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Redox probe-free readings of a β-amyloid-42 plastic antibody sensory material assembled on copper@carbon nanotubes

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ABSTRACT

This research work describes the synthesis of a new mediator-free electrochemical sensor, containing an electrochemically active ingredient at the carbon-working electrode. For this purpose, carbon nanotubes were modified with copper nanoparticles (CNT-CuO) and casted on the carbon-area. This electroactive film also acted as substrate to assemble the biorecognition element.

As proof-of-concept, the 3-electrode system was made sensitive to the peptide β-amyloid42 (Aβ-42), by assembling a plastic antibody on top of the electroactive film. The plastic antibody was obtained by eletropolymerizing aniline (ANI) at neutral pH, under the presence of the template (Aβ-42). Next, the template molecule was removed from the polymeric network by acidic treatment. The vacant sites so obtained preserved the shape of the imprinted protein and were able to rebind new peptide molecules.

SEM, XRD and RAMAN analysis were performed in order to control the surface modification of the carbon electrode. The ability of the biosensor to rebind Aβ-42 was monitored by square wave voltammetry (SWV). Redox peaks were centred at +0.4 V and peak currents decreased for an increasing concentration of Aβ-42. The reproducibility of the analytical signal was 8.37%, expressed in terms of the relative standard deviation (RSD, n = 3) of an Aβ-42 standard solution of 1.0 ng/mL. The detection limit was 0.4 (±0.03) pg/mL. The application of the device was tested in serum samples, spiked with Aβ-42 from 1.0 to 66.0 ng/mL. The obtained recovery data ranged from 88 to 93%.

The greatest achievement of this work relates to the elimination of a redox probe reading-stage in electrochemical biosensing, by incorporating the electroactive element within the working electrode. Overall, this approach opens doors for direct sample readings, avoiding the incubation of active redox elements on the sensing area for analytical purposes. In addition, the developed biosensor showed excellent proprieties in terms of response time and simplicity, showing a remarkable potential for on-site application in medical research and clinical diagnosis.

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1. Introduction

Electrochemical biosensors may turn out a promising alternative to conventional methods reported in the literature for biochemical analysis. These biosensors offer fast response time, portability and simple equipment requirement, which in turn allows point-of-care analysis [1]. In general terms, electrochemical biosensors are simple devices that combine a conductive substrate (A) with a suitable biorecognition element (B). This biorecognition element (B) is responsible to interact with the target compound, in a sensitive and selective way, by incubating the sample on it for a given period of time. The resulting interaction may be monitored directly, when the target is electroactive, or indirectly, by placing a standard redox probe on top and using it to check any alteration upon a given electrical property out coming from such interaction. As few compounds hold electroactivity features, most interactions are monitored indirectly. Iron(III) hexacyanoferrate(II) and Ruthenium hexamine are common probes used for this purpose. These are used in diluted solutions, casted over all electrodes after the sample incubation. Creating a redox probe-free device means that this step would no longer required, even if the target compound would not have electroactivity. For this purpose, the redox-probe

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could be included directly into the substrate, thereby avoiding its external addition after sample incubation.

Regarding the conductive substrate (A), there are many electrical-based biosensors employing carbon based nanomaterials, such as carbon nanotubes (CNTs), due to their remarkable features in terms of size (high surface/volume ratio), conductivity and chemical stability [2–4]. Metallic nanoparticles (NPs) are also employed for the same purposes. These rely mostly in gold, platinum, or silver, but may also be of palladium, nickel, and copper [5]. Biosensors combining both CNT and metallic NPs also show several advantages in terms of overall analytical features due to the amplified surface area and mass transport [5–11]. This was proven by the high sensitivity and fast electrochemical detection features obtained, promoted by the increase in the electrocatalytic active area and enhancement of electron transfer in the oxidation reactions especially in biosensing devices. But such NPs are not employed as redox probe. This possibility is explored herein, by including redox active nanoparticles among a CNT support and checking their electrochemical signal after interaction between target compound and biorecognition element. As proof-of-concept, this principle is tested for copper nanoparticles and for Aβ-42 as target compound, a biomarker in Alzheimer Disease (AD).

AD is a progressive and degenerative disease of the central nervous system leading to memory loss and dementia [12]. AD diagnosis during life is a hard task and still relies in clinical examination [13]. To increase the accuracy of diagnosis, researchers are trying to find novel AD biomarkers. Preference is given to biomarkers allowing non-invasive procedures (urine and blood), in early AD condition and differential diagnosis [14]. In this scenario, the detection of Aβ-42 in serum is a strong candidate among several AD candidate biomarkers. Several methods established for this purpose include enzyme-linked immunosorbent assay [15,16], radioimmunoassay [17–19], fluorescence [20–25], chemiluminescence [26], surface plasmon resonance spectrometry [27,28], cell-based assay [29], and two-photon scattering assay [30] and electrophoresis [31–37]. Nevertheless, all these show advantages and limitations. Antibodies as a natural biorecognition element are simple and effective, but expensive, limited use, lack in stability, cost, response time and need a redox probes for all electrochemical measurements. Synthetic biorecognition element as oligopeptides with amino acid sequence and aptamers demonstrates good operational features in terms of LOD and selectivity. However, these assays are costly, laborious in terms of synthesis process and in addition need redox probe reading-stage in electrochemical biosensing.

As far as we know, only one recent paper has been reported in literature based on plastic antibodies for Aβ oligomers detection in POC by Moreira and co-authors [38]. The sensor was fabricated by peptide biomarker (Aβ1-42) imprint using a sugar monomer (α-cyclodextrin, α-Cd) as polymeric matrix. The device showed good overall analytical performance with LOD 0.20 ng/mL (SWV) assay [38]. Comparing to this, the present work shows improvements in terms of LOD (0.4 ± 0.03 pg/mL) and no need of incubating an active redox element on the sensing area.

This approach is simple and effective, but expensive and of limited use. Tailoring plastic antibodies by molecularly-imprinted technology is a promising alternative to antibodies produced in nature, typically of an animal source. Such biomimetic materials are typically very sensitive, selective, and resistant to pH and temperature variations [39–41].

Thus, this work relies in the synthesis of a new redox probe-free biosensor making use CNTs doped with copper NPs (CNT-Cu) as substrate and a plastic antibody as biorecognition element, applied to an AD biomarker. The plastic antibody was obtained by electropolymerizing ANI under neutral pH and in the presence of the Aβ-42. The electroanalytical features and the feasibility of its application to the determination of Aβ-42 are detailed next.

2. Experimental section

2.1. Apparatus

The electrochemical measurements were conducted with a potentiostat/galvanostat from Metrohm Autolab and a PGSTAT302N. SPEs were purchased from DROPSENS (DRP-C110) having working and counter electrodes made of carbon and reference electrode and electrical contacts made of silver. The diameter of the working electrode was 4 mm. The electrical reading of the carbon SPEs (C-SPEs) was made by placing these a switch box (BioTID/Porto-Portugal) interfacing the electrical contacts of the SPE with the electrical connections of the potentiostat/galvanostat.

The CNTs were modified by an ultrasonication bath with 50 W power and 40 kHz. Raman spectroscopy data was generated by a Thermo Scientific DXR Raman spectroscope, equipped with a confocal microscope and a 532 nm laser. A 5 mW laser power at sample was allowed for 25 μm slit aperture. Scan-electron microscopy (SEM) studies were performed on an FE-CryoSEM/EDS, from JEOL JSM 6301F, Oxford INCA Energy 350, Gatan Alto 2500 microscope, operating at 15 kV and 9.9 mm working distance.

2.2. Reagents

All chemicals were of analytical grade and water was deionized or ultrapure Milli-Q (MQ) laboratory grade. β-amyloid-42 (Aβ42) was obtained from GenSript; oxalic acid (Oac), potassium chloride (KCl), dimethylformamide (DMF), aniline (An) and PBS buffer tablets from Fluka; synthetic Cormay Serum HN (CSHN), sulfuric acid (H_2SO_4), nitric acid (HNO_3), ferrocene, toluene ($C_6H_5CH_3$),copper(II) sulphate anhydrous ($CuSO_4$), hydrazine monohydrate (N_2H_4 64–65%)were obtained from Sigma;

Stock solutions of Aβ-42 oligomer 0.5 mg/mL were prepared in PBS buffer pH 7.4. Less concentrated standards were obtained by accurate dilution of the previous solution in PBS buffer or in CSHN, diluted 10 times in PBS buffer pH 7.4. Electrochemical assays were performed in KCl 0.1 mol/L.

2.3. Synthesis of the CNT-Cu composites

CNTs were synthesized by spray-assisted chemical vapor deposition (CVD). The solution used for this purpose was 5% ferrocene made in toluene. This solution was nebulized by an Ar flow rate (2.2 L/min), through a quartz tube heated at 850 °C, by 30 min [42]. The CNTs so produced were further functionalized with carboxylic acid groups (COOH) by ultrasonication in a nitric acid/sulfuric acid (3:1) solution for 24 h. After the sonication procedure, the sample was washed several times with deionized water until pH ~7 and dried at 80 °C. To decorate CNTs with CuO nanoparticles, a suspension of 1 mg/mL of carbon nanotube functionalized (CNTs-COOH) was dispersed in 1% $CuSO_4$ solution prepared in water. The available Cu^{2+} was reduced by a 0.1 M N_2H_4 solution, gently added to the mixture under stirring, and maintained at 90 °C in a water reflux for 1 h.

2.4. Design of the plastic antibody on the carbon electrode

As amount of 1 mg of CNT-Cu composite prepared as described was dispersed in 1 mL of DMF and then immersed in an ultrasonic bath for 4 h until a black homogeneous suspension was obtained. About 5.0 μL of the final dispersion was applied in the working electrode area (carbon working electrode). After drop-casting, the

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