Development of Polymeric Enzymatic Electrodes for Ethanol Oxidation

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ABSTRACT

Enzymatic fuel cells are devices in which enzymes are located in the electrode in order to catalyze fuel oxidation. These biological catalysts, unlike inorganic ones, have the advantage of being selective, renewable and clean. The enzymes are supported onto an electronically conducting material, compatible with the enzymes, for example conductive polymers such as polypyrrole. In this project, polymeric anodes are prepared by immobilizing the enzyme alcohol dehydrogenase from S. cerevisae in polypyrrole, potentiostatically electrodeposited onto carbon paper. The applied enzymatic immobilization procedures are both direct adsorption and crosslinking with glutaraldehyde. The characterization of electrodes is made by cyclic voltammetry using a phosphate buffer solution with ethanol and β-NAD^+ as supporting electrolyte. Tests show that reversible ethanol oxidation and reduction occurs at around $0V_{SCE}$ for the polypyrrole electrodes, recorded current values due to polymer oxidation and reduction are higher by two orders of magnitude than those recorded for ethanol oxidation in carbon enzyme electrodes. Furthermore, the polymeric enzymatic electrodes crosslinked with glutaraldehyde show higher current values than those with adsorbed enzyme, which reflects a better retention of the protein in the electrode. Also, electrodes with crosslinked enzyme preserve catalytic activity for longer times than those with adsorbed enzyme. Spectrophotometric and fluorescence measurements are performed in order to determinate enzymatic activity and quantify protein, respectively. Fuel cell performance will be presented for an enzymatic direct ethanol fuel cell.

1. Introduction

Hydrogen is considered as a clean and safe energy source. The oxidation reaction of this element is object of study as part of the hydrogen technology, which uses the direct electrochemical conversion of hydrogen to generate electricity and heat in fuel cells.

In a fuel cell, hydrogen is oxidized at the anode releasing electrons and protons. While the electrons pass through an external circuit, the protons cross an electrolyte to reach the cathode where they react with oxygen to produce water. However, some limitations in performances coupled with the fact that these devices use catalysts based on precious metals and their alloys, have led to the development of biofuel cells [1].

Biofuel cells operate in the same way as conventional cells with the difference that the inorganic catalyst is replaced by a biological catalyst that could be an organism producing enzymes or the enzymes themselves [2]. The enzyme-based cells are attractive due to the advances reported recently. In this type of cell, the enzyme is located at the electrode to catalyze the fuel oxidation, being involved directly in the reaction that generates electricity, with the advantage of being a renewable and clean catalyst, selective, flexible in the types of fuel used and able to work at low to medium temperatures [3].

Even though in their own environment the enzymes have a very short lifetime, this life time can be increased by immobilization [4]. The electroconductive polymers are materials compatible with.

Polypirrole can be used as support for enzyme immobilization due to its low oxidation potential, environmental stability, sensitivity and good quality matrix [5]. When both elements are linked, they forme and enzymatic electrode and the performance is related to the level of contact between enzyme and polypirrole.

2. Experimental

Enzymatic activity was determinate by continuous spectrophotometry, following the Sigma quality control test for alcohol dehydrogenase (EC 1.1.1.1), and electrochemically by cyclic voltammetry using a carbon paper electrode as working electrode, in a buffer solution with NAD^+, ethanol and 751units/ml of alcohol dehydrogenase as supporting electrolyte.

For electrode preparation, polypyrrole was electrodeposited onto $1cm^2$ carbon paper by a potentiostatic method, applying 0.7V vs $_{SCE}$ for 420s in a solution of 0.1M monobasic sodium phosphate/0.1M pyrrole. The enzymatic immobilization was made by direct adsorption, adding alcohol dehydrogenase (6mg/ml) onto the polymer, and by crosslinking, adding alcohol dehydrogenase (6mg/ml) onto the polymer followed of the addition of 0.1% glutaraldehyde solution. For drying, in both cases, the electrode was kept in a desiccator at room temperature for approximately 5 hours.

The electrode characterization was performed by cyclic voltammetry in a range of -0.3V to 0.3V vs $_{SCE}$ at 100mV/s in a three electrode cell with phosphate buffer solution at pH8.8, NAD^+ and 5mM ethanol as supporting electrolyte.

In order to quantify the amount of protein immobilized onto the electrode, the NanoOrange Proteín Quantitation assay was perfomed. The NanoOrange reagent produces fluorescence in presence of the protein, so it was added to

enzymatic electrodes and incubated to 95°C for 10 min protected from the light. After that, the electrode was cooled to room temperature and the fluorescence intensity was measured in a spectrofluorometer at 580 nm with an excitation wavelength of 470 nm.

3. Results and discussion

The enzymatic activity in solution measured by spectrophotometry was of 365.45 units/ml enzyme. Results for cyclic voltammetry are shown in figure 1. An oxidation peak corresponding to ethanol oxidation can be observed around 0V vs $_{SCE}$, this peak was better defined at a sweep rate of 100mV/s, with values in the order of μA.

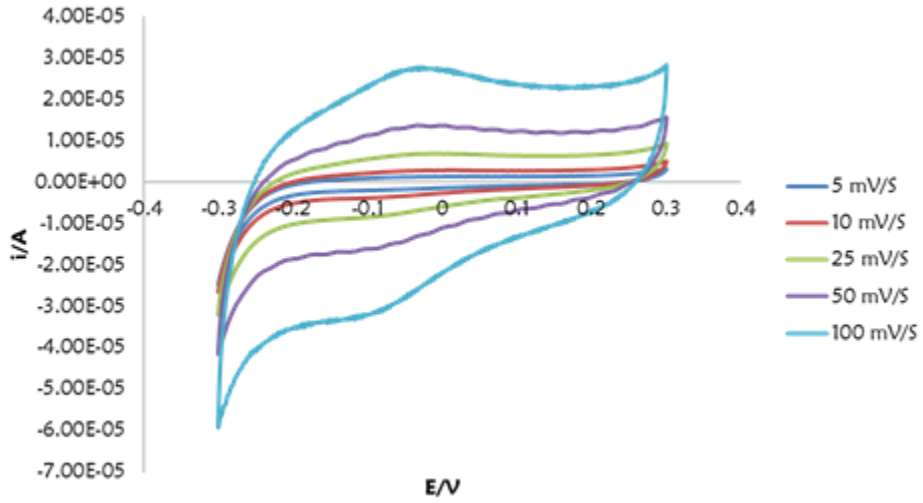

Figure 1 Voltammetry of carbon electrode to different swept potential in phosphate buffer solution 0.1M, pH 8.2, alcohol dehydrogenase 751units/ml, ethanol 2mm and NAD^+ 1.5mM, last cycle.

The amount of polypyrrole deposited was calculated to be 559μg, based on the deposition curve, and its characterization with phosphate buffer solution shows that polymer oxidation and reduction occurs around 0.6V and -1V vs $_{SCE}$, respectively. This process therefore does not interfere in the potential range for enzyme activity.

The polymeric electrode with adsorbed enzyme does not show a defined oxidation peak for ethanol due to the overlap by the polymer oxidation in the same electrolyte conditions generating higher current values than those for the carbon electrode. However, there was an evident change in the performance of polymeric electrodes with and without enzymes in ethanol containing electrolyte, reaching current values in the order of mA for enzyme containing electrodes, related to ethanol oxidation. Moreover, the electrode with crosslinked enzyme generates higher currents than those corresponding to the electrode with adsorbed enzyme, which was related the improved retention of the protein.

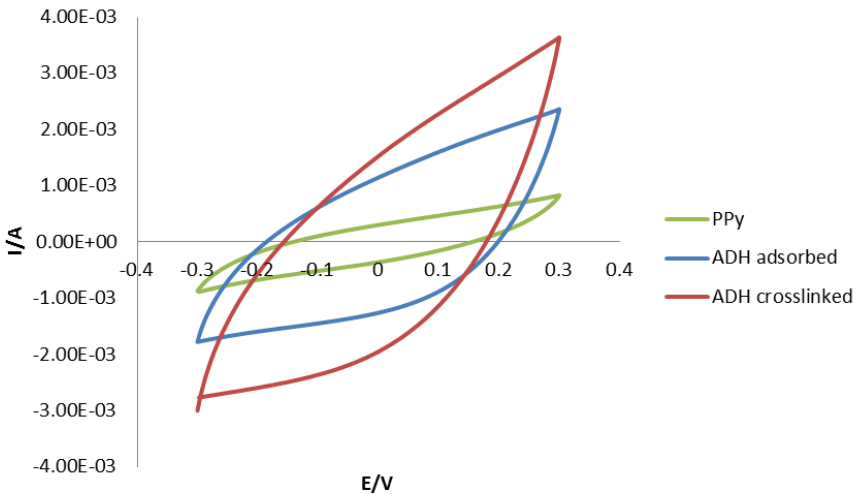

Figure 2 Voltammetry of polypyrrole, enzyme adsorbed electrode and enzyme crosslinked electrode, second cycle.

Cyclic voltammetry was realized over a three day period to determine enzyme degradation. For absorbed enzymatic polymeric electrodes, the current is virtually constant. Small variations probably are result of small temperature change. The current recorded for crosslinked enzyme was more stable.

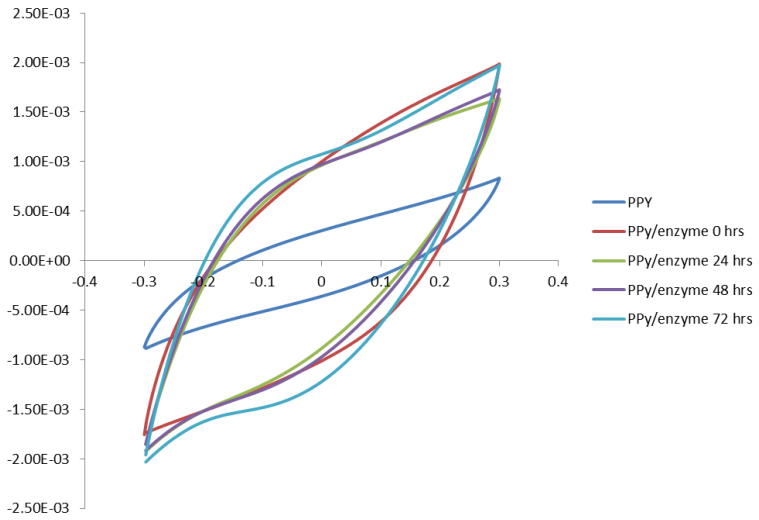

Figure 3 Cyclic voltammetry in different time of adsorbed enzyme electrode, second cycle.

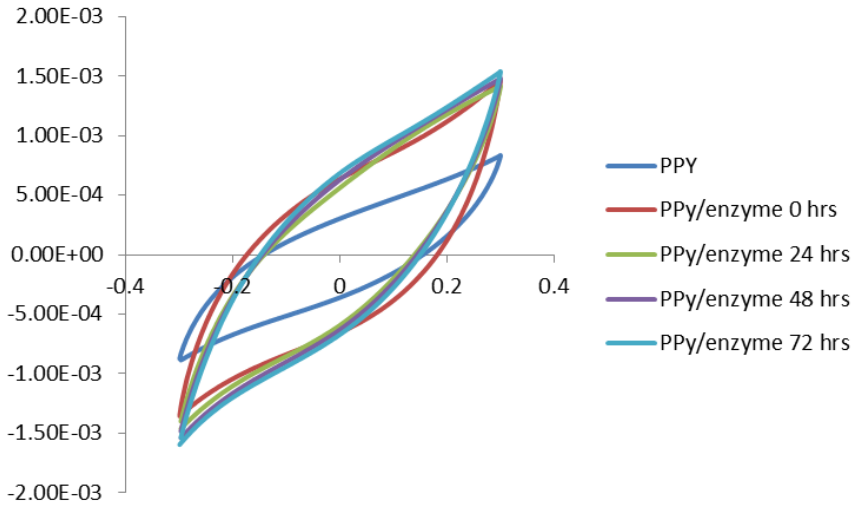

Figure 4 Cyclic Voltammetry in different time of crosslinked enzyme electrode, second cycle.

Finally, it was not possible to quantify the protein by the NanoOrange assay, because of the interaction between the NanoOrange reagent and the polypyrrole, producing higher fluorescence than those emitted by the protein.

4. Conclusions

It was found possible to inmobilize the alcohol dehydrogenase enzyme by direct adsorption and by crosslinking with glutaraldehyde onto polypyrrole electrodes and to maintain biocatalytic activity for ethanol oxidation for several days.

Immobilization of alcohol dehydrogenase onto polymeric electrode results in a better performance than immobilization onto carbon electrode.

The crosslinked immobilization method results in higher stability in enzymatic activity than the direct adsorbed immobilization method.

5. Akcnowledgements

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6. References

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