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Polymeric luminol on pre-treated screen-printed electrodes for the design of performant reagentless (bio)sensors

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ABSTRACT

A performant reagentless electrochemiluminescent (ECL) system for H_2O_2 detection based on electropolymerized luminol is first presented. In this work, polyluminol is formed under near-neutral conditions onto pre-treated screen-printed electrodes (SPEs). Pre-treatment conditions of the working electrode surface have been optimized so as to obtain the best ECL responses to H_2O_2 that were increased by a factor as high as 400. Galvanostatic polymerization has been tested as a new process to form polyluminol films. Good performances were obtained in terms of responses to H_2O_2, with quite the same linear range as the ones obtained under potentiostatic and potentiodynamic modes.

The association of the polyluminol film with an H_2O_2-producing oxidase has also been investigated, using choline oxidase as a model enzyme. Silica glasses obtained by the sol–gel process have been employed for biomolecule immobilization. Polymeric luminol has been coupled with choline oxidase-immobilizing gel under bilayer or monolayer configurations. In the first case, enzyme has been immobilized in a silica gel formed on a polyluminol film. In the second case, the enzymatic gel formed on SPE was soaked in a solution containing monomeric luminol units, allowing them to diffuse and penetrate throughout the porous gel. Then, electrodeposition was performed to polymerize the luminophore within the silica matrix. In both cases, choline could be detected down to micromolar concentrations.

1. Introduction

Electrogenerated chemiluminescence (ECL) of luminol is widely used to develop very sensitive biosensors allowing the detection of analytes at low concentrations and over a wide range. Such an optical detection system is mainly based on the presence of luminol in solution. In order to develop easy-to-use reagentless systems that avoid conventional luminophore addition, its immobilization on a transducer surface appears attractive [1,2]. Based on the structural analogy between aniline and luminol, electropolymerization of luminol on a glassy carbon electrode has been previously reported in an acidic (0.5 M H_2SO_4) solution [3]. Luminol electropolymerization appeared to be compatible with ECL reaction and quite high H_2O_2 concentrations (linear range between 10^{-5} and 10^{-3} M) could be detected with polyluminol formed under such drastic acidic conditions [4].

Recently, our group proposed an original polyluminol film preparation, under mild near-neutral buffered conditions, so as to be suitable for enzyme activity preservation [5]. We demonstrated that polyluminol formed under a potentiodynamic or a potentiostatic mode in a pH 6 buffered solution is able to act as an electrochemiluminescent luminophore, like luminol monomer does when it is more classically used in solution. Under these conditions, H_2O_2 detection was possible in a wide linear range, extending from 7.9×10^{-8} to 1.3×10^{-3} M.

Until now, luminol electropolymerization reported in the literature was carried out on conventional electrodes such as ITO [4,6], glassy carbon [3,6–8], gold [6–10] or Pt [6,8,11]. Our group has described for the first time the polymerization of luminol on non-conventional screen-printed electrodes (SPEs) [5]. SPEs can combine ease-of-use and portability with simple, inexpensive fabrication techniques. The modest cost of SPEs has further enhanced their desirability because it allows the devices to become disposable.

Screen-printing technology involves the printing and curing of various inks onto planar ceramic or plastic supports. Commercial carbon ink formulations are commonly used for printing the working electrode, whereas silver-based formulations are used for obtaining the reference electrode. Carbon inks are particularly attractive because they are relatively inexpensive and lead to low background currents and a wide potential range. Compared to solid graphite electrodes, the printing inks for SPEs contain some mineral binders or insulating polymers to improve the adhesion onto the substrate.

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Simple electrochemical surface treatment methods have been applied to SPEs in order to remove organic ink constituents or contaminants and to increase surface roughness or functionalities. In the first part of this work, the effect of various electrochemical surface treatment methods on the ECL responses of the polyluminol-based sensor to H_2O_2 has been examined.

Electropolymerization processes are commonly performed by using potentiodynamic, potentiostatic or galvanostatic modes. This latter is used here for the first time and the performances of the sensors are compared in terms of hydrogen peroxide detection according to the polymerization mode.

Silica glasses obtained by the sol–gel process have been widely employed for biomolecule immobilization [12,13]. The use of sol–gel glasses as an enzyme immobilization matrix has many advantages, i.e. the ability to entrap large amounts of enzymes, simplicity of preparation without covalent modification, and flexibility of controlling pore size and geometry. Moreover, many enzymes have been immobilized within these matrices while maintaining their native properties. The sol–gel method has been widely employed for the development of electrochemical biosensors [14,15]. Silica is also well-suited for fabrication of optical sensor membranes because this material does not absorb in the near UV and visible [16]. Nevertheless, it can be noticed that only few papers deal with the use of this method for fabrication of electrochemiluminescent biosensors [17].

In this work, choline oxidase (ChOD)-immobilizing silica gel was associated to polymeric luminol film to develop a polyluminol-based biosensor allowing choline detection. Two configurations have been tested. The first one is a bilayer configuration. In this case, luminol is first polymerized at pH 6 onto a SPE. Then, ChOD is immobilized in a sol–gel membrane formed on the above modified working electrode. In the second case, a monolayer configuration has been developed using a “diffuse and polymerize” process that has been mainly described with polyaniline [18–22]. In this strategy, non-conductive silica sol–gel porous films have been used as template for the polymer electrosynthesis. Aniline [18–22] or thiophene [23] diffused into silica gel and polymerized inside the porous structure. In the work presented here and based on the same principle, luminol is shown to be able to polymerize into a porous sol–gel film by soaking the planar supported sol–gel film in a solution of monomer, allowing it to penetrate and diffuse throughout the porous structure. Electrochemical polymerization is then used to grow the polymer within the silica gel.

2. Experimental

2.1. Reagents

Hydrogen peroxide, veronal (sodium diethylmalonylurea), sodium carbonate, sulphuric acid, potassium hydrogen phosphate and potassium hexacyanoferrate (III) were obtained from Prolabo. Potassium chloride was supplied by BDH. Luminol (3-aminophthalhydrazide), sodium bicarbonate, choline oxidase from *Alcaligenes species* and choline were purchased from Sigma. Tetramethoxysilane (TMOS) was obtained by Fluka. Nafion and potassium hexacyanoferrate (II) were supplied by Aldrich. DEAE (diethylaminoethyl)-Sepharose (average particle size 45–165 μm) fast flow was obtained from Pharmacia. All buffers and aqueous solutions were prepared with double-distilled demineralised water. A 5.5 mmol L^{-1} luminol stock solution was prepared in 10^{-2} M KOH.

2.2. Electrode fabrication

The SPE, comprising a carbon working electrode (area: 19.6 mm^2) and a Ag/AgCl reference electrode, was mass-fabricated in-house by a multi-stage screen-printing process using a DEK 248 screen-printing machine (DEK, UK). A polyester monofilament fibre screen (DEK, UK) characterized by a mesh size of 260 counts per inch and a thickness of 13 μm was used to print the different inks onto a plastic. The basal tracks were printed first using silver-based ink (Electrodag 418 SS, Acheson, USA) and dried 15 min at 100 °C (Scheme 1A). In the second step, the carbon paste (Electrodag PF-407A, Acheson, USA) was printed onto the basal tracks and cured 15 min at 100 °C (Scheme 1B). Then, the Ag/AgCl paste (Electrodag 6037 SS, Acheson, USA) was printed onto one of basal tracks and dried at 100 °C (Scheme 1C). Finally, an insulating ink was deposited (Minico M7000, Acheson, USA) which was cured 20 min at 100 °C (Scheme 1D).

2.3. Electrode pre-treatment

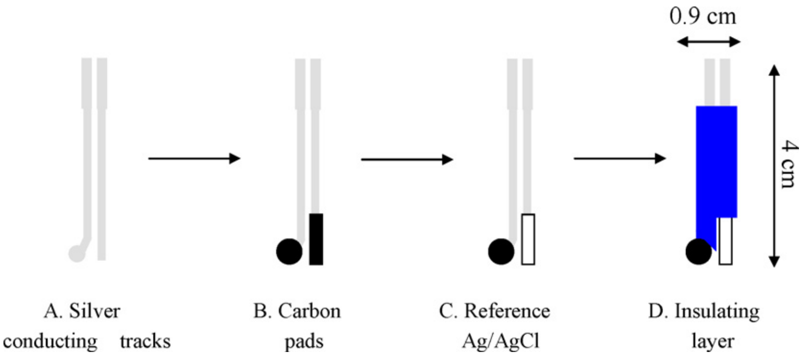
Screen-printed electrodes were pre-treated in different ways:

(i) Electrochemical pre-treatments of SPEs were carried out by applying anodic potentials in various electrolyte solutions (1 M H_2SO_4, 1 M $NaHCO_3$, and saturated KCl or Na_2CO_3) for different periods or until a given charge.
(ii) A single voltammetric cycle was carried out in 1 M H_2SO_4 between −1200 and +1500 mV vs. Ag/AgCl at a scan rate of 100 mV s^{-1}.
(iii) Ultrasonic bath during 7 min.

After activation, SPEs were rinsed with de-ionized water.

2.4. Luminol polymerization and electrochemical characterization

The electrochemical formation of polyluminol films was performed using a Voltalab PGZ 301 potentiostat (Radiometer Analytical, Villeurbanne, France). An auxiliary platinum electrode



Scheme 1. Screen-printing electrode fabrication process.