Bull Mater Sci (2020)43:283
https://doi.org/10.1007/s12034-020-02268-3

Electrochemical studies of V_2O_5/GOx for glucose detection

F A DE SOUZA, R C F DA ROCHA, N S VIEIRA, D T CESTAROLLI and E M GUERRA*
Departamento de Química, Biotecnologia e Engenharia de Bioprocessos, Ouro Branco CEP 36420-000, Brazil
*Author for correspondence (elidiaguerra@ufsj.edu.br.com)

MS received 23 May 2020; accepted 22 July 2020

Abstract. A component of biosensor was prepared based on vanadium pentoxide xerogel (V_2O_5) and glucose oxidase (GOx). GOx was immobilized on V_2O_5 to obtain V_2O_5/GOx thin film. V_2O_5/GOx thin film was deposited onto an indium-tin-oxide-coated polyethylene terephthalate film as substrate. The immobilization of the enzyme GOx on V_2O_5 was cross-linked by covalent bonds. After immobilization of GOx, an increase in total charge was observed. The rugosity factor indicated a 0.125 of electrochemically active surface of V_2O_5/GOx with low porosity. V_2O_5/GOx thin film presented a sensibility in different concentrations of glucose as well as a good linear correlation.

Keywords. Glucose; biosensor; V_2O_5/GOx; electrochemical studies.

1. Introduction

In recent years, the biotechnology has shown a breakthrough in the processing and development of new materials. In the field of clinical medicine, chemistry and industrial processes, for example, the scientific studies have had a great need to identify and quantify metabolites quickly, inexpensively and reliably and have specificity for the determination of substances in biological fluids. Thus, the development of biosensors became fundamental [1,2]. The necessity for determination of substances in biological fluids, such as blood glucose, cholesterol, lactate, urea, creatine, hemoglobin, has led to the demand for methods that have high selectivity, reliability and inexpensive. Besides, a real-time analyses are explored then the application of biosensors became necessary, especially in clinical analysis [3,4]. Blood glucose monitoring is of utmost importance for diabetes control. According to the International Diabetes Federation (IDF) [5], diabetes is a chronic disease that occurs when the pancreas is no longer able to make insulin, or when the body cannot make good use of the insulin it produces. Besides, insulin is a hormone produced by the pancreas, which acts as a key that allows food glucose to pass from the bloodstream to the body's cells, producing energy. The non-production of insulin or its effective use leads to elevated blood glucose (known as hyperglycemia), long-term serious damage to the body. Commercially available glucometers require greater sensitivity and lower costs. Currently, there are several materials proposed in the literature that help reduce cost, increase sensitivity and have conductive properties, such as some oxides such as vanadium pentoxide (V_2O_5) [3], tungsten trioxide (WO_3) [4,6], zinc oxide (ZnO) [7], V_2O_5 nanowires [8] and titanium dioxide (TiO_2) [9]. It is possible to highlight V_2O_5, which has high stability and conductivity, making it possible in science applications, such as electrochromic devices [10,11] and biosensors [1,3,12–14]. Due to these properties, vanadium pentoxide becomes quite promising in the production of biosensors. Biosensors have a biological component that is attached to the surface of a transducer. When this component is an enzyme, it identifies and relates specifically to the analyte of interest, thus chemical and physical changes occur [15]. Then, they are called enzymatic biosensors. Enzymatic biosensors have the objective of detecting glucose in the blood, thus the biological component of the biosensor is the enzyme glucose oxidase (GOx) which, when compared to other enzymes, has a low cost of production, coupled with the fact that it is highly specific and stable [16]. In this context, the purpose of this research was to contribute to the study on the introduction of new materials (V_2O_5/GOx) and the development of a biosensor for glucose detection that is more sensitive and less expensive compared to those available.

2. Experiments

2.1 *Preparation of vanadium pentoxide by sol–gel process*

Vanadium pentoxide gel was prepared *via* acidification of an aqueous 0.1 mol l^{-1} of sodium metavanadate solution and was percolated on an acid-form ion exchange resin column to obtain the polyvanadic acid solution, which when freshly prepared has an orange colouration [17]. After passing through an acid ion exchange resin, the solution was aged for 7 days at room temperature. Thus, the oxide was

Published online: 27 October 2020

polymerized by a self-catalytic process and obtained a dark-red viscous solution, characteristic colour of V_2O_5.

2.2 *Immobilization route*

The solution of V_2O_5 *via* sol–gel was deposited on the polyethylene terephthalate (PET)/indium-tin-oxide (ITO) electrode by casting method in an area of 1 cm^2. The sample was dried at room temperature for 24 h. Subsequently, the enzyme GOx, 1.5 mg ml^{-1} in sodium phosphate solution (PBS), was added to the film, together with a 5% v/v glutaraldehyde solution. A ratio of 1:1 was used for addition of the sample, adding 100 µl of the mixture. The film was dried using a cooler with an average temperature of 4°C, which the GOx enzymatic activity could be maintained. PBS was prepared at pH = 6 by adding monosodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4), both at 0.1 mol l^{-1} concentration. Cyclic voltammetries were performed varying the pH, in different scan rate and concentrations of glucose in PBS solution for the best biosensor calibration and identification of redox reactions during the electrochemical process.

2.3 *Characterization methods*

The cyclic voltammetry (CV) study was performed to analyse the behaviour of the V_2O_5 film before and after GOx immobilization in the same film. The CV experiments were performed on a computer interfaced Potentiostat/Galvonostat AutoLab III. The reference electrode used was Ag/AgCl together with the platinum counter electrode and the PET/ITO as working electrode. As electrolyte, was prepared a PBS solution, in pH = 6, containing glucose 4.4 mmol l^{-1}. Other CV tests were performed at different glucose concentrations after choosing the ideal pH for the biosensor according to the PET/ITO results.

3. Results and discussion

The modified electrode response to glucose was explored from CV using the V_2O_5 film enzyme-free and with enzyme. Initially, for the first test, the electrolyte containing PBS, pH = 6, glucose 4.4 mmol l^{-1} was used. This concentration was chosen from the series of concentrations to be tested, as described in the experimental procedure.

The CV study was performed with the aim of analysing the behaviour of the V_2O_5 film before and after the immobilization of GOx in the same electrode, in which they were cycled 30 times. Due to the insertion and disinsertion of ions into the film, the number of cycles increased, and the current density decreased in function of structural accommodation (figure 1). Thus, after several cycles, the V_2O_5 film achieves structural stability due to mechanical stress.

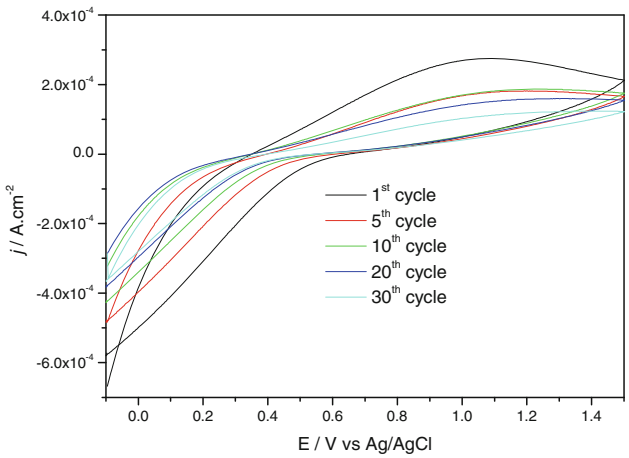

Figure 1. Cyclic voltammogram of the V_2O_5 film obtained during the 30 cycles before GOx immobilization, v = 20 mV s^{-1}, PBS, pH = 6, glucose 4.4 mmol l^{-1}.

After the immobilization of the enzyme, the film V_2O_5/GOx presented a new voltammetric profile due to the electrocatalytic activity of GOx and the glucose present in the medium. The new material (V_2O_5/GOx) has a new electrochemical characteristic, which can be observed because the voltammogram area increases and there is also the displacement of anodic peaks.

From the CV of V_2O_5 before and after immobilization of GOx, it is observed that charge variation decreases as the number of cycles increases. This can be explained because charge transfer reactions tend to be stable due to V_2O_5 restructuring with ion insertion/disinsertion at different active sites, achieving a dynamic equilibrium on the electrode surface, causing an increase in structural stability as a function of total charge as well as achieving structural accommodation after several cycles, allowing for better performance [6].

In figure 2, the cyclic voltammetry refers to V_2O_5 before the immobilization of GOx and after immobilization of this

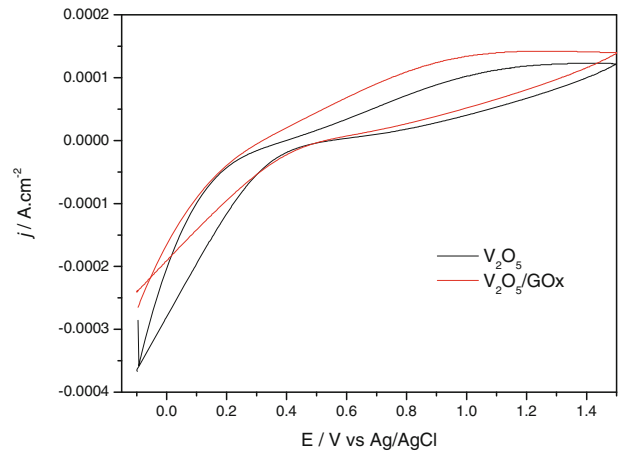

Figure 2. Cyclic voltammograms of V_2O_5 and V_2O_5/GOx, v = 20 mV s^{-1}, PBS, pH = 6, glucose 4.4 mmol l^{-1}.

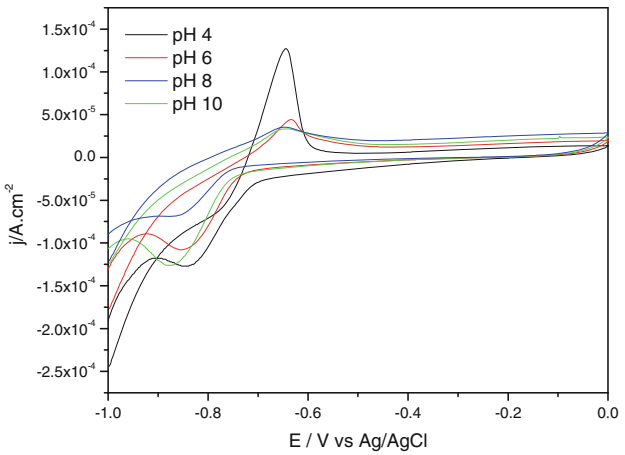

Figure 3. V_2O_5/GOx voltammograms, in 0.1 mol l^{-1} PBS buffer, with 4.4 mol l^{-1} glucose, v = 20 mV s^{-1}, with pH range 4 to 10.

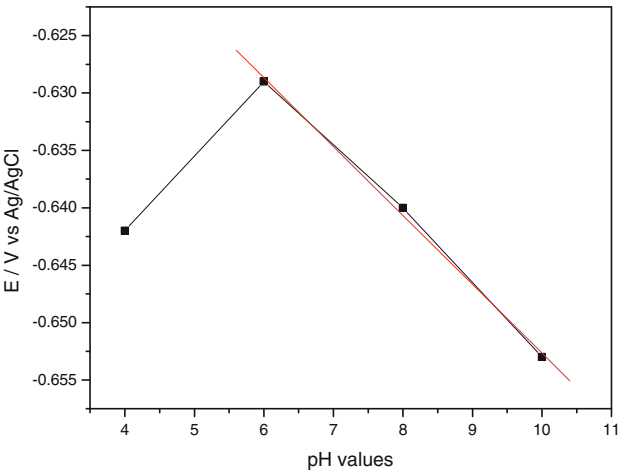

Figure 4. Potential variation as a function of different values of pH.

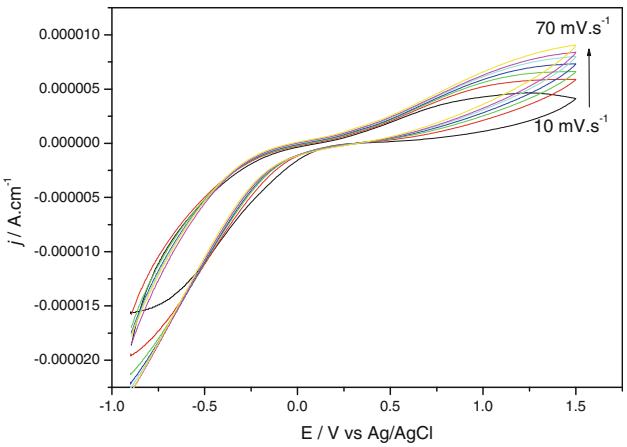

Figure 5. V_2O_5/GOx film at pH 6, glucose 4.4 mol l^{-1}.

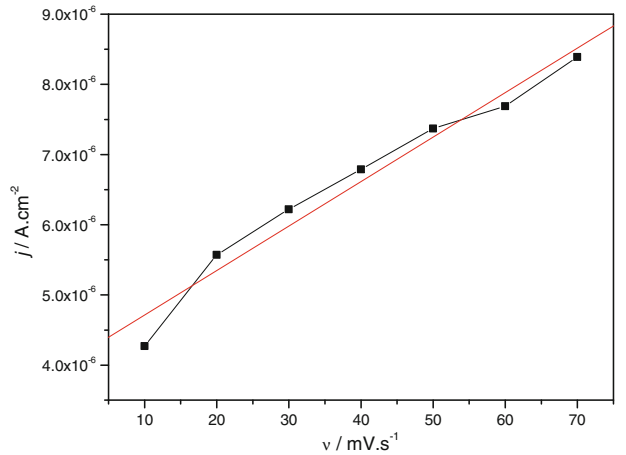

Figure 6. Dependence of current density as a function of scan rate. The values were collected on transition region.

enzyme. Considering the possibility of structural stabilization of the film before immobilization, the changes observed in the CV after the addition of GOx indicate that the enzyme may have immobilized on the substrate.

From figure 2 it is possible to note that the adding GOx slightly increases the total charge. This is because the immobilization of the enzyme GOx is probably a cross-linking between the enzyme molecules by covalent bonds, thus making it difficult to lose mass and expose the film's inner layers. This improvement in structural stability helps confirm the enzyme's immobilization effectiveness [1].

In order to analyse the modified electrode dependence, CV studies were performed. To test the biosensor sensitivity, four different pH values were chosen (4.0, 6.0, 8.0 and 10.0). Thus, the pH of the medium used for glucose detection measurements from V_2O_5/GOx films was varied from the use of a 0.1 mol l^{-1}, PBS buffer solution. In figure 3, it is possible to observe anodic and cathodic peaks. The total area of the voltammogram decreases in function while the pH increases. This can indicate that the enzyme has higher activity between pH 4 and 6, and above 6 the enzyme may be initiating the denaturation process reducing the catalytic activity of the enzyme.

According to Nernst's equation, the slope value of the line E *vs.* pH is 59.2 mV/pH [18]. However, the study result was 6.0 mV/pH, which is far from the expected value, as shown in figure 4. Thus, it is concluded that the pH variation in this case influences the catalytic activity. Therefore, it is not possible to do a sensitivity study using the Nernst equation in this case.

In figure 5, it is possible to observe current peaks that increase linearly with the scan rate, v, which was used between 10 and 70 mV s^{-1}. The separation between the peaks increased considerably with this varying.

According to figure 6, anode peak currents vary as a function of scan rate. Regarding the calculation of the correlation coefficient, an R value of 0.997 was calculated, with v ranging from 10 to 70 mV s^{-1}. This indicates that

there is a proportional linear correlation between scan rate and current density.

CV can provide a very wide range of information, such as location of redox transitions, number of electrons involved, anodic charge, and especially to correctly estimate the electrochemically active area of the electrode surface. To calculate the electrochemically active area, two parameters are evaluated: the apparent total capacitance, which is commonly, referred to as the electric double layer capacitance (C_{dl}) and the roughness factor (RF). These parameters, combined with other information obtained by cyclic voltammetry, can provide a good estimate of what happens on the electrode surface. To obtain the C_{dl}, the voltammetry curves were observed from figure 5. The current measured outside the electronic oxide transitions (i.e., the capacitive region of the cyclic voltammogram) was plotted as a function of scan rate variation. Assuming that the charging of the electric double layer is solely responsible for the process, a linear behaviour is obtained, and the angular coefficient gives the C_{dl}. Then, the roughness factor can be obtained by equation (1)

$$\mathrm{RF} = C_{dl}/C \tag{1}$$

where C is capacitance using 60 μF cm^{-2} for oxide as suggested by Trasatti and Petrii [19]. Then, the slope of the linear region of figure 7 can provide C_{dl} [20,21]. Under the experimental conditions which the studies were performed, a linear relationship was obtained when the current density, j, was plotted against the scan rate variation, represented in figure 7.

From figure 7, it was observed that an excellent linearity is a good indication that redox transitions contribute little to the chosen potential. Then, the roughness factor of V_2O_5/GOx was calculated as being 0.125. Based on literature [20], values below 0.3 indicate that the electrodes surface have a low electrochemical porosity and, consequently, a low electrochemical active area. This result can refer to the fact that the enzyme has a large surface area per unit and with few active sites and influencing the final electrode porosity. This method should then be considered as empirical, and it is not entirely reliable to compare different oxide systems. However, this method provides a good estimate of the surface area of an oxide electrode when used in this format and considering its limitations [18–20,22].

From the results obtained and in order to compare the results obtained in both techniques, new CV tests were performed at the pH that best responded to the biosensor. Fixing the pH at 6, the glucose concentrations were varied in the PBS solution of 0.1 mol l^{-1}. The concentrations chosen were 4.4, 4.9, 5.4, 6.0 and 6.6 mmol l^{-1}. The values of 4.4 and 6.6 mmol l^{-1} represent borderline values associated with hypoglycemia and hyperglycemia, respectively [1]. Other concentrations were chosen to represent more values for biosensor sensitivity studies and to decrease the standard deviation value if there is a linear correlation close to 1. Therefore, these values are within the concentration variation of a healthy individual without hypoglycemia or hyperglycemia. Figure 8 shows the variations in concentrations in the V_2O_5/GOx film. In all results, it was noted that the current intensity increased concomitantly with the glucose concentration.

According to figure 9, anode peak currents vary as a function of glucose concentration. Regarding the calculation of the correlation coefficient, an R value of 0.99 was observed, with a concentration ranging from 4.4 to 6.6 mmol l^{-1}. This indicates that there is a proportional linear correlation between the variation of concentrations and current density.

The square wave voltammetry was used, as it is a faster and more sensitive voltammetric technique. Subsequently, we used the same glucose variations (4.0; 4.4; 5.9 and 6.6 mmol l^{-1}) to be analysed (figure 10). As compared with cyclic voltammograms, the square wave voltammetry presented an increase in current density as a function of the concentration increase of glucose. The linear correlation observed (figure 11) gave an R-value of 0.986. From

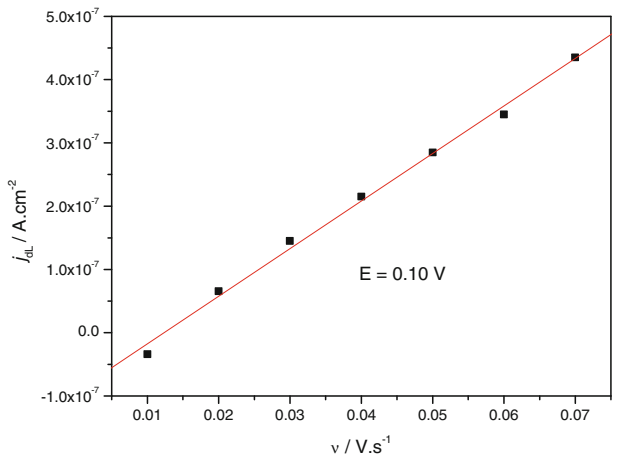

Figure 7. Variation of current density in double layer region as a function of scan rate.

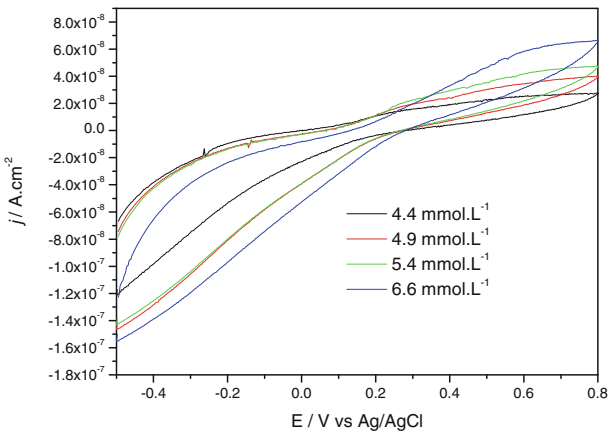

Figure 8. V_2O_5/GOx voltammograms, in 0.1 mol l^{-1} PBS buffer, pH 6, v = 20 mV s^{-1}, with varying concentrations of glucose.

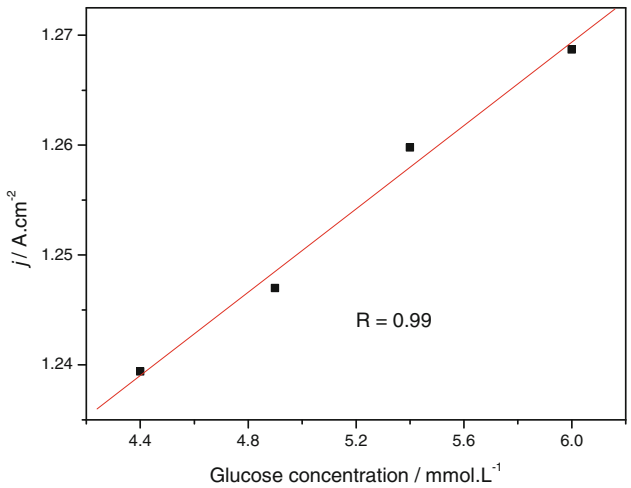

Figure 9. Correlation coefficient of glucose concentration in function of current density from cyclic voltammogram data.

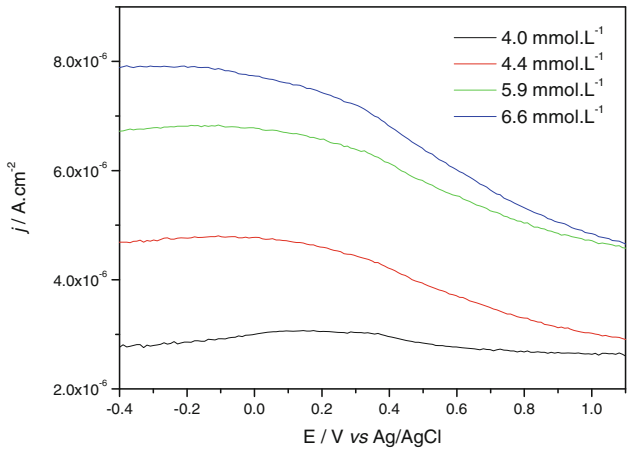

Figure 10. Square wave voltammogram using V_2O_5/GOx, in PBS, pH = 6, v = 20 mV s^{-1}, depending on the variation in glucose concentrations.

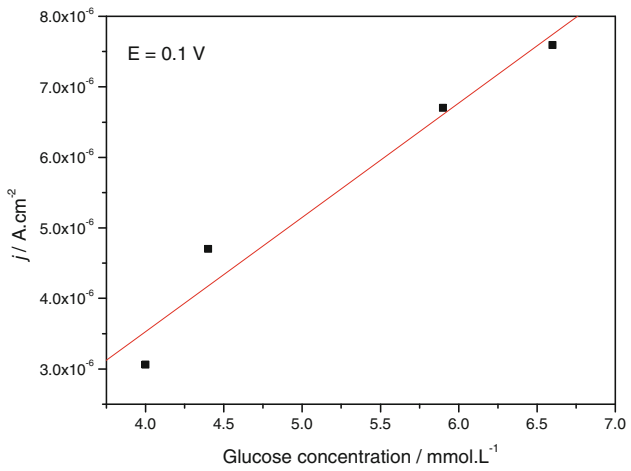

Figure 11. Electrochemical studies of glucose detection sensitivity from square wave voltammetry data.

figure 11, it is possible to obtain the sensitivity and it was calculated using the slope of the current *vs.* concentration divided by the active surface area of V_2O_5/GOx. Therefore, the slope obtained was 1.62 μA (mmol $l^{-1})^{-1}$ and the sensitivity of the V_2O_5/GOx sensor was calculated as being 1.62 μA (mmol l^{-1} $cm^{-2})^{-1}$. The sensitivity of V_2O_5/GOx electrode is considered good when compared with recent literature [23–27].

4. Conclusion

From the use of V_2O_5/GOx it was possible to construct an enzymatic glucose electrochemical biosensor. The presence of GOx onto the V_2O_5 displayed a slightly increase in the total charge that caused a better performance in detection of glucose. A linear relationship from current density and different scan rate were 0.99 for V_2O_5/GOx in the presence of glucose. A low electrochemically active surface of sensor was 0.125. PET/ITO plays an important role in inducing the transfer of charge carriers at the interfaces of the V_2O_5 and GOx. The sensitivity of the V_2O_5/GOx sensor was 1.62 μA (mmol l^{-1} $cm^{-2})^{-1}$, which is considered as good. Moreover, a new material V_2O_5/GOx enhances the biosensor performance, demonstrates to have a sensibility of glucose variation, and might be applied as an important component, such as biosensor.

Acknowledgement

This study was supported by INEO, FAPEMIG, RQ-MG/FAPEMIG, CNPq and CAPES.

References

[1] Felix A T, Mulato M and Guerra E M 2018 *J. Electron. Mater.* **47** 6016
[2] Alfaya A A S and Kubota L T 2002 *Quimica Nova Nova* **25** 835
[3] Guerra E M, Silva G R and Mulato M 2009 *Solid State Sci.* **11** 456
[4] Guidelli E J, Guerra E M and Mulato M 2012 *ECS J. Solid State Sci. Technol.* **1** N39
[5] IDF. International Diabetes Federation—Home. *International Diabetes Federation*. Retrieved from https://www.idf.org/
[6] Guidelli E J, Guerra E M and Mulato M 2011 *Mater. Chem. Phys.* **125** 833
[7] Batista P D and Mulato M 2005 *Appl. Phys. Lett.* **87** 143508
[8] Qu K, Shi P, Ren J and Qu X 2014 *Chem.— Eur. J.* **20** 7501
[9] Guerra E M and Mulato M 2014 *Mater. Sci. Appl.* **05** 459
[10] Wei Y X, Ma Y B, Chen M, Liu W M, Li L and Yan Y 2017 *J. Electroanal. Chem.* **807** 45
[11] Yong Zhang S, Wu Y, Tang K, Zhang Y, Yingdi Shi, Cui J *et al* 2018 *J. Mater. Chem. C* **6** 12206

[12] Guerra E M, Cestarolli D T and Mulato M 2018 *Int. J. Electrochem. Sci.* **13** 9905
[13] De Campos R C, Cestarolli D T, Mulato M and Guerra E M 2015 *Mater. Res.* **18** 15
[14] Guerra E M and Mulato M 2009 *J. Sol–Gel Sci. Technol.* **52** 315
[15] Graça J S and Ferreira M 2015 *Rev. Virtual Quim.* **7** 1552
[16] Villalba P, Ram M K, Gomez H, Kumar A, Bhethanabotla V and Kumar A 2011 *Mater. Sci. Eng. C* **31** 1115
[17] Guerra E M, Ciuffi K J and Oliveira H P 2006 *J. Solid State Chem.* **179** 3814
[18] Cheng Y, Gao F, An L, Lan J, Li X, Wang G *et al* 2015 *Res. Chem. Intermed.* **41** 1741
[19] Trasatti S and Petrii O A 1993 *J. Electroanal. Chem.* **321** 353
[20] Lipkowski J and Ross P N 1994 *Electrochemistry of novel materials* vol 3 (New York: VCH Weinheim)
[21] Soares C O, Carvalho M D, Melo Jorge M E, Gomes A, Silva R A, Rangel C M *et al* 2012 *J. Appl. Electrochem.* **42** 325
[22] Da Silva L M, De Faria L A and Boodts J F C 2001 *Electrochim. Acta* **47** 395
[23] Gougis M, Ma D and Mohamedi M 2015 *Int. J. Nanomed.* **10** 2939
[24] Das S and Saha M 2014 *J. Pharm. Anal.* **4** 35
[25] Njoko N, Louzada M, Britton J, Khene S, Nyokong T and Mashazi P 2020 *Colloids Surf. B: Biointerfaces* **190** 110981
[26] Yang Q, Zhu G, Singh L, Wang Y, Singh R, Zhang B *et al* 2020 *Optik* **208** 164536
[27] Vieira N S, Souza F A, Rocha R C F, Cestarolli D and Guerra E M 2020 *Mater. Sci. Semicond. Process*. **121** 105337