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Amperometric Sensing of Hydrogen Peroxide Using Graphene Oxide-Alizarin Modified Glassy Carbon Electrode

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A sensitive and selective amperometric sensor for hydrogen peroxide was constructed by mixing graphene oxide (GO) and alizarin red (Ali) at certain amounts, and dropping it onto a glassy carbon electrode (GC). The freshly prepared electrode (Ali-GO/GC) was activated by voltage scans at the range of -0.6 V and 0.9 V for ten cycles. Activated Ali-GO/GC has possessed a reversible redox activity in both anodic and cathodic directions. Ali-GO/GC exhibits a suitable electrocatalytic activity against hydrogen peroxide towards the anodic redox region with a linear range from 3.6 μM to 513 μM of H_2O_2 and a detection limit of 1.1 μM. Ali-GO/GC displays selectivity, relatively high sensitivity, and reproducibility at the amperometric analysis of hydrogen peroxide.

Keywords: Hydrogen peroxide, Alizarin, Graphene oxide; Glassy carbon electrode.

1. INTRODUCTION

Hydrogen peroxide is an oxidation product of oxidase type enzymes. Hydrogen peroxide which forms as a enzymatic product gives a current signal by making an $2e^-$ transfer reaction upon the electrode. Although the electrochemical reaction of hydrogen peroxide varies according to temperature, the type of the electrode and ions in the solution its most basic form is as in Equation 1 [1-5].

$$H_2O_2 \xrightleftharpoons{\text{electrode}} O_2 + 2e^- + 2H^+ \qquad (1)$$

In order to prepare the enzyme electrodes for the accurate determination of hydrogen peroxide catalytic noble metals such as Pt and Au; metal oxides such as ZrO_2 and ZnO have been employed [6-9]. The biosensor studies using dyestuffs such as prussian blue [10], pyrocatechol red [11] and congo red [12] as the mediator have been drawing great interest in recent years. These materials are very popular not only for their cost effectiveness and availability but also their ability of improving electrode's stability, reproducibility, working range and selectivity. Alizarin Red (Ali) is an electroactive hydroquinone derivative. Due to its electrochemical activity it is used as the mediator in order to facilitate the electron transfer mechanism on the electrode surface for the determination of many biologically significant molecules [13]. Ali owes its redox capacity to the hydroxyl and quinone functional groups it has in its structure. That is why it is one of the very few compounds which gives cathodic and anodic reversible peaks in both anodic and cathodic directions. The reduction and oxidation mechanisms of alizarin red were found to be strongly dependent on the pH of the medium. This situation is presented in Figure 1. Electrochemical sensors are the most widely used in enzyme electrode [14-17]. Amperometric biosensors are more attractive due to their low detection limit as enzyme stabilization can easily be achieved [18, 19].

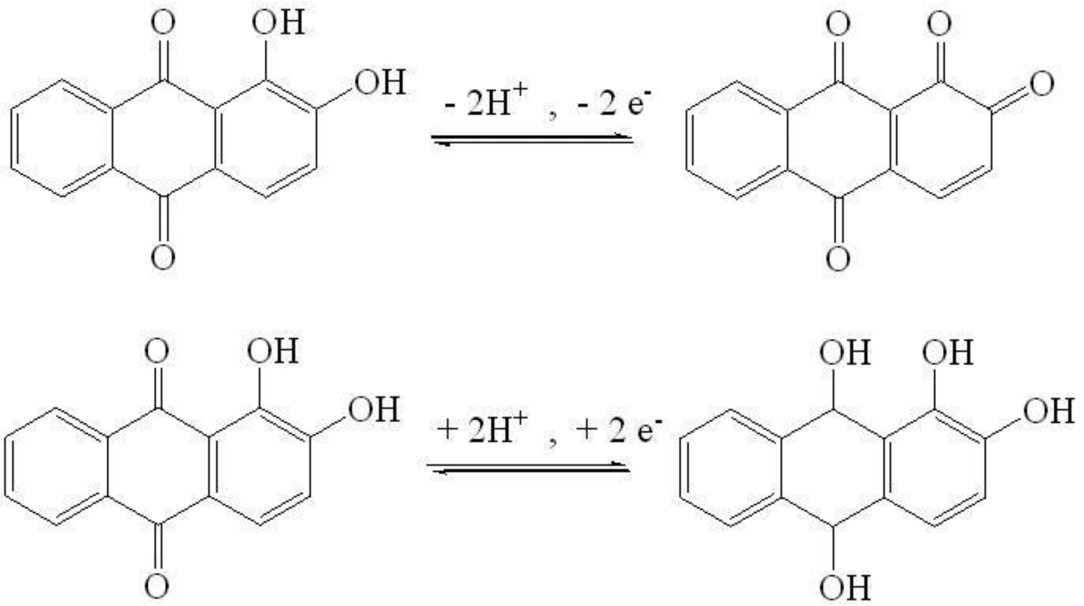

Figure 1. The oxidation and reduction equilibria of alizarin red

Alizarin red S (ARS) is a water soluble sulpho derivative of alizarin red. Tedoradze et al [20] reported that ARS is reduced with a $2e^-$ transfer mechanism at pH < 2.8 and a $4e^-$ transfer mechanism at pH > 2.8 . Dai et al 1 [21] found that ARS is well adsorbed on pre-anodized glassy carbon electrode and the reversible peaks disappeared when the pH is brought to pH 5 from the more acidic values. These features make Ali and ARS very attractive for electrochemists. Apart from that, the chelating ability of Ali makes it very suitable for the adsorption and the stripping of metals ions such as aluminum [22, 23].

Graphene is a material with an extremely high surface area such as 2630 m^2/g 1 [1]. Due to its excellent electrical conductivity and very large surface area graphene has become a very popular modifier for the electrochemical analyses [24, 25]. The electrochemical analyses of DNA bases with the graphene mobilized electrode with electrochemically deposited Ali have been reported [26, 27] carried out the analyses of four bases at sub μM levels of concentration without any interference.

In this work, alizarin-graphene oxide (Ali-GO) mixture freshly prepared in water was dropped on a glassy carbon (GC) electrode, and it was activated by a potential scan at pH 2. The activated electrode was observed to give good response to hydrogen peroxide. This study deals with the performance of the activated electrode in the amperometric determination of hydrogen peroxide.

2. EXPERIMENTAL

2.1. Reagents:

Synthetic flake graphite was purchased from Sigma-Aldrich. All solutions were prepared by deionized water supplied from Millipore Simplicity 185. Also, graphene was washed and freshly prepared with deionized water. The supporting electrolytes were 1 M KNO_3 at pH 2.0 (adjusted by adding 1.0 M HCl) and 0.10 M phosphate buffer solution (PBS) at pH 7.0 . PBS was prepared by using Na_2HPO_4 and NaH_2PO_4 reagents purchased from Sigma-Aldrich.

2.2. Preparation of Ali-GO coated GC electrode:

GO was prepared by Hummers method [28] by treating graphite with strong oxidants as $KMnO_4$ and H_2SO_4. After purification, the solid GO was dried at 60 ^oC in vacuum oven. Solid GO was transferred into a beaker and added a certain amount DMF to obtain a suspension at 1 mg GO / 1 mL solvent. 50 mL of GO suspension in DMF was homogenized in sonic bath for 2 hours, and then 25.0 mg of alizarin red was added into the homogenized suspension with through stirring. Ali containing GO (Ali-GO) suspension in DMF was sonicated again for 2 hours.

Ali-GO was centrifuged at 5300 rpm, and the precipitate of Ali-GO was carefully separated. Then deinozed water was added onto the solid Ali-GO to obtain same volume of suspension in water. Finally, Ali-GO was dispersed in water in such a way that the concentration of suspension was 1 mg GO per 1 mL water. Ali-GO mixture in water was sonicated for 5 min, and 5 μL of Ali-GO dispersion freshly prepared in water was dropped on GC electrode, and modified GC electrode was dried at room temperature. (Caution: Further sonication of Ali-GO in water may cause the aggregation, and Ali-GO must be freshly prepared in water).

2.3. Apparatus:

All electrochemical experiments were performed using CHI 660B potentiostat and a three-electrode cell with a Ag/AgCl (3 M NaCl) reference electrode and a Pt wire as auxiliary electrode. The working electrode was Ali-GO modified GC electrode (Ali-GO/GC) with a radius of 3 mm. 10 mL of support solutions of 1.0 M KNO_3 at pH 2 and PBS buffer at pH 7.0 were separately placed into the voltammetric cell and purged with argon for 5 min.

2.4. Activation of Ali-GO/GC electrode:

Ali-GO/GC was dipped into the support solution of pH 2.0, and potential was scanned linearly at the potential range between -0.6 V and 0.9 V for 10 cycles. After 10 cycles, the electrode response was almost stable. The voltammograms were overlaid and presented in the section of results and discussion.

3. RESULT AND DISCUSSION

3.1. Characterization of Ali-GO:

GO was prepared by the chemical oxidation of graphite by the use of very popular method developed by Hummers. The preparation method of Ali-GO mixture was described above in detail. The AFM result of the sample of Ali-GO taken on the silicon wafer cleaned with Piranha solution was presented in Figure 2. It was observed that the mixture of Ali-GO had a significant height due to penetration of Ali into the layer of GO. This proves that Ali is mixed well with the GO due to abundant amount of functional groups it contains.

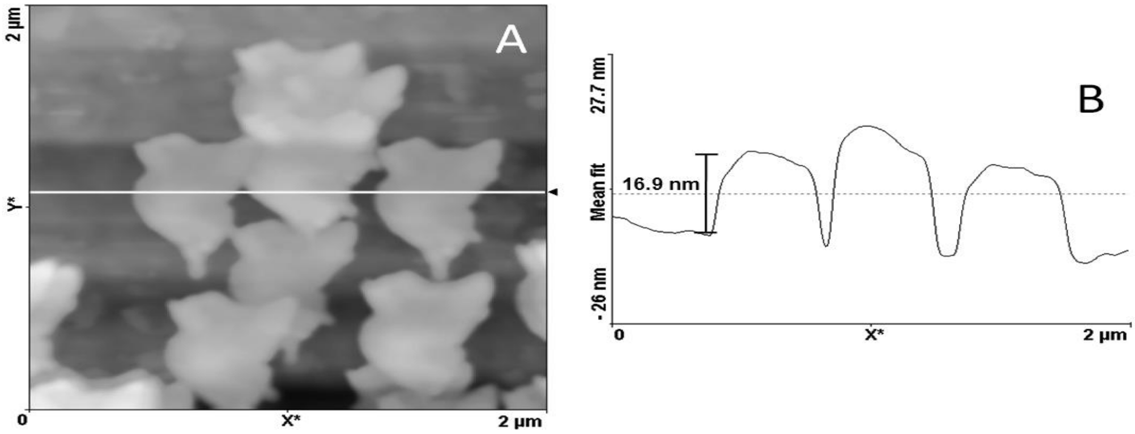

Figure 2. AFM images of Ali-GO with their height profiles

Figure 3 shows the scanning electron micrographs (SEM) Ali-GO/GC and GO/GC which show that Ali-GO is homogeneously mixed and dispersed on the electrode surface.

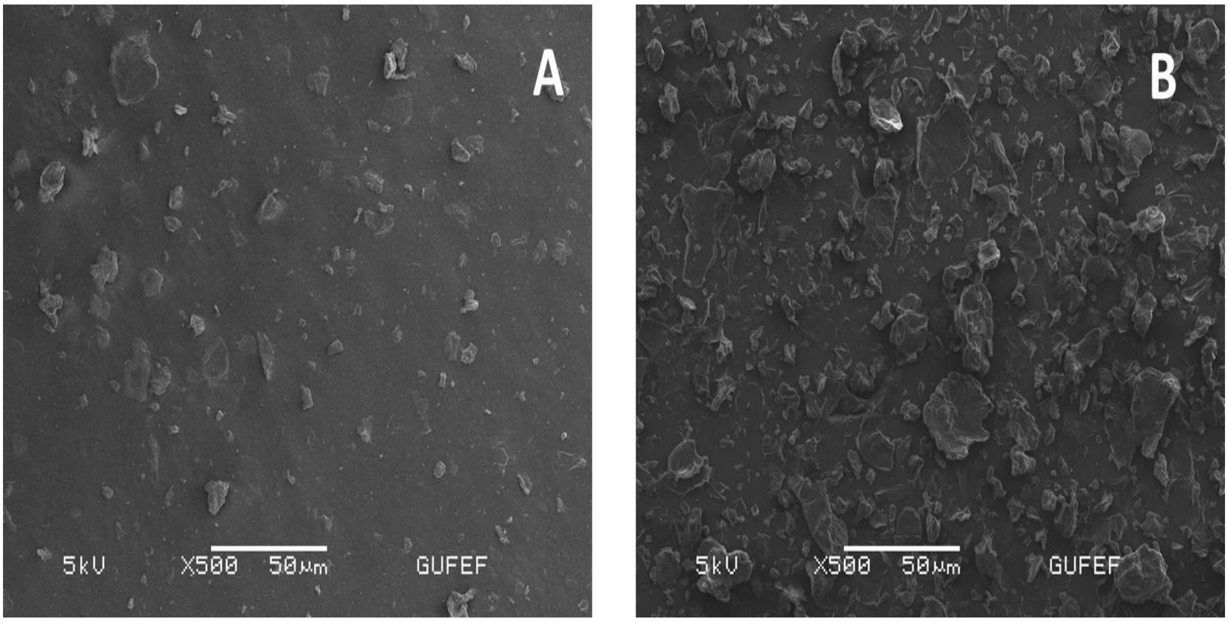

Figure 3. Scanning electron micrographs of GO (A), and Ali-GO (B) on GC

3.2. Electrochemical behavior of Ali-GO/GC in pH 2:

Ali has both quinone and hydroxyl functional groups in its structure. These groups with increasing the redox capacity and chelating ability of Ali make it a perfect material for the specific adsorption of heavy metals [21-23] found that ARS is strongly adsorbed upon the surface of pre-anodized glassy carbon electrode and gives two reversible peaks in both anodic and cathodic directions in acidic media. However if the electrode is over-oxidized by scanning up to 1.0 V at pH 5 the peaks belonging to ARS are observed to disappear. The Ali-GO coverage on GC was subjected to 10 successive scans starting form 0 V to between 0.9 V and -0.6 V at pH 2. The resulting scans were overlaid and presented in Figure 4. The voltammograms in Figure 4 are in good compliance with the results obtained to elucidate the electrochemistry of Ali. The anodic peaks obtained for Ali-GO/GC differs from those obtained for adsorbed Ali [21] as being the equal. This shows that there is a significant improvement in redox features of Ali homogeneously distributed in GO with its adsorption properties. The functional groups which facilitate the adsorption of Ali upon the pre-oxidized GC electrode changes –OH → –C=O during the oxidation and –C=O → –OH during the reverse scan. This change naturally affects the adsorption of Ali on pre-oxidized GC surface, but there was no negative influence on the adsorption of Ali on to Ali-GO. Another interesting observation was the fact that the oxygenated functional groups of GO do not adversely affect the redox capacity of Ali.

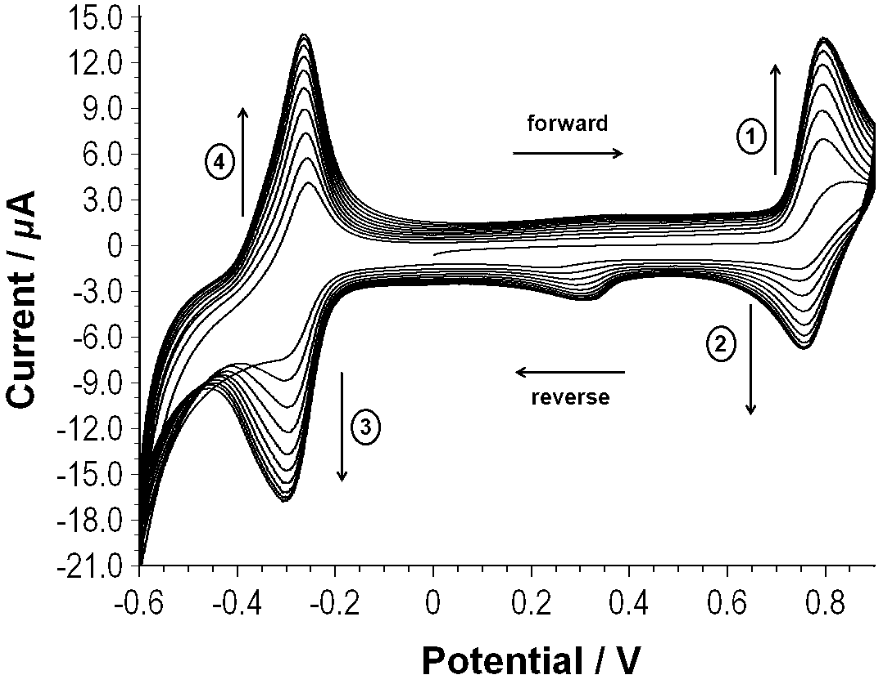

Figure 4. The electrochemical behavior and activation of Ali-GO/GC through cyclic voltammetry obtained with the scan rate of 50 mV/s and ten cycles in 1.0 M KNO_3 at pH 2.

The peak (1) in Figure 4 which appears at 0.797 V corresponds to oxidation of Ali, and its reverse peak (2) occurs at 0.756 V. The peak (3) which appears at -0.302 V during the cathodic scan corresponds to reduction of Ali which gives a corresponding reversible peak (4) at -0.266 V. After the successive scans the increasing electrocatalytic activity results a broad peak around 0.30 V is due to the redox active groups which appear as formation of oxygen-containing quinone-like functionalities upon the GC electrode after the activation process [29]. The difference between the forward and reverse peaks (ΔE_p) in the anodic sweep is 41 mV. The corresponding difference between the forward and the reverse peaks in the cathodic direction is 36 mV. For a totally reversible systems is $\Delta E_p = 58/n$ mV at 25 °C. Therefore the transferred electron number (n) for both the reversible peaks at the anodic direction is about 1.5 which can be approximated to 2.

After the activation process the Ali-GO/GC electrode was found to possess a very good redox activity with a reversible behavior in both anodic and cathodic directions. Figure 5 displays the over imposed forms of the voltammograms which show a reversible behavior around 0.7-0.8 V at pH 2 with Ali-GO/GC electrode at different scan rates. The plot of I_p vs. $v^{1/2}$ shows a linear change up to scan rates as high as 0.5 V/s. At higher scan rates the linearity is distorted as shown in the inset in Figure 5 due to the insufficient diffusion rate of H^+ ions [30-32].

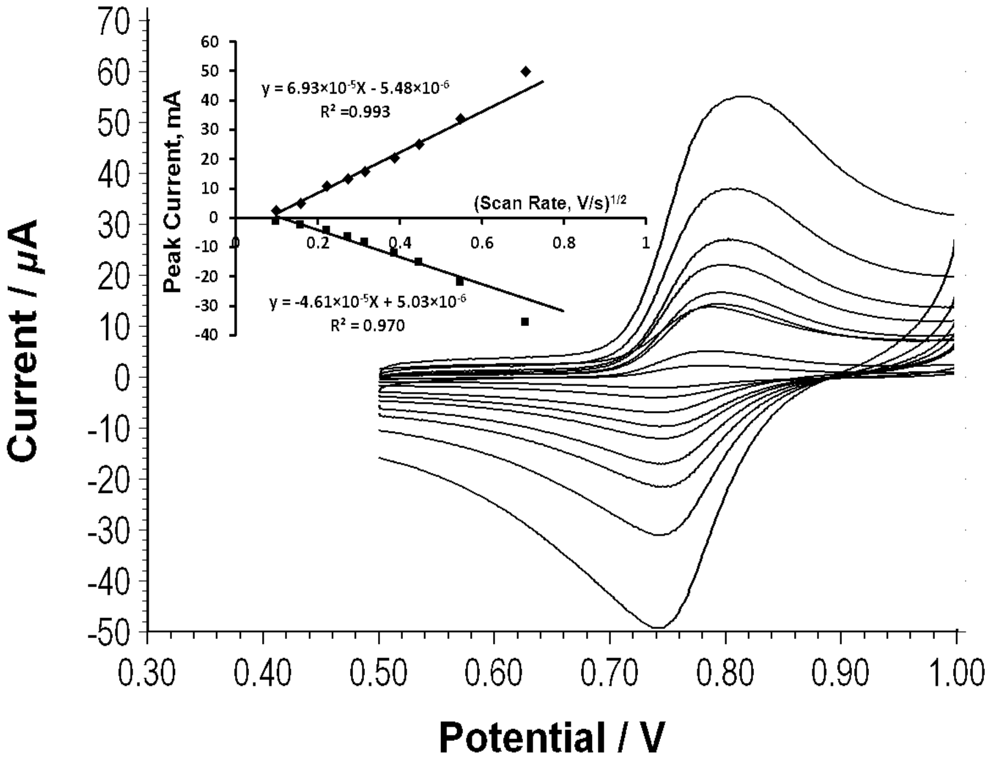

Figure 5. The change of anodic reversible peaks of Ali-GO/GC obtained in 1.0 M KNO_3 at pH 2 with various scan rate.

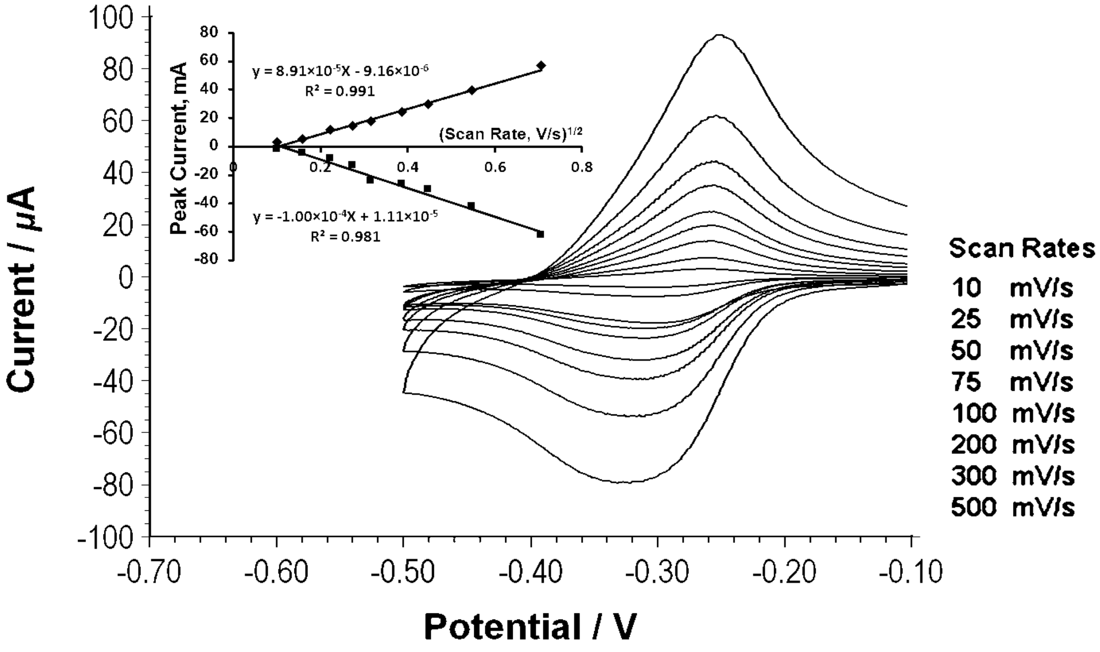

Figure 6. The change of cathodic reversible peaks of Ali-GO/GC obtained in 1.0 M KNO_3 at pH 2 with various scan rate.

Figure 6 shows the corresponding change for the cathodic peaks. The backwards peak and the Nernstian reversible process appears to be broader than the anodic case. ΔE_p was 43 mV which gave the *n* value of 1.4 for the cathodic case which was in close approximation of the anodic case. The discrepancy between the theoretical value of 2 and experimental value of 1.4 is resulted from high solution resistance. This behavior can be attributed to the fact that Ali between the GO layers with much inferior electrical conductivity cannot respond to the electron transfer as fast as it should.

3.3. Electrochemical behavior of hydrogen peroxide in PBS pH 7:

It is well known that the electrochemical activation of carbon electrodes produces quinone-like molecular goups on the electrode surface, which increase the electrochemical performance of the surface against analyte(atıf). Ali modification of the GC electrode decorated the electrode surface with the electroactive carboxylic groups that has an activity in both the anodic and cathodic regions. Ali-GO/GC was shown to give both anodic and cathodic reversible peaks during the voltammetric scans at pH 2. The catalytic response of the Ali-GO/GC electrode activated at pH 2 was investigated towards hydrogen peroxide at pH 7 PBS buffer. The catalytic responses of the bare GC and Ali-GO/GC against different concentration of hydrogen peroxide were recorded. The catalytic efficiency of Ali-GO upon the electrochemical oxidation of hydrogen peroxide was clearly demonstrated in Figure 7. Ali-GO showed a similar current response against the oxidation of hydrogen peroxide in tenfold lower concentration compared to the GC electrode. This shows that the Ali-GO/GC can enable us to carry out the determination of hydrogen peroxide at lower concentrations.

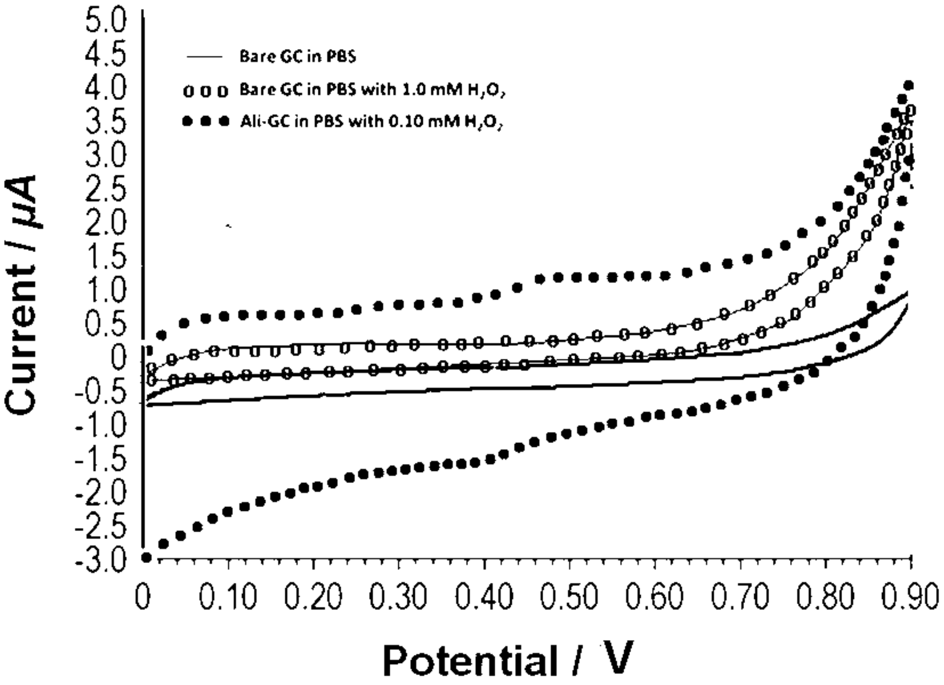

Figure 7. The voltammetric response of bare GC in PBS buffer pH 7 (—), in 1.0 mM hydrogen peroxide at PBS buffer pH 7 (o o o), and Ali-GO/GC in 0.1 mM hydrogen peroxide in PBS buffer pH 7 (•• •).

Figure 8 shows the increase in the oxidation current with the increasing hydrogen peroxide concentration. There is an apparent increase starting from 0.7 V with the increasing hydrogen peroxide concentration in Figure 8. The fact that this increase takes place at the same potential with the anodic peak of Ali-GO/GC in pH 2 shown in Figure 5 clearly proves the catalytic effectiveness of Ali upon the electrochemical behavior of hydrogen peroxide. Although the increase in the current response corresponding the increasing hydrogen peroxide concentration does not have a peak formation, the change of the baseline subtracted currents measured at 0.9 V show a linear increase with the hydrogen peroxide concentration. This shows that the sensitive determination of hydrogen peroxide is possible on Ali-GO/GC.

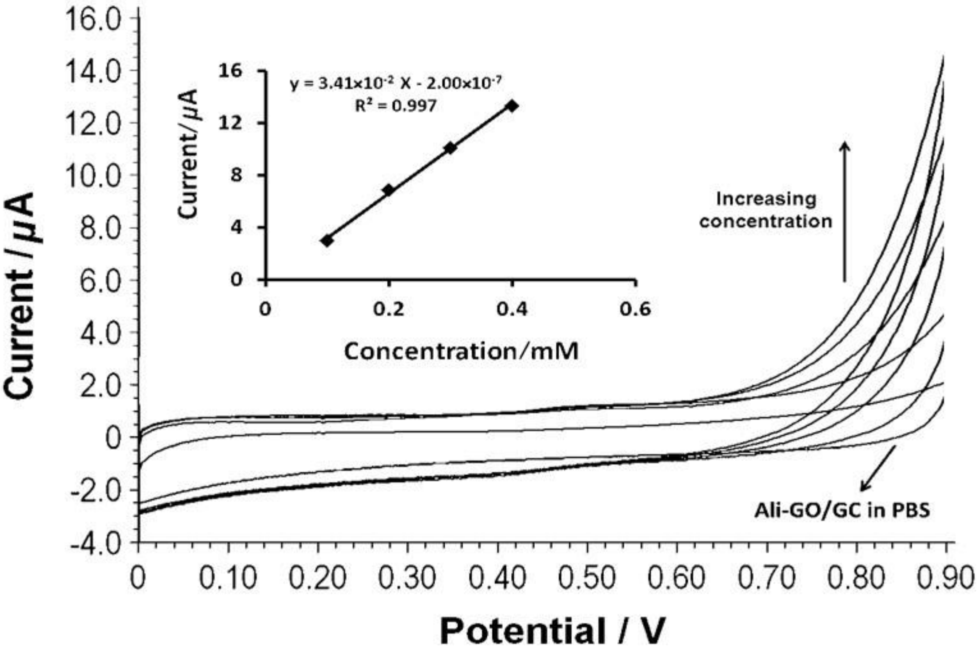

Figure 8. The cyclic voltammograms obtained at various hydrogen peroxide concentrations (0.1 mM → 0.4 mM). Inset: The change of current (measured at 0.9 V) with the concentration.

Following all these observations the amperometric detection was carried out by measuring the change in the current values obtained with Ali-GO/GC in PBS buffer at pH 7 at the peak appeared at 0.9 V with the increasing hydrogen peroxide concentrations. The solution was mildly stirred throughout the measurement. Figure 10 shows the concentration-current graph obtained from Figure 9. The results obtained at very low hydrogen peroxide concentrations were given as inset both in Figure 9 and Figure 10.

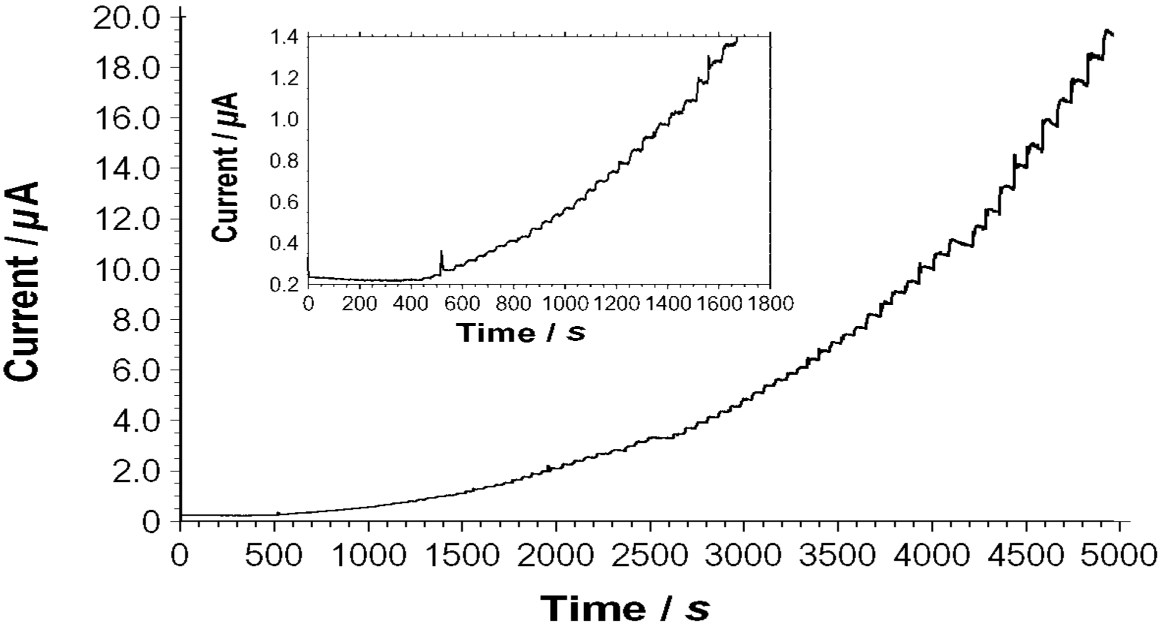

Figure 9. Amperometric response of Ali-GO/GC in PBS buffer pH 7 at the constant voltage of 0.9 V. (Inset: Current responses at lower concentrations of H_2O_2)

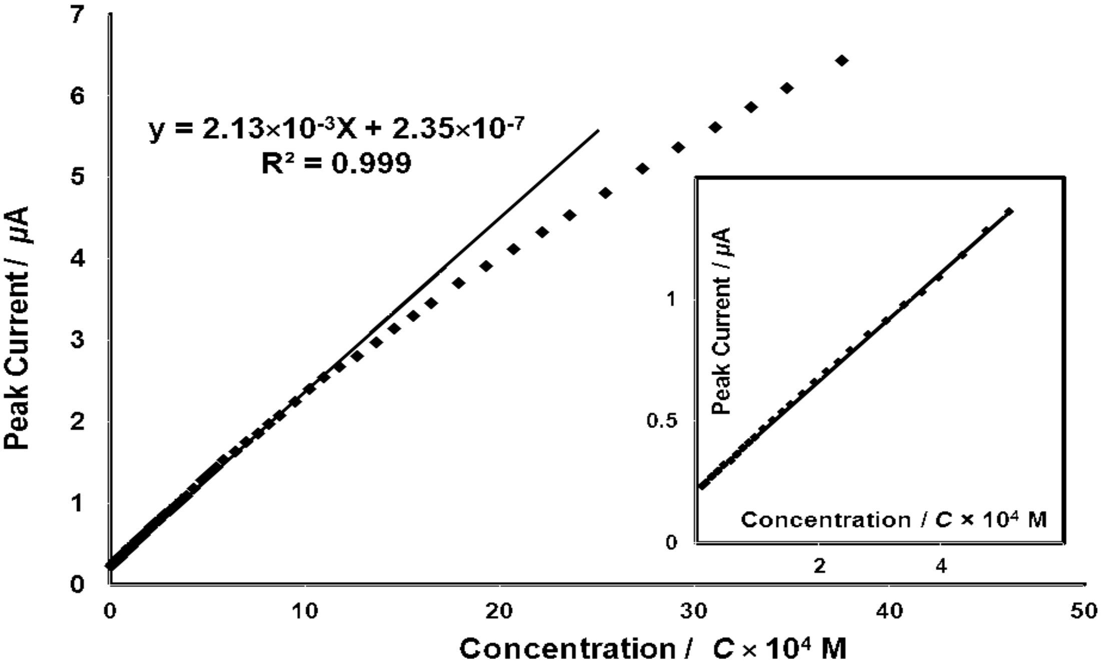

Figure 10. The change of current with hydrogen peroxide concentration obtained by amperometry shown in Figure 9 (Inset: Plot at linear change of current response against lower concentration of H_2O_2)

The linear range in Figure 10 was observed between 3.6×10^{-6} M and 5.13×10^{-4} M of H_2O_2 (I=$2.13\times10^{-3}\,C + 2.35\times10^{-7}$). The sensitivity was found to be 2.13 μA/mM from the plot in Figure 10.

The detection limit was calculated as 1.1 μM taking into account the signal/noise ratio was 3. The selectivity of Ali-GO/GC was determined by the addition of ten folds more uric and ascorbic acid in to the medium than the hydrogen peroxide. No obvious current change was detected. It was concluded that Ali-GO/GC possesses high selectivity and sensitivity towards hydrogen peroxide and can conveniently be used as a sensor in its detection at low concentration through the literature survey result presented in Table. 1. Here, the higher performance of Ali-GO/GC compared to pyrocatechol violet modified electrode was attributed to the improvement of electron transfer and catalytic efficiency due to the addition of graphene oxide although they have similar functional groups,

Table 1. Comparison of analytical performance of Ali-GO/GC for the electrochemical determination of hydrogen peroxide.

Sensor surfaces	**Linear range, μM**	**LOD, μM**	**Reference**
PCV/CCE	7.8-160	4.0	33
AP-Ni-MOF/CPE[1]	4–6.0×10^4	0.9	34
[PB/WV–Pt@Pd]$_6$ [2]	0.40-2.7×10^4	0.10	35
GR-MWCNTs	5–145	0.83	36
Cu_2O/PANI/rGO	0.8-12.8×10^4	0.5	37
Ali-GO/GC	3.6-513	1.1	In this work

[1] Pyrocatechol violet (PCV) modified carbon ceramic electrode
[2] Nickel-based MOF in carbon paste
[3] Pt@Pd alloy and Prussian blue NPs decorated heteropolyanion $PW_9V_3O_{40}^{3-}$ on ITO electrode
[4] Flower-like MoS_2 nanostructure on graphene and carbon nanotubes
[5] Cuprous oxide/polyaniline/reduced graphene oxide

3.4. Reproducibility and stability:

The reproducibility of Ali-GO/GC electrode was determined to be 5.1% by taking the RSD value of the current values taken at the same calibration range with the independently prepared Ali-GO/GC electrodes. There was not a significant change in the hydrogen peroxide detection concentration of the Ali-GO/GC electrode which had been kept in the pH 7 PBS for a week at room temperature.

4. CONCLUSIONS

The electrode of Ali-GO/GC with redox capacity of both the anodic and cathodic regions was produced by the activation of the mixture of the Alizarin red and graphene oxide. The anodic scans showed that the electrode had a linear response in the anodic direction against the increase of the hydrogen peroxide concentrations. It was concluded that Ali-GO/GC electrode is very suitable and stabile electrode in the detection of hydrogen peroxide with its large range of linear response, high reproducibility and good selectivity.

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References

1. S.B. Hall, E.A. Khudaish, A.L. Hart, *Electrochim Acta*, 45 (2000) 3573.
2. S. Cete, Ö. Bal, film *Artificial cells., nanomedicine, and biotechnology*, 41(6) (2013) 414.
3. S. Cete, A. Yaşar, F. Arslan, film' *Artificial cells, blood substitutes, and biotechnology*., 35(6) (2007) 607.
4. Ş. Kalaycı, G. Somer, G. Ekmekçi, *Talanta*, 65 (2005) 87.
5. Ş. Kalaycı, American Journal of Anal. Chem., 11 (2020) 205.
6. H. Tang, J. Chen, S. Yao, L. Nie, G. Deng, Y. Kuang, *Anal Biochem*., 331 (2004) 89.
7. J. Wang, L. Wang, J. Di, Y. Tu, *Talanta*, 77 (2009) 1454.
8. S. Liu, Z. Dai, H. Chen, H. Ju, *Biosens Bioelectron*., 19 (2004) 963.
9. X. Zhu, I. Yuri, X. Gan, I. Suzuki, G. Li, *Biosens Bioelectron*., 22 (2007) 1600.
10. A.A. Karyakin, E.E. Karyakina, L. Gorton, *Anal Chem*., 72 (2000) 1720.
11. B. Fang, N. Zhang, W. Zhang, A. Gu, G. Wang, *J Appl Polym Sci*., 112 (2000) 3488.
12. C. Hu, X. Chen, S. Hu, *J Electroanal Chem*., 586 (2006) 77.
13. M. Şen, U. Tamer, N.P. Özçiçek, *J Solid State Electrochem*., 16 (2012) 457.
14. B.O. Özer, S. Cete, *Artificial cells, nanomedicine, and biotechnology*,. 45(4) (2017) 824.
15. M. Görgülü, S. Cete, H. Arslan, A. Yaşar, *Artificial cells, nanomedicine, and biotechnology*., 41(5) (2013) 327.
16. F. Yıldırımoğlu, F. Arslan, S. Çete, A. Yaşar, *Sensors*, 9(8) (2009) 6435.
17. S. Cete, M. Ozyurt, E. Yildirim, D. Akin, *Chemical Papers*, 74(3) (2020) 799.
18. E. Erol, E. Yildirim, S. Cete, *Journal of Solid State Electrochemistry*, 24 (2020) 1695.
19. S.M. Muhammet, S. Cete, F. Arslan, A. Yaşar, *Artificial Cells, Blood Substitutes, and Biotechnology*., 37(6) (2009) 273.
20. G. A. Tedoradze, D.S. Leibman, E.M. Savenko, *Elektrochemistry*, 12 (1976) 832.
21. H.P. Dai, K.K. Shiu, *Electrochim Acta*, 43 (1998) 2709.
22. J. Di., S. Bi, T. Yang, M. Zhang, *Sensor Actuator B-Chem*., 99(2-3) (2004) 468.
23. Y.H. Li, Y.X. Wang, M.H. Huang, *Electroanal*., 20 (2008) 1440.
24. S. Park, R. S. Ruoff, *Nature Nanotech*., 4 (2009) 217.
25. M.A. Özbek, A. Yasar, S. Cete, E. Er, N. Erk, *Journal of Solid State Electrochemistry*, 25(5) (2021) 1601.
26. Y. Shao, J. Wang, H. Wu, J. Liu, I.A. Aksay, Y. Lin, *Electroanal*., 22 (2010) 1027.
27. X. Ba, L. Luo, Z. Zhang, Y. Chu, B. Wang, *Anal Chim Acta*, 752 (2012) 94.
28. W.S. Hummers, R.E. Offeman, *J Am Chem Soc*., 80 (1958) 1339.
29. H.P. Dai, K.K. Shiu, *J Electroanal Chem*., 419 (1996) 7.

30. Q. Sheng, H. Yu, J. Zheng, *Electrochim Acta*, 52 (2007) 7300.
31. D. Cakmak, T. Bulut, D. Uzun, *Electroanalysis*, 32 (2020) 1559.
32. G. Baysal, D. Uzun, E. Hasdemir, *J. Electroanal.Chem.*, 860 (2020) 113893.
33. Q. Sheng, H. Yu, J. Zheng, *Electrochimica Acta*, 52 (25) (2007) 7300.
34. B. Sherino, S. Mohamad, S.N.A. Halim, N.S.A. Manan, *Sensors and Act. B*, 254 (2018) 1148.
35. D. Zhu, J. Zuo, H. Pang, H. Ma, *New Journal of Chemistry*, 43 (2019) 1053.
36. M. Govindasamy, V. Mani, S. Chen, R. Karthik, K. Manibalan, R. Umamaheswari, *Int. Journal of Electrochemical Science*, 11 (2016) 2954.
37. J. Liu, C. Yang, Y. Shang, P. Zhang, J. Liu, J. Zheng, *Microchimica Acta*, 185 (2018) 172.