Application of a nanostructured platform and imprinted sol-gel film for determination of chlorogenic acid in food samples

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ABSTRACT

Chlorogenic acid (CGA) is a polyphenol derivative that widely exists in higher plants like fruits, vegetables, black teas, and some traditional Chinese medicines. In this work, we have proposed a sensitive and selective electrochemical sensor for detection of CGA. The sensor was based on a glassy carbon electrode (GCE) modified with a functional platform by grafting vinyltrimethoxysilane (VTMS) in multiwalled carbon nanotubes (MWCNTs) and covered by a molecularly imprinted siloxane (MIS) film prepared using the sol-gel process. The VTMS was grafted onto the surface of the MWCNTs via *in situ* free radical polymerization. The MIS was obtained from the acid-catalyzed hydrolysis/condensation of a solution consisting of tetraethoxysilane (TEOS), phenyltriethoxysilane (PTEOS), (3-aminopropyl)trimethoxysilane (APTMS), and CGA as a template molecule. The modification procedure was evaluated by differential pulse voltammetry (DPV) and scanning electron microscopy (SEM). Under optimized operational conditions, a linear response was obtained covering a concentration ranging from $0.08 \ \mu mol \ L^{-1}$ to $500 \ \mu mol \ L^{-1}$ with a detection limit (LOD) of $0.032 \ \mu mol \ L^{-1}$. The proposed sensor was applied to CGA determination in coffee, tomato, and apple samples with recoveries ranging from 99.3% to 108.6%, showing a promising potential application in food samples. Additionally, the imprinted sensor showed a significantly higher affinity for target CGA than the non-imprinted siloxane (NIS) sensor.

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1. Introduction

Chlorogenic acid (CGA) is a well-known substrate of polyphenol oxidases and it has antioxidant, free radical scavenging, mutation suppression, and anti-tumor properties [1]. CGA is found in plants, fruits, and vegetables such as coffee beans, apples, pears, tomatoes, blueberries, potatoes, peanuts, and eggplant [2,3]. Therefore, the determination of CGA is an important factor in different areas and in various foods. Thus, there is a need for new and more versatile analytical methods, since the methods that have already been reported, such as infrared spectrometry [4], thin layer chromatography [5], spectrophotometry [6], ^{1}H NMR spectroscopy [7], liquid chromatography [6], gas chromatography [6], and chemiluminescence [8], are time-consuming, laborious, expensive, and require more complicated instrumentation.

On the other hand, electrochemical sensors are becoming important tools in medical, food, biological, and environmental analysis due to their simplicity, high sensitivity, and relatively low cost [9,10]. Furthermore, the sensitivity and specificity of these systems can be improved considerably with the use of suitable electrocatalysts, which improve the interfacial electron transfer. Therefore, a carbon nanotube (CNT) is one type of material that has been broadly recognized as playing a key role in the process of charge transfer. This material can improve interfacial properties providing unique properties to the electrode, including high electroactive surface area [11], low charge transfer resistance [12–14], and the possibility of functionalization [15,16]. The functionalization factor is very important when the immobilization of enzymes, selective recognition elements, and/or redox mediators in the carbon nanotube structure is needed. One strategy that has been used for functionalization is the method of "grafting from" polymers. The "grafting from" approach involves the growing of polymers from the CNT surfaces via *in situ* polymerization of monomers initiated by chemical species immobilized on the CNT

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sidewalls and the CNT edges [17,18].

The monomer type used in the polymerization reaction allows the nanocomposite to be employed in the construction of platforms, modified, for instance, with molecularly imprinted polymers (MIP), forming a nanostructured platform.

However, the coupling of MIP with these transducers is still at an early stage. Most of the already reported molecularly imprinted materials are formed by acrylate or acrylic-based polymers, which present a drawback related to the slow diffusion of the imprinted analyte in the material [19]. In this sense, several types of material have been developed for imprinted films [20,21], and one of the most promising are the siloxane-based materials, which are prepared by the sol-gel method [22,23].

Siloxane-based materials can be prepared by hydrolysis and condensation of one or more silane monomers, and they are usually catalyzed by acid or base, using the sol-gel process. Siloxane-based sensors present excellent physical rigidity, chemical inertness, and thermal stability [24,25]. Therefore, the combination of the molecular imprinting technique with the sol-gel process offers an attractive alternative to constructing electrochemical sensing devices [26–29]. Indeed molecularly imprinted siloxane (MIS) possesses numerous significant advantages over conventional MIP, such as easy preparation and gelation at room temperature, which is particularly important for preserving the weak interactions with the target molecule. In addition, these materials exhibit significantly higher porosity and surface area than conventional MIP, as well as negligible swelling in organic solvents [30–32].

The aim of this work was the determination of CGA in real samples with a new sensor based on a nanostructured platform and the molecularly imprinted technique, using a sol-gel siloxane network.

2. Experimental procedure

2.1. Materials

Tetraethoxysilane (TEOS), (3-aminopropyl)trimethoxysilane (APTMS), phenyltriethoxysilane (PTEOS), vinyltrimethoxysilane (VTMS), chlorogenic acid (CGA), caffeic acid, gallic acid, 2-ethoxyethanol, anhydrous ethanol, hydrochloric acid, 2,2′-azobis-isobutyronitrile (AIBN), N,N-dimethylformamide (DMF), and multiwalled carbon nanotubes (MWCNTs) ($\geq$ 90% purity, 10–15 nm external diameter, 0.1–10 μm length) were purchased from Sigma-Aldrich. All the other chemicals were purchased from Merck (Darmstadt, Germany). All the chemicals were of analytical-reagent grade. The ultrapure water used in this work was provided by a reverse osmosis-based system.

2.2. Equipment

All the electrochemical measurements were carried out using an Autolab PGSTAT30 potentiostat/galvanostat, from Eco Chemie (Utrecht, The Netherlands), connected to a PC running GPES 4.9 software. A conventional three-electrode system, consisting of a modified glassy carbon electrode (GCE) as a working electrode, an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode, was employed. Scanning electron micrographs were obtained using a Magellan 450 scanning electron microscope (SEM) from FEI (Hillsboro, OR, United States).

2.3. Preparation of the nanostructured surface and imprinted sol-gel film on the GC electrode

In order to obtain the vinyltrimethoxysilane-modified MWCNTs (MWCNTs-VTMS), a procedure adapted from Qin and co-workers [33] and Côrrea and co-workers [34] was employed. Therefore, 50 mg of MWCNTs and 70 mL of DMF were mixed and stirred for 20 min under a nitrogen atmosphere. Then, 8.0 mL of VTMS and 80 mg of AIBN were added to the previous mixture. The flask was placed in a thermal oil bath at 70 °C, under stirring, for 48 h. After this period of time, the resulting mixture was cooled to room temperature, vacuum-filtered through a 0.20 μm PTFE membrane, and washed 10 times with DMF. The resulting solid was then dried and stored.

For the preparation of the CGE modified with MWCNTs-VTMS, 10 mg of MWCNTs-VTMS were dispersed into 1 mL of DMF by ultrasonic stirring for 30 min to give a homogeneous suspension. Then, 4 μL of the resulting suspension was dripped onto the CGE surface and the solvent was allowed to evaporate at room temperature (Fig. 1). The GCE was previously polished with 1.0 μm, 0.5 μm, and 0.3 μm alumina slurry to a mirror finish, and then it was rinsed and sequentially sonicated in doubly-distilled water and ethanol (5 min each).

For the preparation of the imprinted sensor, a mixture of 400 μL (1.8 mmol) of TEOS, 65 μL (0.27 mmol) of PTEOS, 65 μL (0.37 mmol) of APTMS, and 3.0 mL of 2-ethoxyethanol was prepared and stirred for 10 min Next, 100 μL of 0.1 mol L^{-1} HCl and 90 μL of H$_2$O were added to that mixture and stirred for 2 h to obtain a homogeneous sol at room temperature (solution 1), which is the NIS. Another solution was prepared by diluting 12.35 mg of CGA in 2-ethoxyethanol (solution 2). Then, 1 mL of solution 1 was mixed into 350 μL of solution 2 (solution 3). Therefore, solutions 1 and 3 were stirred for an additional 2 h, leading to the formation of the MIS in solution 3. Finally, solution 3 was used for the preparation of the CGA imprinted sensor, while solution 1 was used to prepare the reference sensor. For that, the electrode modified with MWCNTs-VTMS (MWCNTs-VTMS/GCE) was immersed in either solution 1 or solution 3 and rotated at 1500 rpm for 15 min The decorated sensor was left to deposit and dry overnight at room temperature. The doped CGA was extracted from the imprinted film (MIS/MWCNTs-VTMS/GCE) by repetitive immersion in a solution of methanol containing 10% acetic acid, and then air-dried for 24 h.

2.4. Electrochemical measurements

The modified MIS/MWCNTs-VTMS/GCE was dipped for 15 min in the rebinding solution (solutions for different test molecules at 100 μmol L^{-1} or sample solutions). The incubation time for rebinding experiments was selected in such way that the high sensitivity and stability of the current response were obtained. Then, differential pulse voltammograms were taken in 10 mL of phosphate buffer solution (PBS) as a supporting electrolyte, using a potential range of -0.3 V to 0.7 V, a pulse amplitude of 50 mV, and a scan rate of 20 mV s^{-1}. The PBS was a 0.1 mol L^{-1} KH$_2$PO$_4$ solution, whose pH was adjusted to about 7.0 with NaOH. All the measurements were performed at room temperature.

2.5. Sample preparation

Three samples (ground coffee, tomatoes, and apples) were obtained from local supermarkets in Rio de Janeiro (Rio de Janeiro, Brazil). For the coffee sample, a coffee drink was prepared by mixing 3.0 g of the ground coffee sample with 50.0 mL of hot water, followed by filtration. The tomato and apple samples were wholly ground (with segments and skin). Next, all of the samples were diluted with PBS. For the electrochemical measurements, 2 mL of the sample solutions were used in the incubation step.