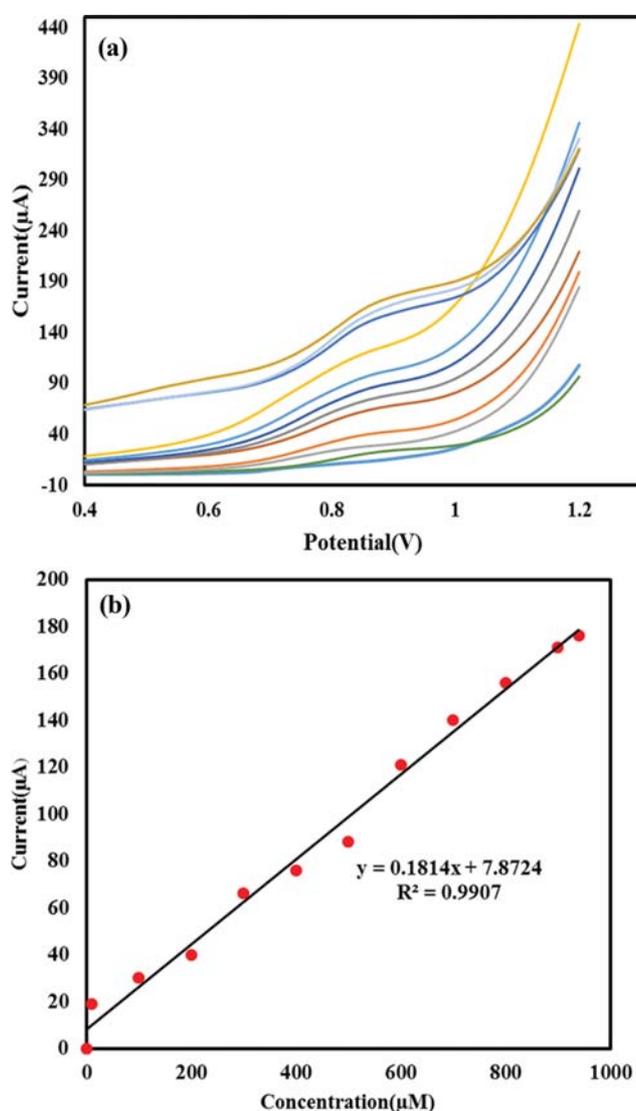


Fig. 9. (a) Differential pulse voltammograms in different concentrations of thiourea at Ag NPs/AYP/GCE surface and (b) current changes with increasing concentration of thiourea.

Table 1. Comparison of the results of the use of modified electrodes in the detection and measurement of TU

Electrode type	LOD (μM)	Linear range (μM)	Ref.
Ionic liquid/graphene/Ag NPs	0.7	1-3,000	[24]
CuO NPs modified	20	-	[25]
Alumina modified Pt	4.8	25-7,000	[26]
CuO/Cu	-	1,000-8,000	[29]
B doped diamond	-	4-8,000	[30]
DNA and MnO NPs modified	-	50-3,000	[31]
Cyclic voltammetry of oxidation peak 1	14	50-3,000	[31]
Cyclic voltammetry of oxidation peak 2	22	4-3,000	[31]
Differential pulse voltammetry of oxidation peak 1	7	4-3,000	[31]
Differential pulse voltammetry of oxidation peak 2	1	4-2,000	[31]
Graphite pencil	1.29	6.3-30	[32]
Carbon based composite	20	100-1,000	[33]
Ag NPs/AYP/GC (CV)	63.3	190-1,100	Present study
Ag NPs/AYP/GC (DVP)	3.3	10-940	Present study

correlation.

Based on the calibration curve and Eq. (1), the sensitivity and detection of limit obtained was $0.048 \mu\text{A}\mu\text{M}^{-1}$ and $63.3 \mu\text{M}$, respectively. Based on the results, the oxidation and reduction mechanism of TU is proposed as Eq. (2).

$$(\mu\text{A})=0.048 [\text{TU}] \mu\text{A}\mu\text{M}^{-1}+6.1386 \mu\text{A} \quad (1)$$



To obtain the low detection limit, differential pulse voltammetry (DPV) was used. The differential pulse voltammograms of modified electrode in the presence of various concentration of TU were investigated in 0.1 M NaOH buffer solution. As can be seen from Fig. 9, by increasing the concentration of TU, the intensity of the anodic peak increased. A linear dependence of the catalytic currents vs TU concentration can be calculated based on Eq. (3) and $R^2=0.9907$. Limit of detection, sensitivity, and linear range were calculated as $3.3 \mu\text{M}$, $0.181 \mu\text{A}/\mu\text{M}$, and 10-940 μM , respectively.

$$(\mu\text{A})=0.1814 [\text{TU}] \mu\text{A}\mu\text{M}^{-1}+7.8724 \mu\text{A} \quad (3)$$

4. Comparison of the Proposed with those of Previous Electrochemical Methods

The modified electrode was compared with several other procedures applied to TU determination. The results are presented in Table 1. As shown, the linear dynamic range and lower detection limit of this electrode are better or comparable with those of other techniques.

5. Interference Effects

To investigate the selectivity of the Ag NPs/AYP/GCE, the influence of some inorganic ions was studied in NaOH 0.1 M containing 200 μM TU. The results showed that CN^- , NO_3^- , SO_4^{2-} , IO_3^- , F^- , I^- , Cl^- , Br^- anions did not interfere with the determination of TU up to 50 times of TU concentration.

6. Repeatability and Stability

The repeatability of response current of the modified electrode was investigated at a TU concentration of 600 μM . The variation coefficient (R.S.D) was 2.2% for three successive assays. The stabil-

Table 2. Determination of TU in real water samples using Ag NPs/AYP/GCE

Sample	Added (μM)	Found (μM)	Recovery (%)
1	600	590.53	98.42
2	600	618.23	103.03
3	600	621.30	103.55

ity and lifetime of the sensor were investigated by measuring the response current of the sensor after one and two weeks. The observed decreases in the electrode response were 3 and 11.5% of initial current after one and two weeks, respectively. Therefore, it was confirmed that the Ag NPs/AYP/GCE is reusable and has good reproducibility and stability.

7. Analytical Application

The applicability of the modified electrode was tested for the measurement of TU from three river water samples via a recovery study according to the above-described analytical procedure. The river water sample concentration was measured using the modified electrode, and the results are presented in Table 2. As can be seen, it produced satisfactory results with the recoveries ranging from 98.4 to 103.5%.

CONCLUSION

Glass carbon electrode modified with Ag NPs and alizarin yellow polymer was designed to determine low concentrations of thiourea, and the electrochemical behavior of thiourea on the modified electrode was investigated using cyclic voltammetry and differential pulse voltammetry techniques. Use of Ag NPs and alizarin yellow polymer strengthened the conductivity of the nanosensor fabricated and led to an increase in the electroactivity of thiourea and the formation of larger oxidation peaks of thiourea by facilitating the electron transfer between the electrode and the analyte. The results of this study show the capability of the designed sensor to detect and determine the low concentration of thiourea in environmental samples.

ACKNOWLEDGEMENTS

This manuscript was extracted from the Master's dissertation of the first author approved by Ethic Committee under Research Project No IR.MUK.REC.1397/292 with the financial support of the Research V.C. of Kurdistan University of Medical Sciences. Hereby the authors express their gratitude to Kurdistan University of Medical Sciences. This study was also partially supported by International Affairs Division of Khon Kaen University (International Visiting Scholar, 2019).

REFERENCES

1. M. J. Janssen, *Rcc. Trav. Chim.*, **79**, 650 (1960).
2. T. P. Ruiz, C. M. Lozano, V. Tomas and R. Casajus, *Talanta*, **42**, 391 (1995).
3. M. R. Smyth and J. G. Osteryoung, *Anal. Chem.*, **49**, 2310 (1977).
4. A. N. de Oliveira, H. de Santana, C. T. B. V. Zaia and D. A. M. Zaia, *J. Food Compos. Anal.*, **17**, 165 (2004).
5. K. Ziegler-Skylakakis, J. Kielhorn, G. Konnecker, J. Koppenhofer and I. Mangelsdorf, World Health Organization: Geneva; Document 49 (2003).
6. D. Amin, *Analyst*, **110**, 215 (1985).
7. M. Sarwar and R. J. Thibert, *Anal. Lett.*, **1**, 381 (1968).
8. C. P. K. Pillai and P. Indrasenan, *Talanta*, **27**, 751 (1980).
9. H. J. Bowley, E. A. Crathorne and D. L. Gerrard, *Analyst*, **111**, 539 (1986).
10. A. K. Abd El-Kader, M. Abd El Mottalab, M. G. Abd El Wahed and M. Ayad, *Anal. Lett.*, **17**, 2291 (1984).
11. M. Fedoronko, O. Manousek and P. Zuman, *Chem. Listy*, **49**, 1494 (1953).
12. V. Stara and M. Kopanika, *Anal. Chim. Acta*, **159**, 105 (1984).
13. A. Trojanek and M. Kopanika, *J. Chromatogr. A*, **328**, 127 (1985).
14. Z. He, F. Wu, H. Meng, L. Ling, L. Young, Q. Lu and Y. Zeng, *Anal. Sci.*, **15**, 381 (1999).
15. A. Raffaelli, S. Pucci, R. Lazzaroni and P. Salvadori, *Rapid Commun. Mass Spectrom.*, **11**, 259 (1997).
16. N. Amini, M. Shamsipur and M. B. Gholivand, *J. Mol. Catal. A: Chem.*, **396**, 245 (2015).
17. Y. He, R. Wang, C. Sun, S. Liu, J. Zhou, L. Zhang, T. Jiao and Q. Peng, *ACS Omega*, **5**, 3689 (2020).
18. R. Wang, X. Yan, B. Ge, J. Zhou, M. Wang, L. Zhang and T. Jiao, *ACS Sustainable Chem. Eng.*, **11**, 4521 (2020).
19. Y. He, R. Wang, T. Jiao, X. Yan, M. Wang, L. Zhang, Z. Bai, Q. Zhang and Q. Peng, *ACS Sustainable Chem. Eng.*, **7**, 10888 (2019).
20. K. Chen, J. Li, L. Zhang, R. Xing, T. Jiao, F. Gao and Q. Peng, *Nanotechnology*, **29**, 445603 (2018).
21. X. Q. Lin, J. Chen and Z. H. Chen, *Electroanalysis*, **12**, 306 (2000).
22. P. Chen and R. L. Mccreey, *Anal. Chem.*, **68**, 3958 (1996).
23. K. Zhang, N. Zhang, L. Zhang, J. Xu, H. Wang, C. Wang and T. Geng, *Microchim. Acta*, **173**, 135 (2011).
24. A. Safavi, R. Ahmadi, F. Aghakhani and M. Tohidi, *Sens. Actuators, B*, **207**, 668 (2015).
25. L. Tian, Y. Gao, L. Li, W. Wu, D. Sun, J. Lu and T. Li, *Microchim. Acta*, **103**, 607 (2013).
26. D. Nematollahi and M. Rafiee, *Sensors*, **3**, 534 (2003).
27. B. D. Malhotra, A. Chauby and M. Gerard, *Biosens. Bioelectron.*, **17**, 345 (2002).
28. X. Luo, A. Morrin, A. J. Killard and M. R. Smyth, *Electroanalysis*, **4**, 319 (2005).
29. F. Manea, J. Radovan and J. Schoonman, *J. Appl. Electrochem.*, **36**, 1075 (2006).
30. N. Spataru, T. Spataru and A. Fujishima, *Electroanalysis*, **17**, 800 (2005).
31. A. Maleki, H. Daraei and N. Amini, *J. Colloid Interface Sci.*, **504**, 579 (2017).
32. A. Levent, E. Keskin, Y. Yardim and Z. Senturk, *Rev. Anal. Chem.*, **30**, 45 (2011).
33. I. Corb, F. Manea, C. Radovan, A. Pop, G. Burtica, P. Malchev, S. Picken and J. Schoonman, *Sensors*, **7**, 2626 (2007).