Synthesis and characterization of V_2O_5/urease for a biosensor of urea

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Abstract

Vanadium pentoxide (V_2O_5) was used as support material to immobilize the urease enzyme. V_2O_5 was prepared from a sol–gel process, and the immobilization of urease was done onto the surface of the PET/ITO/V_2O_5 film. Different concentrations of urea were tested to study the sensitivity of the biosensor. Tests with pH variation were carried out, and it was noticed that the total charge in the cyclic voltammograms decreases due to the increase in pH that influences the catalytic activity of the enzyme. From cyclic voltammograms, with a different scan rate, for linear correlation testing, it was observed that the anodic currents varied as a function of the scan rate. Based on the variation in the urea concentration, the current increased with the increase in the urea concentration, indicating the sensitivity of the material. The use of V_2O_5/urease showed favorable electrochemical responses for application in biosensors as well as being sensitive to the variation in the urea concentration and easy to obtain and prepare. Volume 0, Number 0, Pages 1–6, 2020

Keywords: *biosensors, sensitivity, urea, V_2O_5/urease*

1. Introduction

Since the development of improved new technologies and low cost computation, there is now a significant demand for new biosensors in search of better precision in the results of real-time analysis, applied in the field of medicine, clinical chemistry, and industry and the control of pollutants. In addition, there is a growing need to identify and quantify metabolites quickly, specifically and in very small sample quantities, favoring the development of biosensors [1]. Biosensors are sensors modified with a biological material attached to the surface of a transducer capable of converting a chemical reaction into an appropriate signal. The biological component recognizes the substance of interest by means of a chemical reaction generating a signal that may result from a variation in the concentration of protons, release of gases, emission or absorption of light, emission of heat, variation of mass, change of oxidation state, etc. The transducer converts this signal into a measurable response, such as current, potential, temperature variation, among others [2]. The necessity to determine substances in biological fluids, such as, blood glucose, cholesterol, lactate, urea, creatine, and hemoglobin, has led to the demand for methods that are cheap and have high selectivity, reliability, and capability of real-time analysis. Then, the application of biosensors has become necessary mainly in clinical analysis [3, 4]. In the clinical area, there are several reports on kidney diseases and, as a result, there is also a growing demand for rapid and accurate diagnoses, considering that the evaluation of renal function is one of the oldest challenges to be resolved [5]. In Brazil, official sources indicate that about four million people are affected with chronic renal failure [6]. In the United States of America, the disease affects around 20 million individuals [7]. It is worth adding that the incidence of individuals with kidney diseases is extremely high, and it is recognized as a complex disease that requires multiple approaches to its treatment. These pathologies require great care, since their control is not easily detected, and greater control is essential to avoid causing complications. Clinically, the most used method to obtain information about these diseases is the creatinine clearance, with urine collection over 24 H. Unfortunately, creatinine clearance does not meet the criteria of an ideal marker, since creatinine is excreted not only via glomerular

Abbreviations: *Glu, glutaraldehyde; ITO, indium tin oxide; PET, polyethylen terephthalate; V_2O_5, vanadium pentoxide.*

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Highlights

- Biosensor for urea
- Immobilization of urease onto V_2O_5
- Characterization of V_2O_5/urease

filtration but also via secretion in the proximal tubule. Another problem with creatinine clearance is the need to collect urine for 24 H, something that is inconvenient for some patients and, therefore, collections are often inaccurate. This is particularly true in some clinical situations (e.g., very elderly or cognitively impaired individuals). Among the most effective routine tests for assessing renal function are urea measurements [8, 9]. Normal urea values in human blood in an adult are around 10–40 $mg \cdot dL^{-1}$, and in urine around 9–24 $mg \cdot dL^{-1}$. Values above this limit may indicate renal failure, severe dehydration, heart failure, high protein diet, liver disease, among others. For values between 180 and 400 $mg \cdot dL^{-1}$ of blood urea, the patient urgently needs hemodialysis [10]. The excessive concentration of urea in the body (in serum or urine) is an indicator of these kidney diseases. However, medical samples are often mixtures that require the use of complex techniques to detect regulatory substances in the bloodstream, such as urea. Therefore, the development of biosensors has been a key element in analytical instrumentation, since they are able to determine the amount of urea in the body through a specific enzyme [11]. The medical samples available in the market are mixtures that demand the use of complex techniques for detecting urea. Currently, several materials such as, vanadium pentoxide (V_2O_5) [4, 12, 13], tungsten trioxide (WO_3) [14–16], zinc oxide (ZnO) [17], and titanium dioxide (TiO_2) [18, 19] have beenproposed in the literature as material for biosensor, which may reduce costs, increase sensitivity, and possess conductive properties. V_2O_5 is one of the oxides that has been shown to be quite promising due to its remarkable characteristics such as thermal and chemical stability, biocompatibility, and conductivity, which allows its application in multiple and different areas [22,23]. One of the best way to obtain V_2O_5 films is via sol–gel. The use of synthesis by the sol–gel route can be easily recognized, as it deals with the synthesis of materials in which, at a certain moment, a transition from the sol system to a gel system occurs. This synthesis has attracted considerable interest from researchers, since it presents ease of manufacture, flexibility of the synthesis project, and the fact that the enzymes included in the sol–gel matrix maintain their respective catalytic activity. In addition, it is a relatively simple and inexpensive process, when compared to other methods with the same purpose [24]. Through a brief search using the keywords "V_2O_5 urease biosensor" in Web of Science, we find only one article using V_2O_5 nanorods with urease immobilized [20]. Thus, it can be highlighted that there is still more study to be done on this subject. Based on this context, the present study is aimed to develop an amperometric biosensor for the detection of urea, using the enzyme urea immobilized in vanadium pentoxide films.

2. Experimental Procedure

2.1. Synthesis of V_2O_5 by sol–gel process

To obtain the V_2O_5 by the sol–gel process, an aqueous solution of 0.1 $mol \cdot L^{-1}$ sodium metavanadate was used, and it was percolated on an ion exchange resin column in an acid form, to obtain the polyvanadic acid solution, which when freshly prepared it has an orange coloration [27]. After passing through an acidic ion exchange resin, the solution was left to stand for 7 days at room temperature. Therefore, the oxide was polymerized by a self-catalytic process and obtained a viscous solution with a dark red color, a characteristic of V_2O_5.

2.2. Immobilization route

After characterization, the V_2O_5 solution via sol–gel was deposited on the PET/ITO electrode by the casting method in an area of 1 cm^2. The sample was dried at room temperature. Subsequently, 5.0 mg of urease was dissolved in sodium phosphate solution (PBS) solution and was added with a 5% v/v solution of glutaraldehyde (Glu) onto the V_2O_5 film. The film was dried at room temperature for 24 h.

The PBS was prepared at pH = 7 by adding monosodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4) both in 0.1 $mol \cdot L^{-1}$. Cyclic voltammetry tests were performed by varying the pH, variation in the scan rate, and different concentrations of urea in PBS solution for the best calibration of the biosensor and identification of the redox reactions in the electrochemical process.

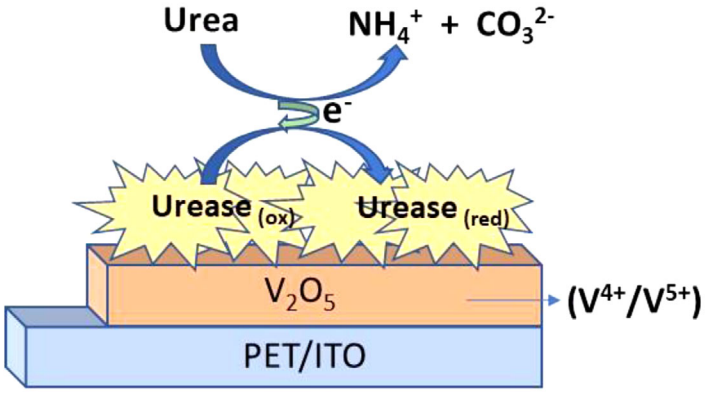
2.3. Characterization methods

The cyclic voltammetry study was carried out with the objective of analyzing the behavior of the V_2O_5 film before and after urease immobilization in the same film. Cyclic voltammetry is an electroanalytical technique that is based on the phenomenon that occurs at the interface between the surface of three electrodes (reference electrode, work, and counterelectrode).

The cyclic voltammogram experiments were performed on a Potentiostat/Galvonostat AutoLab III interfaced to a computer. The reference electrode used was Ag/AgCl, together with the platinum counterelectrode and the PET/ITO working electrode. The electrolyte used for the first test was PBS buffer, pH = 7, and urea at a concentration of 25 $mmol \cdot L^{-1}$. Other cyclic voltammogram tests were performed at different concentrations of urea after choosing the ideal pH for the biosensor according to the results obtained. A schematic mechanism of bioelectrode with the sensing mechanism is shown in Scheme 1.

3. Results and Discussion

The response of the V_2O_5 film to urea was explored from cyclic voltammograms using the enzyme-free film. Initially, for that purpose, the electrolyte containing PBS, pH = 7, and urea was



SCHEME 1 *Schematic mechanism of the electron exchange occurring at the surface of PET/ITO/V_2O_5/urease in the presence of redox species*

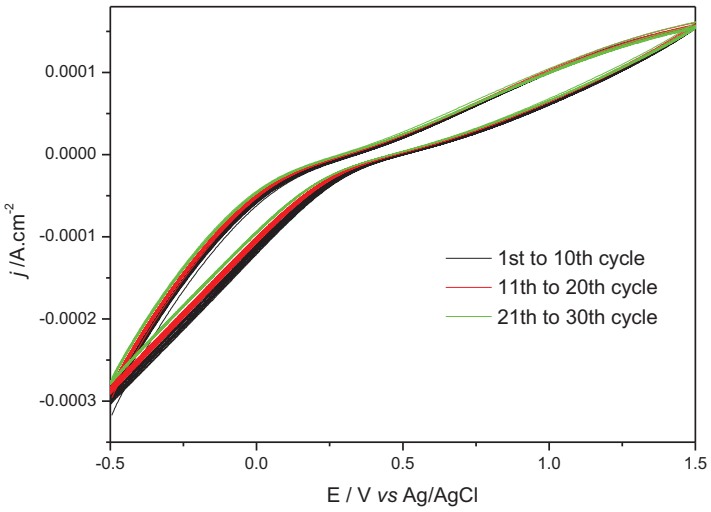


FIG. 1 *Cyclic voltammogram obtained during the 30 cycles of the V_2O_5 film, $\nu = 10\ mV \cdot s^{-1}$, PBS, pH = 7, urea in 25 mmol·L^{-1} vs. Ag/AgCl.*

used, 25 mmol·L^{-1} vs. Ag/AgCl. This concentration was chosen from the series of concentrations to be tested corresponding to the normal limits of urea in the human body.

The cyclic voltammogram study was carried out with the objective of analyzing the behavior of the V_2O_5 film before and after the immobilization of the urease in the same film, in which each cycle was cycled 30 times. Figure 1 shows cycles 1–30 in the absence of urease. The area covered by V_2O_5 onto PET/ITO electrode was approximately 1.0 cm^2, and PBS, pH = 7, with a fixed concentration of urea 25 mmol·L^{-1} was used as electrolyte. Due to the insertion and de-insertion of ions in the film, the total charge decreases as the number of cycles related to structural accommodation increases (Fig. 1). Besides, from the cyclic voltammogram of the V_2O_5 film before urease immobilization, the total charge decreases when the number of cycles increases because the charge transfer reactions tend

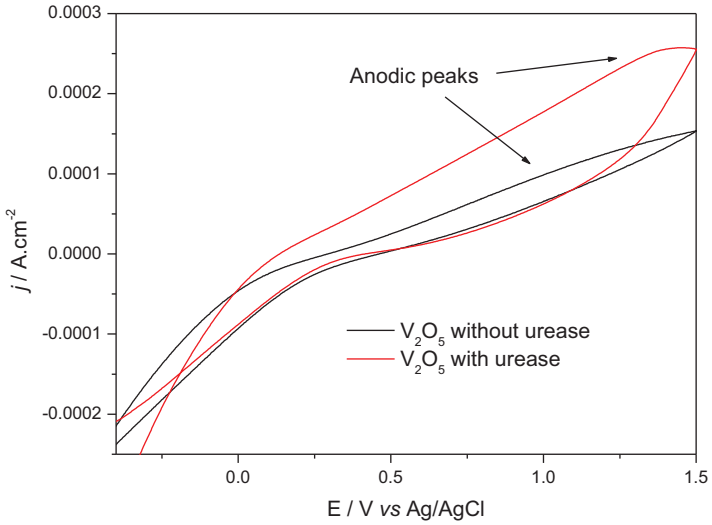


FIG. 2 *Cyclic voltammogram of the 30th cycle of V_2O_5 and the first cycle of V_2O_5/urea, $\nu = 10\ mV \cdot s^{-1}$, PBS, pH = 7, urea in 25 mmol·L^{-1} vs. Ag/AgCl.*

to be stable due to the restructuring of V_2O_5 caused by the insertion/de-insertion of ions reaching a dynamic balance on the electrode surface. Then, the dynamic balance cause an increase in voltammetric stability as a function of the total charge as well as reaching a structural accommodation, after several cycles and allowing a better performance [5].Thus, after several cycles, the film reaches structural stability due to mechanical stress and the immobilization was carried out onto the electrode.

After the immobilization of the enzyme, the V_2O_5/urease film presented a new voltammetric profile due to the electrocatalytic activity of the urease in urea present in the medium (Fig. 2). The new material (V_2O_5/urease) starts to present new electrochemical characteristics that can be observed as the voltammogram's area increases, and there is also the displacement of anodic peaks, as shown in Fig. 2. In Fig. 2, the cyclic voltammogram refers to the 30th cycle before enzyme immobilization and the other voltammogram refers to the first cycle after the enzyme is immobilized. Besides, with the structural stabilization of the film before immobilization, the changes observed in the voltammogram after the addition of urease indicate that the enzyme may have been immobilized to the substrate. In addition, after adding of urease, there is a subtle increase in the total charge (total area of the voltammogram). This can be attributed to the immobilization of the urease enzyme that can be done by covalent cross-linking, thus making it difficult to lose mass and expose the film's internal layers. This improvement in structural stability proves the effectiveness of immobilizing the enzyme.

In order to analyze the sensitivity of the component together with the enzyme, cyclic voltammetry studies were carried out, making it possible to observe anodic and cathodic peaks, shown in Fig. 3. That one may have to study the

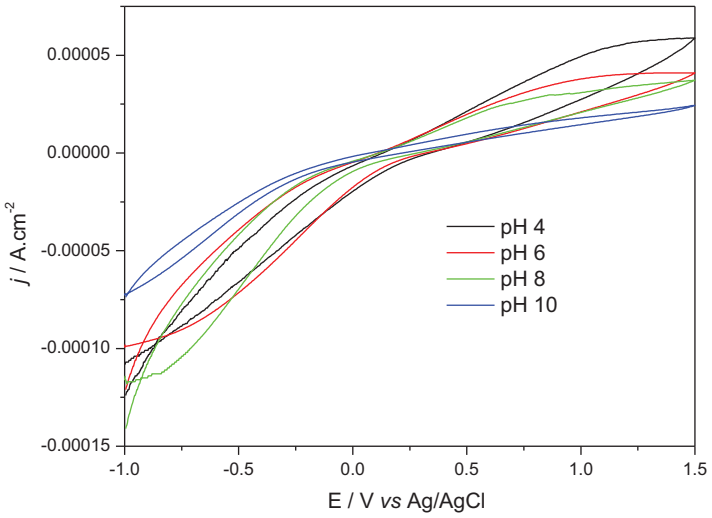


FIG. 3 *V_2O_5/urease cyclic voltammogram with pH range 4–10, $\nu = 10$ mV·s^{-1}, PBS, urea in 25 mmol·L^{-1} vs. Ag/AgCl.*

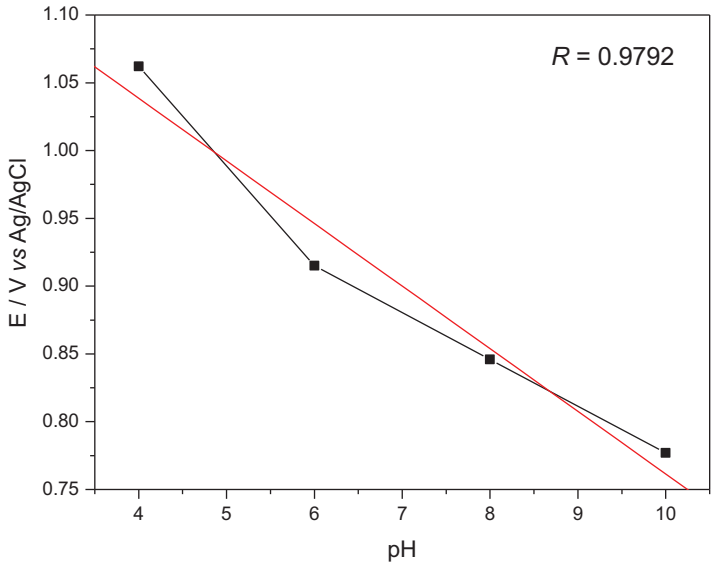


FIG. 4 *Variation of potential as a function of different pH values.*

effect of the pH on the reaction medium on the activity of the enzyme immobilized and biosensor sensitivity; therefore, four different pH values (4.0, 6.0, 8.0, and 10.0) were chosen for the electrolyte buffer solutions. At room temperature, the best enzyme activity occurs at pH 7 [21]. Thus, the pH of the medium used in the measurement of urea from V_2O_5/urease films was varied to verify the electrochemical behavior changes using a PBS 0.1 mol·L^{-1} buffer solution.

In all pH values, it was noted that there is a slight change in values of redox potentials. However, the total area of the voltammogram decreases as the pH increases. This may indicate that the enzyme has greater activity between pH 4 and 8, and above 8 the enzyme may be starting the denaturation process, reducing the catalytic activity of the enzyme. According to the Nernst equation, the slope value of the line E vs. pH should be 59.2 mV·pH^{-1} [28]. However, the result of the study was 46.2 mV·pH^{-1}, which represents a value relatively close to the theoretical one (Fig. 4). However, good linearity has been observed and it can be appropriate as a device for biosensors.

Cyclic voltammograms at different scan rates are shown in Fig. 5. It is possible to notice that the current peaks that increase linearly with the scan rate, ν, in which 10–200 mV·s^{-1} were used. The separation between the peaks increased considerably with the variation in the scan rate.

Figure 6 shows a correlation between the anode peak currents as a function of the scan rate. Regarding the calculation of the correlation coefficient, an R value of 0.9962 was observed, with ν ranging from 10 to 200 mV·mol·L^{-1}·s^{-1}. This indicates that there is a proportional linear correlation between the scan rate and current density.

Finally, the investigation of the variation in urea concentrations in the electrolyte was evaluated (Fig. 7). New cyclic voltammetry tests were performed at a fixed pH of 7, and the

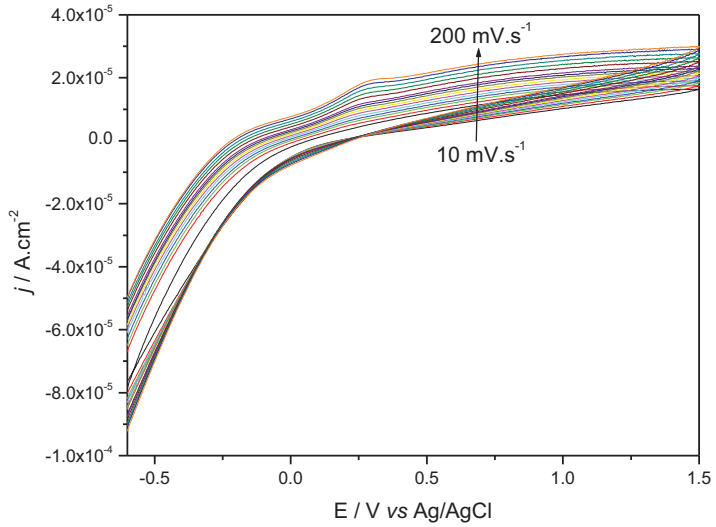


FIG. 5 *Cyclic voltammogram of V_2O_5/urease, PBS, pH = 7, urea in 25 mmol·L^{-1} vs. Ag/AgCl*

chosen concentrations were 20, 40, 60, and 80 mmol·L^{-1}. The values of 20 and 80 mmol·L^{-1} represent borderline values associated with the kidney disease. The other concentrations were chosen to present more values for studies of sensitivity of the biosensor and to decrease the value of the standard deviation if there is a linear correlation close to 1. Furthermore, these values are within the concentration range of a healthy individual without considerable changes in blood urea.

Figure 7 shows the variations in concentrations in the V_2O_5/urease film. In all results, it was noted that the current intensity increased concomitantly with the urea concentration.

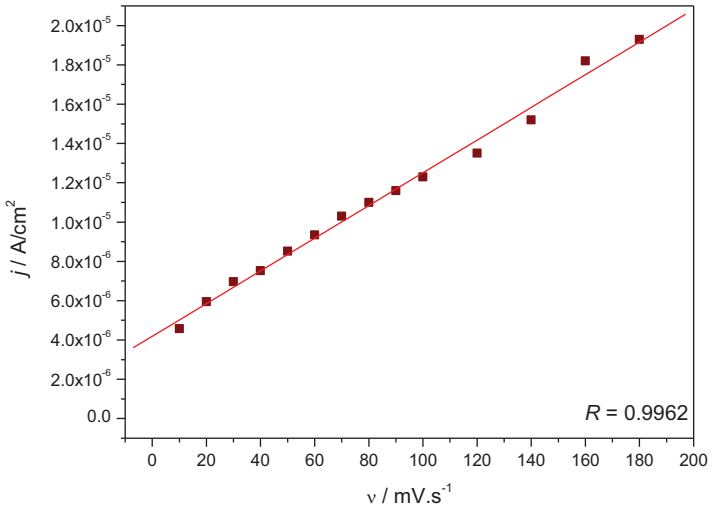


FIG. 6 *Dependence of the anodic peak current as a function of the scan rate in the charge transfer region.*

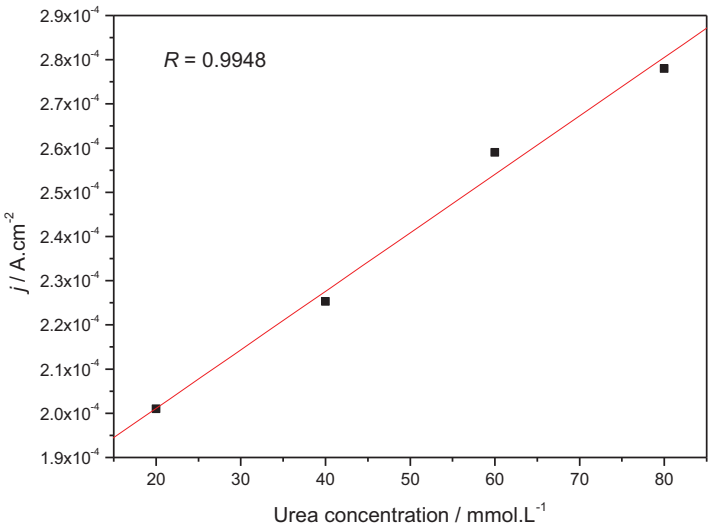


FIG. 8 *Dependence of the anodic peak current with the concentration variation.*

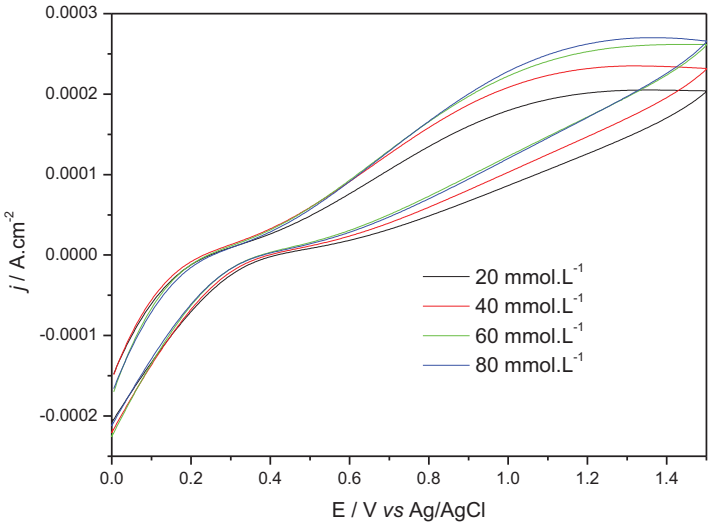


FIG. 7 *Cyclic voltammograms of V_2O_5/urease with variation in the urea concentration from 20 to 80 mmol·L^{-1} vs. Ag/AgCl, $\nu = 10$ mV·mol·L^{-1}·s^{-1}, PBS, pH = 7.*

Figure 8 shows a correlation between anode peak currents as a function of urea concentration. Regarding the calculation of the correlation coefficient, an R value of 0.9948 was observed, with the concentration ranging from 20 to 80 mmol·L^{-1}. This indicates that there is a proportional linear correlation between the variation in the concentration and current density. The sensitivity was calculated using the slope of the current versus concentration divided by the active surface area of V_2O_5/urease. Then, the slope was 1.32 μA·(mmol·L^{-1})$^{-1}$ and the sensitivity of the V_2O_5/urease sensor was calculated as being 1.38 μA·(mmol·L^{-1}·cm^{-2})$^{-1}$. The sensitivity of V_2O_5/urease electrode is considered low when compared with the literature [22–25].

TABLE 1 ***Comparison of the presented work with other type of urea biosensors***

Structure	*Sensitivity [$\mu A\cdot(mmol\cdot L^{-1}\cdot cm^{-2})^{-1}$]*	*Reference*
ZnO nanorods	41.64	[26]
SnO_2	18.90	[27]
CeO_2	3.70	[28]
V_2O_5/urease	1.38	This work

Based on the literature and data observed in Table 1, it is possible to note different values of sensitivity for urea sensors using different oxides. Comparing with works presented in Table 1, the sensitivity in this work is low but still high enough to detect the urea. The largest advantage of our V_2O_5/urease as component in the urea sensor is that the material is biocompatible with high reproducibility, low cost, effectiveness, and easy handling.

4. Conclusion

The use of the V_2O_5 film as an immobilization matrix of the enzyme urease by cross-covalent bonding with glutaraldehyde and the development of enzymatic electrochemical biosensors were performed successfully. Cyclic voltammetry was an extremely important tool and demonstrated that the immobilization occurred satisfactorily through characteristic peaks of the cyclic voltammograms of each material as well as the variation of the total area. The cyclic voltammetry tests

were performed by varying the pH of the support electrolyte, and it was observed that the total area of the voltammogram decreases as a function of the increase in pH influencing the catalytic activity of the enzyme. The optimum pH range of the immobilized enzyme was found to be between pH 6 and 8. When the scan rate variation was carried out, it was observed that there was a proportional linear correlation. In the cyclic voltammetry tests, at different concentrations of urea, it was noticed that the intensity of the current increased concomitantly with the increase in the concentration of urea indicating the sensitivity of the material. The studies carried out revealed that the V_2O_5/urease material proved to be sensitive in the detection of urea, in addition to being relatively simple and sensitive to the analyte being a potentially interesting for application in this area.

5. Acknowledgements

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6. Conflict of Interest

The authors declare that they have no conflicts of interest.

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