

PREPARATION, CHARACTERIZATION AND APPLICATION OF ALKANETHIOL SELF-ASSEMBLED MONOLAYERS MODIFIED WITH TETRATHIAFULVALENE AND GLUCOSE OXIDASE AT A GOLD DISK ELECTRODE

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In recent years, one of the most widely employed procedures to immobilize enzymes onto solid electrodes is the binding of these proteins to self-assembled monolayers (SAMs) constituted by bifunctional compounds. Thus, different electrode materials, specially gold, have been coated with SAMs containing sulphur compounds, such as alkanethiols, by spontaneous adsorption onto the electrode surface. Then, the well-ordered monolayers formed can be used to immobilize enzymes close to the electrode surface with a high degree of control over the molecular architecture of the recognition surface.

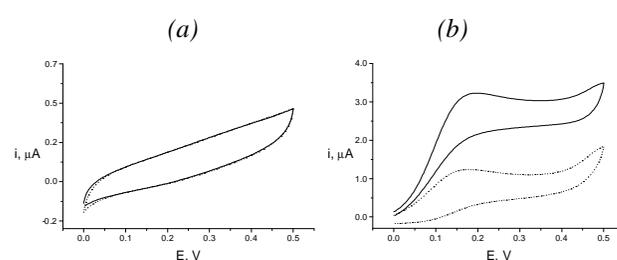
The use of SAMs is associated with various advantages in electroanalysis and particularly in the development of biosensors, such as the dramatical reduction of the double-layer charging current, thus increasing the sensitivity due to the lower background current obtained [1]. Consequently, SAMs modified electrodes are nowadays being increasingly used in the development of amperometric enzymatic biosensors for the determination of different analytes, really constituting a promising research field.

In this work, the results obtained with a gold disk electrode modified with alkanethiol SAMs and glucose oxidase (GOD), and the redox mediator tetrathiafulvalene (TTF) immobilized atop are presented, together with the optimization of the modified electrode preparation procedure, and the characterization of the binding of the SAM to the gold electrode.

Previous to the SAM deposition, the gold electrode needs to be cleaned for which several polishing and washing procedures were tested. The chosen chemical pretreatment consist of first polishing the electrode with diamond powder (3 μm) for 1 min, then washing it by sonication in deionized water for 1 min, followed by a 1 h immersion in a hot 2 mol L⁻¹ KOH solution; then the electrode is rinsed with H₂O, immersed in H₂SO₄c for 10 min, rinsed with H₂O, immersed in HNO₃c for 10 min and rinsed once more with deionized water. The electrochemical surface of this electrode was determined by applying the Randles-Sevcik equation to a cyclic voltammogram of 0.5 mM Fe(CN)₆³⁻/Fe(CN)₆⁴⁻, as 0.112 cm².

SAMs produced from alkanethiols with different length chains give different characteristics [2], so mercaptopropionic acid (MPA), mercaptoundecanoic acid (MUA), and mixtures of both were tested in the formation of SAMs. The association of these SAMs to the gold electrode was evaluated by chronopotentiometry and cyclic voltammetry using 1 mM Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ as redox probe. Stable SAMs were obtained in all cases, although the best analytical signals were achieved, as expected, for MPA SAMs. These SAMs are formed by immersion of the clean electrode in a 40 mM MPA solution in EtOH/H₂O 75/25 (v/v) for 15 h. This SAM is stable for more than 40 days when kept in dry conditions.

Two enzyme immobilization procedures atop the SAM were assayed. The first one consisted of covalently binding the enzyme to the terminal acid groups of the MPA, previously activated in a N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (ECD) solution in phosphate buffer (pH 7.4) for 1 h. No good responses were observed for glucose in the presence of TTF in solution due to the low signal-to-noise ratio obtained. The second immobilization procedure allows the attachment of both, enzyme and mediator, by cross-linking with glutaraldehyde; thus, the SAM modified gold electrode was covered with 12.5 U of GOD and 1.5 μmol of TTF and then it was immersed in a 25 % glutaraldehyde solution for 1 h. The modified electrode obtained gave a lower background noise and better amperometric responses (more sensitive and more reproducible) signals to successive additions of glucose than using the covalently binding method. Figure 1 shows cyclic voltammograms obtained with this electrode.



Cyclic voltammograms for 2.5 mmol L⁻¹ glucose (—) in 0.05 mol L⁻¹ phosphate buffer (pH 7.4) (- -) on a MPA-AuE (a), and on a MPA-AuE modified with GOD and TTF by cross-linking with glutaraldehyde (b).

Linear calibration curves in the range 5.0 10⁻⁶-1.0 10⁻² mol L⁻¹ glucose with a detection limit of 1.3 10⁻⁶ mol L⁻¹ have been obtained by amperometry in stirred solutions for an applied potential of +0.20 V. A RSD of 6.3 % (n=10) was obtained at a concentration level of 5.0 10⁻⁴ mol L⁻¹, and no leaching of the enzyme and mediator is observed during the whole working day. The modified electrode is stable in dry conditions for 24 h. The influence of various potential interferents on the amperometric glucose signals is now being studied in order to apply the developed electrode to the determination of this compound in soft drinks.

[1] S.E. Creager and K.G. Olsen; *Anal. Chim. Acta*, **307** (1995) 227.

[2] J.J. Gooding and D.B. Hibbert; *Trends in Anal. Chem.*, **18** (1999) 525