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Enhanced electrochemical response of structurally related antioxidant at nanostructured hybrid films

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ABSTRACT

In this work, the redox behaviour of structurally related antioxidants, such as catechol, hydroxycinnamic acid and flavonoid derivatives, has been investigated at electrode surfaces modified with different gold nanoparticles (AuNPs)–chitosan films. Our findings demonstrate that the chemical structural features of the antioxidants play a key role in the interaction with modified electrodes that strongly affect the electrochemical response. In particular, a better response was observed for molecules with two hydroxyl groups in ortho position of the catechol ring and with a low steric hindrance. However, the redox behaviour cannot be explained only on these bases, since the nanostructure and surface functional groups of AuNPs–chitosan modified electrodes have also to be considered. The presence of interconnected metal nanoparticles into chitosan film strongly affects the electron transfer properties, whereas the surface functional groups can promote the interaction with selected antioxidants acting as scavenger. The understanding of the key factors that affect the electrochemical response of antioxidants at electrode surface modified with nanostructured hybrid films is a key issue and could significantly contribute to the development of highly efficient electrochemical sensors.

1. Introduction

Natural antioxidants are species of great interest in many areas, as food chemistry, health care and clinical applications. They have beneficial effects on human health and could play an important biological role in the prevention and treatment of many pathologies (as cardiovascular disorders, cancer, diabetes, inflammation, arthritis, etc.) and to protect from oxidative stress [1–6]. The classification of antioxidants is commonly carried out on the basis of the chemical structure that determines their reactivity. However, their antioxidant action is also strictly related to the redox properties and consequently the knowledge of the latter is very crucial for a better understanding of antioxidant capacity. To this purpose, an electrochemical study is required to correlate chemical structure, activity and redox behaviour of antioxidants.

The electrochemical methods have been extensively used to investigate the redox properties of various species and as analytical tool for the determination of redox target molecules. At present, the analysis of antioxidants is usually carried out using chromatographic techniques, which require sophisticated equipments and laborious analytical procedures [7–9]. In the past few years, the use of electrochemical methods for analytical purposes is receiving increasing interest [10–18], since they are fast, accurate, sensitive and can be used for the analysis of different and complex matrices with a low cost. In this context, the attention is focused on polyphenols, one of the most important classes of antioxidants. Both electrochemical sensors and biosensors are widely used for their determination. However, the electrochemical responses have been studied only from an analytical point of view, whereas the relationship between the antioxidant chemical structure and electrochemical behaviour has been neglected [19].

The understanding of key factors that affect the electrochemical response of analytes could promote the design of highly efficient sensors. To this aim, the role of the chemical structural features of antioxidants and nature of electrode surface has to be considered. Recently, our group has reported that electrodes modified with nanocomposite films can be successfully used for the analysis of antioxidants, as caffeic acid, in complex matrices [20,21]. These films consist of gold nanoparticles (AuNPs) embedded into chitosan, a biodegradable and biocompatible polymer containing many hydroxyl and amino groups that can interact with the analytes.

Herein we have investigated the electrochemical response of structurally related antioxidants on electrodes modified with different gold–chitosan nanocomposite films. In order to evaluate how the chemical structural features of analytes affect the

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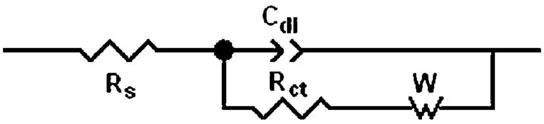

Scheme 1. Randles equivalent circuit.

electrochemical behaviour, we have investigated different types of antioxidants such as catechols, hydroxycinnamic acids and flavonoids, that differs for the functional groups and the steric hindrance.

2. Experimental

2.1. Sample preparation

The synthesis of chitosan-stabilized AuNPs was carried out by using a green route, according to our recently published procedure [20].

Medium-molecular-weight chitosan (5800 g mol^{-1}), composed of β-(1–4)-linked D-glucosamine and N-acetyl-D-glucosamine with a degree of deacetylation of 75–85%, was purchased from Aldrich. In a typical preparation, a suitable amount of chitosan (0.5 wt.%) was dissolved in a 0.1 M aqueous solution of acetic acid (AA), malonic acid (MA), or oxalic acid (OA) by stirring the system overnight.

An appropriate amount of $HAuCl_4$ aqueous solution was added to the chitosan/organic acid solution in order to obtain a 1 mM gold concentration. After the addition of the gold precursor, the solution was heated to 70 °C with reflux under magnetic stirring until the colour turned red. The resulting samples were labelled as AuXX-CHIT, where XX refers to the organic acid that was used in the synthesis. The synthesis with AA was also carried out lowering the concentration of gold precursor from 1 to 0.5 mM and the so-obtained sample was labelled as AuAA-CHIT0.5.

2.2. Electrochemical measurements

2.2.1. Assembling of AuNPs modified gold electrode

After the polishing steps [20], Au electrodes were modified by drop casting 1 μL of AuNPs solution onto electrode surface and subsequent air drying at room temperature. To increase the number of particles at the electrode this procedure was repeated three times.

2.2.2. Procedure

The electrochemical experiments were performed using an Autolab PGSTAT12 potentiostat/galvanostat (Eco Chemie BV, Utrecht, Netherlands). Autolab NOVA software system was used for cyclic voltammetry (CV) and for electrochemical impedance spectroscopy (EIS).

Electrochemical measurements were performed in a conventional two-compartment three-electrode cell with a working electrode (bare or modified gold electrode), an Ag/AgCl electrode as reference and a Pt electrode as counter. Gold electrode (2 mm in diameter,) was purchased from Metrohm Autolab (Utrecht, Netherlands).

AuNPs modified electrode was used in phosphate buffer for the electrochemical investigation of different antioxidants at a concentration of 0.004 M. Phosphate buffer solutions (pH 7.00, 0.1 M) were prepared using sodium dihydrogen phosphate dihydrate (Fluka, Microselect for molecular Biology), adjusting pH with NaOH drops. The buffer solutions were prepared using distilled-deionised water and were kept refrigerated to minimize bacterial growth.

For the fitting of the data obtained by EIS, we used Z-views software (Scribner Associates, Inc.) using the Randles equivalent circuit [22] as shown in Scheme 1.

This equivalent circuit is given as a model for describing the electrolyte/electrode interphase. It consists of R_s (bulk solution resistance) in series with a parallel combination of R_{ct} (interfacial charge transfer resistance), Z_W (diffusion of the analytes in solution), and C_{dl} (double layer capacitance).

EIS was performed in a phosphate buffer solution pH 7.00 0.1 M, containing different catechols at the concentration of 0.004 M, with frequency range from 10^4 to 0.1 Hz and a signal amplitude of 10 mV, at a working potential corresponding to the peak potential of the analyte at the bare and at the modified electrode.

The precision of all the electrochemical data @ the different electrode was evaluated by measuring 0.004 M of each antioxidant using four electrodes ($n = 4$).

2.3. Characterization

UV–vis absorbance spectra of the as-prepared chitosan-stabilized gold solutions were collected using a double beam V-660 spectrophotometer (Jasco).

FE-SEM characterisations were carried out by a high brilliance LEO 1530 field emission scanning electron microscope apparatus, equipped with an energy dispersive X-ray spectrometer (EDS) INCA 450 and a four sectors backscattered electron detector (BSD). FE-SEM images were recorded both in SEI and BSD mode at different acceleration voltage ranging from 3 to 20 kV, according to our procedure previously reported [20]. The samples for the analysis were prepared by deposition of AuNPs–chitosan solutions on a silicon substrate and subsequent drying at room temperature. Before the analysis the surfaces were coated with a thin layer of carbon in order to avoid charging effects.

Fourier transform infrared (FTIR) spectra were collected using a Jasco 4100 FT/IR spectrophotometer equipped with a PRO450-S attenuated total reflectance unit. The samples for the analysis were prepared by depositing gold–chitosan colloidal solutions over a glass substrate and subsequent drying at room temperature.

3. Results and discussion

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) have been used to explore antioxidant molecules, such as catechol, 4-*tert*-butylcatechol, protocatechuic acid (3,4-dihydroxybenzoic acid), caffeic acid, ferulic acid, chlorogenic acid, rosmarinic acid, p-cinnamic acid, rutin, quercetin and catechin. All these species can be considered as structurally related, as shown in Table 1. By using electrochemical techniques, we have explored differences in their redox behaviour, which could be reasonably associated both to the different structures and to different interactions with electrode materials. Consequently, electrodes modified with different gold–chitosan nanocomposite films have been considered in this work.

The formation of a collaborative conductive network of interconnected metal nanoparticles into the polymeric film strongly affects the ability of electron transfer, whereas the functional groups at modified electrode surface are responsible for the chemical interaction with the antioxidants acting as scavengers.

The synthesis of gold–chitosan nanocomposites was carried out according to a recently published green procedure [20] using different carboxylic acids, as acetic, malonic and oxalic acid, and the obtained materials were labelled as AuAA-CHIT, AuMA-CHIT and AuOA-CHIT, respectively. The formation of nanosized AuNPs was confirmed by UV–vis spectroscopy due to the presence of a typical surface plasmon resonance band (see Supplementary Data, Fig. A1 and Table A1). Depending on the synthetic procedure, the size and distribution of AuNPs into chitosan films change significantly