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Electrochemical Behavior of Aminoguanidine and Its Detection by Hybrid Polymer of Theophylline with Copper Oxide Nanoparticles

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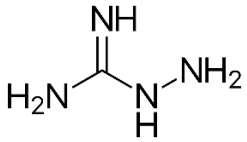
Abstract- Aminoguanidine (AG), as a group of nucleophilic hydrazine compounds, has different pharmacological activities, like reducing the pathological consequence of diabetes. Here, the electrochemical oxidation behavior of aminoguanidine was examined, directly at bare graphite electrode and indirectly, with theophylline hybrid polymer and copper oxide nanoparticles, using cyclic voltammetry (CV) and square wave voltammetry (SWV). The impact of pH, buffer type, time, and concentration were studied in the two methods. Two oxidation peaks were found at (0.773 V, 1.15 V) *vs.* Ag/AgCl in acetate buffer (0.1 M, pH 4.5) and (0.8 V, 1.1 V) *vs.* Ag/AgCl in phosphate buffer (0.1 M, pH 7.0). At graphite bare electrode (GCE), the optimal result was achieved when the phosphate buffer was used, changing the linear range for the detection of aminoguanidine at bare electrode from 299.1 to 990 µM with R^2 of 0.992 and standard deviation of 33.5 µM. At modified graphite electrode with theophylline hybrid polymer and copper oxide nanoparticles (GCE-poly TP/CuO-NPs), a much wider range was obtained for AG, at 9.9 to 610.316 µM with R^2=0.990 the limit of detection of 6.34 µM, and SD of 0.089 µM. Finally, the preparation of modified graphite electrode with nanoparticles (GCE-poly TP/CuO NPs) showed good stability, persisting for more than 2 weeks, and showing a potential to be used as a drug sensor.

Keywords- Aminoguanidine; CuO nanoparticles; Theophylline; Electropolymerization; Graphite electrode

1. INTRODUCTION

Aminoguanidine (AG) belongs to the category of nucleophilic hydrazine compounds, holding a terminal amino group, which is a structural correspondent of L-arginine (Scheme 1). It is a member of guanidine, having one carbon.



Scheme 1. Aminoguanidine structure

Different studies explained the effective role of AG in preventing the creation of progressive glycation end and reactive oxygen species (ROS) products *in vivo* and *in vitro* [1,2]. AG has additional pharmacological functions such as prevention of nitric oxide synthase and semicarbazide-sensitive amine oxidase (SSAO) [3]. Moreover, it is considered a good reactor with biological compounds like pyruvate, glucose, pyridoxal phosphate, malondialdehyde, and others [4].

Practically, studies conducted *in vivo* proved its essential role in diabetic renal pathology through AG interaction with the nitric oxide (NO) pathway [5]. Indeed, AG as the cream may have the ability to reduce the pathological consequences in diabetic rats [6].

According to our search, AG has been studied and determined by very limited literature. Merely, the determination of AG was described by analytical methods, such as ion chromatography [7], UV spectrophotometry [8], visible spectroscopy [9], and HPLC [10,11]. Numerous related electrical electrochemical studies on cyclic voltammetry have been conducted on the derivatives of aminoguanidine at modified electrodes [12] and its complexes [13-15]. To the best of our knowledge, square wave voltammetry (SWV) has not been used for the detection of AG in different pH at the hybrid polymer with nanoparticles.

A polymerization of heterocyclic aromatic compounds has been introduced as very favorable constituents for energy storage and conversion, electronics, electrochromic windows and drugs, and biological composite concentration [16-18].

The modification of electrodes by nanomaterial can be beneficial for the assessment of a wide spectrum of drugs and biological substances, due to their role in the enhancement of electron speed and reduction of the oxidation over the potential of substances [19-22]. Therefore, the nanocomposite matrix attracted much attention in the past years for its special properties [23-25]. There is a kind of compatibility between conducting polymer and nanoparticles form of metal oxides; as a result, a nanocomposite that has a beneficial role in the modification of electrodes can be made [17,26,27].

In electrochemical applications, the nanostructure metal oxide, such as copper oxide has been used, through its surface specified and proper electrochemical action [25,28,29].

In our lab, a conjugated polymer with theophylline (TP) was prepared and its behavior with metals was studied [30]. Herein, we developed a film to be more effective by loading copper nano oxides to prepare graphite-polytheophylline/copper oxide nanoparticles (GCE-poly TP/CuO-NPs). The electrocatalytic characteristics of AG were studied and optimized, and then the determination of AG was indirectly obtained at this modified electrode through its interaction with the CuO-NPs peak. The purpose of this work was to assess the electrochemical performance of AG and to detect it indirectly through the modified nano-composite electrode by CuO-NPs peak, to be used in medical products. Therefore, very interesting linear ranges and detection limits for AG with concurrent resolution were obtained by the improved electrode with polymer layer and nanoparticles.

2. EXPERIMENTAL SECTION

2.1. Apparatus

All voltammetry assessments were carried out, using the 797 VA Computrace stand (Metrohm, Switzerland), connected to a PC and administered by the control software, VA Computrace 2.0. The voltammetry assessments were performed in a glass cell (working volume of 5−10 ml) with a three-electrode detection system, comprising of the working electrode made of a graphite electrode (GCE) with 2 mm diameter. The reference electrode was an Ag/AgCl in 3M KCl, and a 1.5 mm platinum tip was applied as the auxiliary electrode. During the experiments, Hanna pH meter model 211 (manufactured in Romania) was used for pH adjustment.

2.1.1. Materials

Aminoguanidine hydrochloride was purchased from Sigma-Aldrich Chemical Company (Munich, Germany). Copper oxide nanoparticles with size 15 nano and purity of 99% from the Nanoshel Company, England. Theophylline with high-grade was kindly given from the State Company for Drugs Industry and Medical Appliances (N.D.I) Ninavah /Iraq. The components of phosphate and acetate buffer were purchased from Fluka Chemicals Company (Buchs, Switzerland), and used to study the influence of different pH. All chemicals were used with no further purification.

2.2. Preparation of poly TP-CuO NP modified electrode

Before alteration, the bare GCE was refined with 0.05 μm Al_2O_3 powder washed and ultrasonicated in water, then the electropolymerization of TP-CuO nanoparticle was performed in doping solution, which consists of 0.01 M TP solution, prepared freshly by dissolving 0.018 g TP in 10 ml distilled water [30]. About 0.0133 g CuO nanoparticles were dissolved in 1 ml of absolute ethanol, the solution was stirred with a magnetic stirrer at ambient temperature for

30 minutes; subsequently, the two solutions were mixed in a ratio of 1:1, by adding 1 ml of TP and 1 ml of CuO nanoparticles to the measuring cell that contained 10 ml of phosphate buffer solution. Then, electropolymerization was performed (Fig. 1) by cycling the potential between (-0.1 V and 1.1 V) at a scan rate of 0.8 Vs^{-1} for five cycles, using cyclic voltammetry (CV).

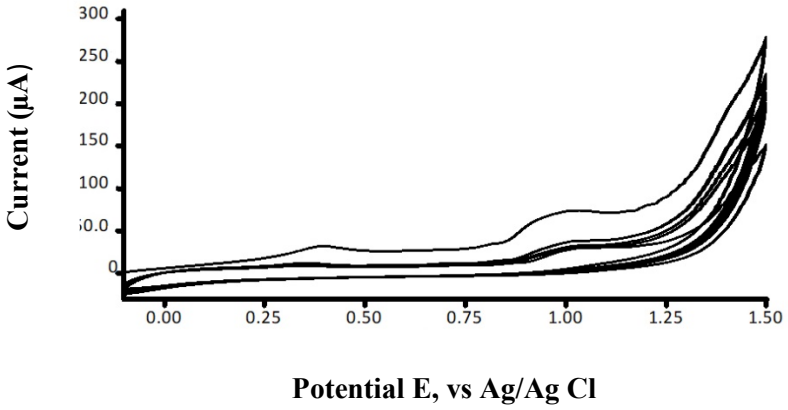


Fig. 1. Electrochemical polymerization of TP

3. RESULTS AND DISCUSSION

3.1. Direct Electrochemical Behavior of AG on GCE

Herein, AG has been assessed directly on GCE and indirectly on (GCE-poly TP/CuO NP), using SWV. AG SW voltammogram was recorded in phosphate buffer solution as a model for the neutral and basic buffer, and for the acidic model, acetate buffer was chosen. First, CV was performed to establish its profile on bare GCE. By using 0.1 M acetate buffer solution pH=4.5, the scan has been applied from 0.4 to 1.5 V for 449.5 µM AG, to avoid any interference of O_2 in the reaction; all experiments were carried out under N_2 gas. As shown in Fig. 2, the value of anodic currents has been assessed, and we can conclude that the oxidation process is irreversible as demonstrated in the voltammogram. Square wave voltammetry (SWV) was applied for 449.5 µM of AG, two peaks were observed one (Ep_1) at 0.77 V and the second peak (Ep_2) at 1.1V vs Ag/AgCl, in the presence of phosphate buffer (0.1 M, pH=7.0), as depicted in Fig. 3. AG is considered as one of the excellent nitrogen donor compounds [31], so its mechanism for the electrochemical oxidation process may be due to the oxidation of the amino group of guanidine, formation of hydroxylamine, so generating the azodicarbamidine. This may hydrolyze in the presence of hydrochloric acid giving rise to carbon dioxide, nitrogen, and hydrazodicarbamide [32].

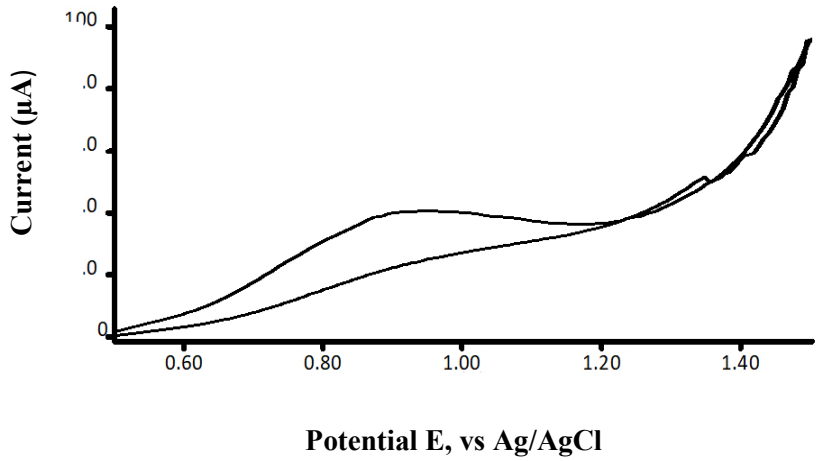


Fig. 2. Cyclic voltammogram of 9090 µM aminoguanidine in acetate buffer pH=4.5, deposition potential 1.4 V, deposition time 80 s, equilibrium time 5 s, sweep rate 0.05 v/s, voltage step 0.008 V.

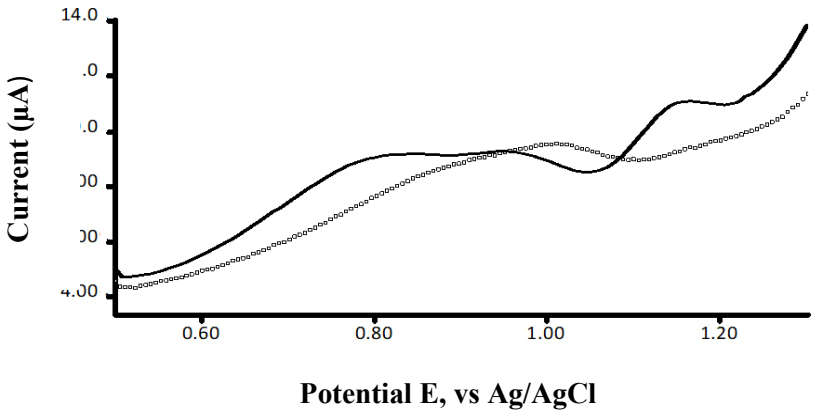


Fig. 3. The SW Voltammogram of 449.5 µM aminoguanidine in phosphate buffer pH=7 (drop line) at default condition (dash line) at optimum condition (voltage step of 0.008 V, the amplitude of 0.03 V, deposition potential of 0.5 V, deposition time of 65 s, equilibrium time of 5 s, and frequency of 50 Hz).

3.1.1. The Impact of pH

The impact of pH on the electrochemical behavior of 596.4 µM AG in 0.1 M acetate and phosphate buffer solutions was assessed, using acetate buffer at pH 3 to 5 and phosphate buffer with pH between 6 to 8 at the bare GCE, using SWV. As shown in Table 1, when the pH increased from 3 to 6, the peak potential moved towards less positive values, which was related to the contribution of proton in the electrooxidation process of AG. The AG anodic peak current reached a maximum value at pH 4.5 and then, decreased gradually with rising pH; even more,

a new peak was observed at 1.15 V in the pH range of 4.5–7.0. On the other hand, AG electrochemical behavior type at pH 7.0 and 8.0 was adsorption, as it is pH-independent.

Table 1. The effect of pH on the peak current of AG

pH	Ep_1 (V)	Ip_1 (µA)	Ep_2 (V)	Ip_2 (µA)
3.0	0.955	1.42		
3.5	0.844	4.5		
4.0	0.797	6.02		
4.5	0.773	6.47	1.15	0.569
5.0	0.749	5.77	1.12	0.764
6.0	0.677	3.56	1.21	2.39
7.0	0.714	2.15	1.14	5.68
8.0	0.944	3.15		

pH values of 4.5 and 7.0 were selected for the construction of calibration curves of AG in acetate and phosphate buffers, respectively.

3.1.2. The Effect of Time on the Stability of Peak Current of AG

The impact of time on the oxidation peak of AG was studied in the phosphate buffer solution (0.1 M, pH 7.0). All measurements were performed at ambient temperature (24±2) ºC, by the presence of 449.5 µM AG. The results demonstrated that the AG peak is time-independent and more stable for 60 min, as depicted in Table 2.

Table 2. The peak current of AG at different time points

Time (min)	Ip (µA)
0	3.59
5	3.56
10	3.62
15	3.62
20	3.7
25	3.70
30	3.51
35	3.69
40	3.68
45	3.57
50	3.57
55	3.70
60	3.71

3.1.3. The Effect of Concentration

After fixing the peak position and pH, the device conditions for SWV were ameliorated to voltage step of 0.008 V, the amplitude of 0.03 V, deposition potential of 0.5 V, deposition time of 65 s, equilibrium time of 5 s, and frequency of 50 Hz. Under these optimum conditions, the

peak current for different concentrations of AG was obtained, the results are presented for the calibration curve in Fig. 4 for both phosphate and acetate solution. The calibration plot obtained for AG determination in phosphate buffer solution, as a straight line over AG concentration, in the range of 299.1– 990 µM with R^2 of 0.992 and standard deviation (SD) of 33.5 µM. The linear regression equation is as follows: IP(A) = 0.0028 X + 1.6907, for phosphate buffer (0.1 M, pH 7). In the meantime, a calibration graph of AG in acetate buffer was represented in the same figure, in the range of 199.6–1080 µM, giving also a linear equation, which is Ip (µA) = 0.0112 X – 0.5809 with R^2 of 0.997 and SD of 12.09 µM. From the correlation coefficient values, we can say that the best calibration curve for AG at bare GCE is in the acetate solution.

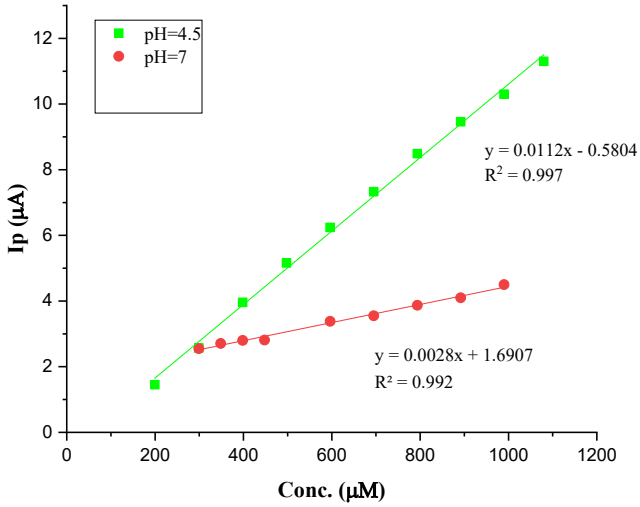


Fig. 4. The calibration curve of aminoguanidine in phosphate buffer pH=7 and acetate buffer pH=4.5

3.2. Indirect Electrochemical Profile of AG on GCE Modified with CuO NP and TP polymer (GCE-poly TP/CuO NPs)

After fixing the peak position of AG at bare GCE, several electrochemical experiments were carried out on the GCE, after covering with a layer of poly TP and CuO NPs (as depicted in the experimental part) to get a unique electrode, which is GCE-poly TP/CuO NPs. It is well known that the roles of conducting polymer, especially in the presence of nanoparticles, enhances the charge movement, by lowering the limit of detection (LOD) and increasing the linear range [33].

3.2.1. AG behavior on GCE Modified with CuO NP and TP polymer (GCE-poly TP/CuO NP)

After elaboration of the electrode with a layer of poly TP, containing CuO nanoparticles, SW voltammogram was recorded in the presence of phosphate buffer (0.1 M, pH 7), as depicted

in Fig. 5. A preliminary experiment showed that a distinct peak was obtained at 0.108 V with 9.42 µA, suggesting that this redox peak is attributed to the reduction of CuO-NP [34].

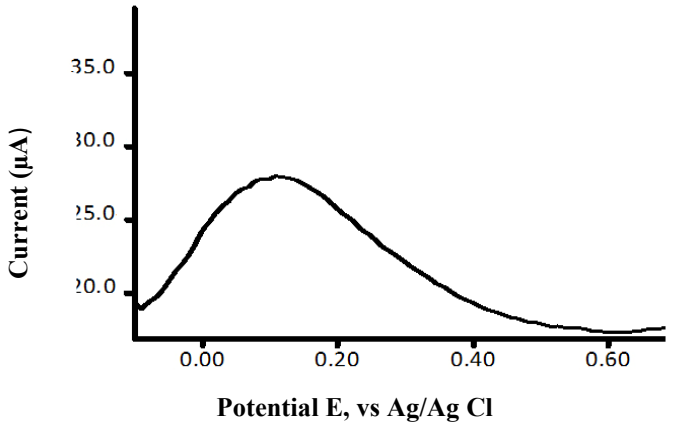
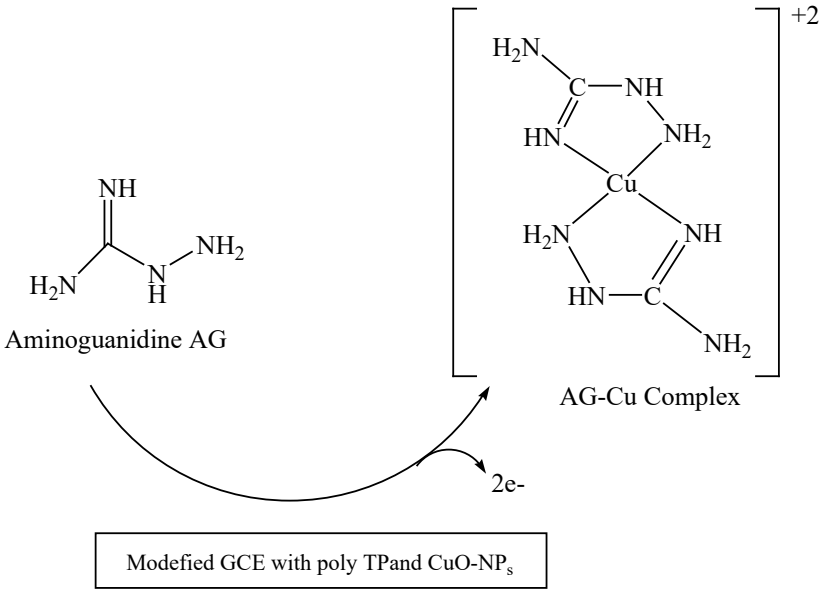


Fig. 5. The SWV of the electrode with a layer of poly TP, containing CuO nanoparticles in phosphate buffer pH=7, deposition potential=-1.3 v, equilibrium time=5 s, deposition time = 60 s, voltage step=0.01, amplitude=0.05v, frequency=80 Hz, and sweep rate=0.8 v/s.

After the addition of 449.5 µM AG, the CuO NPs peak started to decrease, which may be attributed to some interactions that may happen at the modified electrode surface, and the reason behind this phenomenon is the characteristics of AG that interacts with copper and may produce complex scheme 2, as it has a pair of the electron [35].



Scheme 2. Electro-oxidation mechanism of AG in aqueous solution at (GCE-poly TP/CuO NPs)

This interesting result urged us to direct this process to detect the AG in aqueous solutions. At the same time, a peak of AG was recorded at 0.7 V, and it can be noted that the AG peak is shifted to less oxidation potential, as an effect of the nano polymer surface.

Considering the above result, by CuO fixing the peak position, the device conditions for SWV were optimized to voltage step of 0.01 V, the amplitude of 0.05 V, deposition potential of -1.3 V, deposition time of 60 s, equilibrium time of 5 s, and frequency of 80 Hz. Due to this condition, the peak current of CuO-NP reached 9.42 µA, along with a better shape, compared to before. To construct the calibration curve, different conditions including pH, stability, and the scan rate were optimized as follows:

3.2.2. The Impact of pH

The impact of pH was studied, but here, the focus was on the CuO peak at 0.237V vs. Ag/AgCl. The same buffer with the range discussed in section 3.1.1 was studied with GCE-poly TP/CuO NP. The plot of E(v) versus pH indicates linearity in the pH range of 3 to 8, the peak potential of Cu at GCE shifted to less positive (Fig. 6), which is associated with the donation of proton in the electrooxidation process, E(v)= -0.048 pH+0.4861(R^2=0.979).

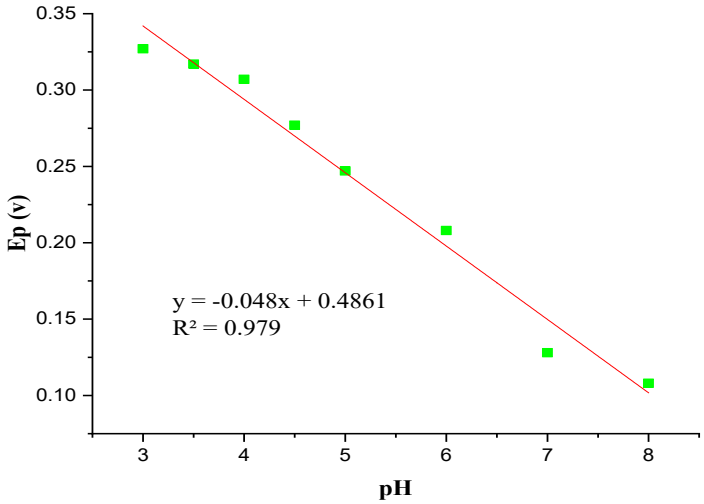


Fig. 6. The effect of pH on the CuO peak on the modified electrode, acetate buffer pH 3–5, phosphate buffer pH 6-8 at optimum condition, deposition potential=-1.3v, deposition time =60 s, equilibrium time=5 s, voltage step=0.01, amplitude=0.05 v, frequency=80 Hz, and sweep rate=0.8 v/s.

3.2.3. The Impact of Time

The impact of time on the voltammetric behavior of CuO NP peak was studied in phosphate buffer solution (0.1 M, pH 7.0). All measurements were performed at ambient temperature 24±2 ºC. The peak current was stable for more than two weeks, from these preliminary results; the fabricated electrode can be considered a perfect sensor for an AG drug.

3.2.4. The Effect of Concentration

Under these optimum conditions, the depletion in the peak current of CuO was measured after different concentrations of AG were indirectly assessed. The results are presented for the calibration curve in Fig. 7, for both acetate and phosphate buffer The plots of concentration vs. Ip gives two straight lines, one in using phosphate buffer at concentration range (9.9 –196) µM with R^2= 0.991 and second at concentration range (196-610.3) µM with R^2=0.994 whereas, in case of Ac. buffer, the plot of concentration vs Ip gives one limited straight line at concentration range (19.96–338.16) µM with R^2=0.990.

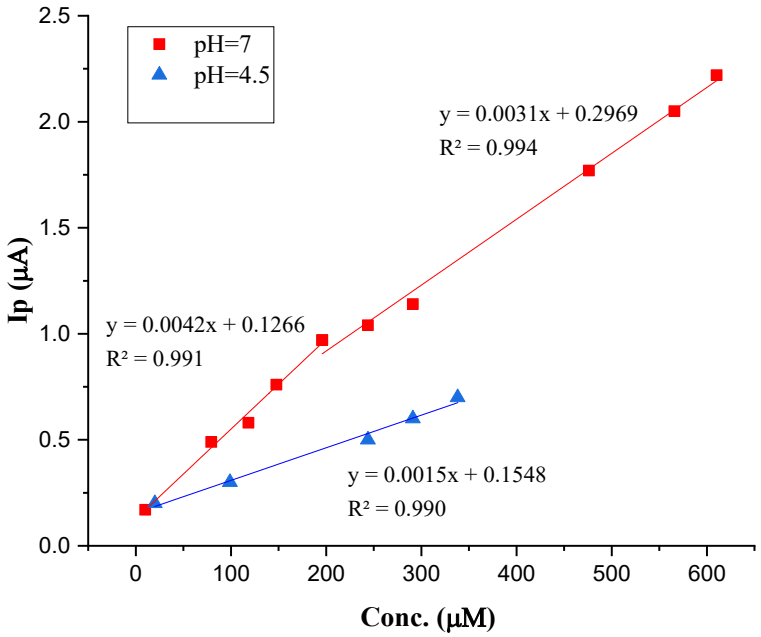


Fig. 7. The calibration curve of aminoguanidine in phosphate buffer pH=7 and acetate buffer pH=4.5 at GCE-poly TP/CuO NPs

Table 3. Comparison of the linear range and the detection limit between the proposed method with other reported analytical methods.

Methods	**Linear range (µM)**	**Limit of detection (µM)**	**References**
Spectrophotometer	1.3–13.5	Not reported	[9]
HPLC with UV-visible detector	0.2–10.1	0.13	[11]
Ion chromatography with a conductivity detector	13–337	4.1	[7]
SWV using modified electrode	9.9–610	6.34	Present work

Several methods are introduced in the ICH guideline to measure the LOD for AG in phosphate buffer (0.1 M, pH 7) at GCE-poly TP/CuO NPs. LOD was computed from the equations of LOD = 3.3 s/m [36,37], using the SD of response(s) and calibration curve. The LOD was equal to 6.34 μM for the designed biosensor.

A comparison of the present work with other analytical methods (Table 3) indicates that the suggested method has a better and wider linear concentration range with acceptable detection of limit.

4. CONCLUSION

AG gives a major and broad oxidation peak on GCE at (0.773) V vs. Ag/AgCl in acidic media and at (0.8) V in neutral one, due to the formation of hydroxylamine (-NHOH), so the novelty of this work involved the indirect determination of AG using electrode modified by CuO-NPs, and theophylline conducting polymer. The purpose method depends on the Cu (II) peak at (0.108) V, the addition of AG caused a decrease in reduction peak current, this may be due to the formation of the Cu-AG complex. The modification of GCE led to an increase in the sensitivity of electrode response towards AG as the electrode surface increased, owing to the existence of CuO NPs; also, the oxidation current increased about three folds, and the best LOD and correlation value were obtained with GCE-poly TP/CuO NPs

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Declarations of interest

The authors declare no conflict of interest in this reported work

REFERENCES

[1] R. Usha, S. M. Jaimohan, A. Rajaram, and A. B. Mandal, Int. J. Biol. Macromol. 47 (2010) 402.

[2] A. A. Booth, R. G. Khalifah, P. Todd, and B. G. Hudson, J. Bio.Chem.272 (1997) 5430.

[3] P. H. Yu, and M. Zuo, Diabetologia 40 (1997) 1243.

[4] R. G. Tilton, T. Kawamura, K. C. Chang, Y. Ido, R. J. Bjercke, C. C. Stephan, and J. R. Williamson, J. Clin. Invest. 99 (1997) 2192.

[5] R. G. Tilton, K. Chang, K. S. Hasan, S. R. Smith, J. M. Petrash, T. P. Misko, and J. R. Williamson, Diabetes 42 (1993) 221.

[6] M. Tian, C. Qing, Y. Niu, J. Dong, X.Cao, F. Song, and S. Lu, AMS. 12 (2016) 179.

[7] V. L. Maddala, P. C. R. Kameswararao Chimalakonda, S. V. S. Koduri, and R. Y. K. Kumar, J. Advan. Chem.11 (2015) 3440.

[8] Y. L. Li, R. Q. Huang, C. H. Luo, W. C. Yang, and X. D. Chinese J. Pharma. 35 (2004) 37.
[9] M. A. Beaven, J. W. Gordon, S. Jacobsen, and W. B. Severs, J. Pharmacol. Exp. Ther.165 (1996) 14.
[10] P. C. Ulrich, and Z.M. Look, Aminoguanidine assay and applications thereof, U.S. Patent No. 5,108,930. Washington, DC: U.S. Patent and Trademark Office (1992).
[11] B. K. Srivastava, D.K. Kushwah, P.Y. Kolhe, and N. Patel. Res J Pharm Technol. (2010) 1058.
[12] M. P. G. da Silva, Y. M. de Oliveira, A. C. L. Candido, J. X. de Araújo-Júnior, É. E. da Silva Rodrigues, K. L. C. Monteiro, and F. C. de Abreu, J. Biomat. Nanobiotech. 11 (2019) 33.
[13] M. M. Lalovic, Lj. S. Vojinovic-Ješic, Lj. S. Jovanovic, V.M. Leovac, V.I. Cešljevic, V. Divjakovic, Inorg. Chim. Acta 388 (2012) 157.
[14] J. Xiang, Q-Q Su, A. L. Luo, and T.C. Lue, Dalton Trans. (2013) 1.
[15] L.S. Vojinovic´ -Ješic´, M.M. Radanovic´, M.V. Rodic, L.S. Jovanovic´, V. I. C˘ešljevic´, M. D. Joksovic, Polyheydron 80 (2014) 90.
[16] S. A. Bhat, M.A. Rather, S.A. Pandit, P.P. Ingole, and M.A. Bhat, J. Electroanal. Chem. 783 (2016) 280.
[17] D. Lan, and L. Zhang, J. Electroanal. Chem. 757 (2015) 107.
[18] C. Y. Yang, Y. Cao, P. Smith, and A. J. Heeger, Synth. Met., 53 (1993) 293.
[19] B. Šljukić, C. E. Banks, A. Crossley, and R. G. Compton, Anal. Chim. Acta 587 (2007) 240.
[20] J. G. Huddleston, A. E. Visser, W. M. Reichert, H. D. Willauer, G. A. Broker, and R. D. Rogers, Green Chem. 3 (2001) 156.
[21] M. Miraki, H. Karimi-Maleh, M. A. Taher, S. Cheraghi, F. Karimi, S. Agarwal, and V.K. Gupta, J. Mol. Liq. 278 (2019) 672.
[22] M. A. Sheikh-Mohseni, and S. Pirsa, Anal. Bioanal. Electrochem. 8 (2016) 777.
[23] A. M. T. Allayla, R. A. Faris, and Z. F. Mahdi. Vibrational Spectroscopy 114 (2021) 103252.
[24] R. H. Al-Hyali and A. T. Al-Taee. Test Eng. Manag. (2020) 2898.
[25] A. Safavi, N. Maleki, O. Moradlou and F. Tajabadi, Anal. Biochem, 359 (2006) 224.
[26] W. Sun, R. Gao, and K. Jiao. J. Phys. Chem B. 111 (2007) 4560.
[27] Q. Wang, Y. Yun, and J. Zheng, Microchim. Acta 167 (2009) 153.
[28] Y. Lin, F. Lu, Y. Tu, and Z. Ren, Nano lett. 4 (2004) 191.
[29] H. Karimi-Maleh, M. Sheikhshoaie, I. Sheikhshoaie, M. Ranjbar, J. Alizadeh, N. W. Maxakatob, and A. Abbaspourrad, New J. Chem. 43 (2019) 2362.
[30] A. M. Al-Hasany, A. T. Al-Taee, and H. A. Al-Wahn, The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM) 7 (2019) 204.

[31] S. Herres-Pawlis, A. Neuba, O. Seewald, T. Seshadri, H. Egold, U. Flo¨rke and G. Henkel, Eur. J. Org. Chem. 22 (2005) 4879.

[32] E. Lieber and G.B.L. Smith, Chem. Rev. 25 (2002) 213.

[33] W. Smirnov, A. Kriele, N. Yang, C.E. Nebel, Diamond Relat. Mater. 19 (2010) 186

[34] G. Zampardi, J. Thöming, H. Naatz, H. M. A. Amin, S. Pokhrel, L. Mädler, and R. G. Compton, Small. 14 (2018) 1801765.

[35] K. Nosratzadegan, M. Mahdavi, K. Ghani, and K. Barati, Propellants, Explosives, Pyrotechnics 44 (2019) 830.

[36] C. M. Riley, T. W. Rosanske, Development and Validation of Analytical Methods, Elsevier: Amsterdam, The Netherlands (1996).

[37] M. E. Swartz, I. S. Krull, Analytical Development and Validation. Marcel Dekker Inc.: New York (1997).