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Procedia Engineering 120 (2015) 1132 – 1136

**Procedia  
Engineering**[www.elsevier.com/locate/procedia](http://www.elsevier.com/locate/procedia)

EUROSENSORS 2015

## Molecularly imprinted high affinity nanoparticles for 4-ethylphenol sensing

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### Abstract

A novel sensor for 4-ethylphenol (4EP) was developed based on molecularly imprinted nanoparticles produced via the solid phase imprinting method. The nanoparticles were immobilised on the surface of a gold electrode previously modified with a self assembled monolayer of lipoic acid coupled to EDC/NHS ((1-ethyl-3-[dimethylaminopropyl]carbodiimide)/N-hydroxysuccinimide). Each step of the process was characterised by electrochemical impedance spectroscopy and cyclic voltammetry. The electrode was used to selectively determine 4EP by differential pulse voltammetry; a quite remarkable sensitivity improvement was observed in the nanoparticle-modified electrode in reference to the non modified bare gold electrode.

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Peer-review under responsibility of the organizing committee of EUROSENSORS 2015

**Keywords:** 4-ethylphenol, electrochemical sensor, molecularly imprinted nanoparticles, solid phase synthesis.

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### 1. Introduction

Natural receptors such as antibodies or enzymes have been widely employed as selective and sensitive recognition elements in the field of Analytical Chemistry in order to develop analytical methods targeted to a certain compound or a group of compounds [1]. Inspired by those that can be found in nature, biological receptors can be mimicked to synthesise smart materials for chemical sensing [2], to this end, the Molecular Imprinting Technology

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offers a versatile approach to develop artificial receptors based on organic polymers. These artificial receptors are synthesised by thermal or photochemical polymerisation that takes place in a mixture containing a template and a functional monomer which previously establish a prepolymerisation adduct in a non-protic solvent known as porogen. This adduct is cross-linked by radical polymerisation employing a cross-linking monomer which will favour the spatial immobilisation of the template-functional monomer complex thanks to the generation of a polymer network around them. Once the polymerisation is complete, the template is removed from the polymer and imprinted sites are released for the latter rebinding or recognition of the target compound.

Usually, Molecularly Imprinted Polymers (MIP) are synthesised in bulk format and they are subsequently ground to obtain irregular micro-sized imprinted particles [1]. One of the main drawbacks of the bulk polymerisation technique is that ground particles need to be sieved obtaining very low yields [3]. Nanosized MIP has constituted a step forward in Imprinting Technology since they overcome most of the drawbacks related with traditional MIP synthesis. On one hand, much higher surface-to-volume ratios are obtained using Molecularly Imprinted Nanoparticles (MIN), and on the other hand, they provide better accessibility to recognition sites and lower mass transfer resistance. Different strategies have been used to obtain MIN such as precipitation polymerisation, emulsion polymerisation or template synthesis approach [1].

Recently, a new method for the synthesis of nanoparticles has been developed, the solid phase synthesis approach. According to this protocol, the template relies immobilised on a solid phase during the synthesis of the polymer instead of being free in a solution; after nanoparticle polymerisation the imprinted particles are purified based on the affinity for the template [4]. Due to the final affinity-based purification step, the number of binding sites is maximised as particles with low or no affinity are removed, lowering non-specific interactions on the final application. Basozabal et al. employed MIN synthesised by solid phase imprinting to develop a potentiometric sensor. The use of high affinity and specificity nanoparticles allowed label-free detection and quantification of histamine in wine and fish samples [5].

In this work, 4-ethylphenol (4EP) specific MIN synthesised using the solid phase approach have been immobilised on a gold electrode surface to improve the performance of the electrode upon 4EP measurement by differential pulse voltammetry (DPV). Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) were used for the characterisation of each step of the process.

## 2. Experimental

### 2.1. Nanoparticle synthesis

The protocol for the solid-phase synthesis with immobilised 4EP was adapted from Moczko et al. [6]. In this work, the 4EP analogue tyramine (TY) was used as template immobilised to the solid phase (glass beads). Immobilisation was carried out through the amino group of TY leaving the phenolic group unbound in order to favour the latter complexation of this hydroxyl group with the functional monomer. The composition of the polymerisation mixture was adapted from [4, 7]. Briefly, the functional monomer 4-vinylpyridine (4ViPy), the cross-linkers ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM), the iniferter *N,N*-diethyldithiocarbamic acid benzyl ester, the chain transfer agent pentaerythritol-tetrakis-(3-mercaptopropionate) and the monomer *N*-(3-aminopropyl) methacrylamide hydrochloride (APMA) were dissolved in acetonitrile (ACN). APMA was used to provide the nanoparticles with amino groups in order to facilitate the subsequent immobilisation on the gold surface. Then the TY derivatised glass beads were added to this mixture and it was left polymerise for 2.5 min under UV energy. After polymerisation, the glass beads were placed in a empty SPE cartridge in order to perform the temperature-based affinity separation of MIN.

### 2.2. Assembly of the MIN on the Gold surface

Prior to immobilisation reactions, the surface of bare gold electrodes was cleaned as describe elsewhere [8]. Then, they were immediately immersed in an ethanol solution of 0.5 mg mL<sup>-1</sup> of lipoic acid, for 24 h at room temperature in order to coat the electrode surface with a self-assembled monolayer (SAM). The carboxylic group of the immobilised LA was linked to EDC/NHS to get a stable active ester. Finally, the electrodes were immersed MIN suspension in phosphate buffer pH 7 for 24 h.

### 2.3. Electrochemical measurements

All electrochemical measurements were performed using a classical three-electrode system, where the MIN modified electrode was used as working electrode, a Ag/AgCl (KCl 3M) as reference electrode and a platinum-wire as counter electrode. All electrochemical measurements were conducted at room temperature using a PGSTAT12 potentiostat/galvanostat controlled by the NOVA 1.8.17 software (Metrohm Autolab B.V., Utrecht, The Netherlands). EIS behaviour of the electrode is determined by analysing the AC voltage and current response at the frequencies ranging from 0.1 Hz to 1 MHz, at an applied potential of 200 mV with an amplitude of 10 mV. CV measurements were carried out from -200 mV to 600 mV on a 10 mV step potential and 100 mVs<sup>-1</sup> scan rate. Characterisation measurements were carried out using an equimolar mixture of [Fe(CN)<sub>6</sub><sup>4-</sup>]/[Fe(CN)<sub>6</sub><sup>3-</sup>] (2 mM) in a KCl 0.1 M solution. 4EP measurements were carried out using Britton-Robinson buffer, modified with NaOH 0.5 M to pH 10, DPV measurements were performed scanning the potential between 0 and 0.5 V, at a scan rate of 10 mVs<sup>-1</sup>.

## 3. Results and discussion

### 3.1. Electrochemical characterisation

Characterisation of the gold electrode after each surface modification step was carried out by CV and EIS. EIS was used to corroborate surface functionalisation of gold electrodes through the evaluation of changes in charge transfer resistance ( $R_{CT}$ ) before and after surface modification. The Nyquist diagrams of the bare gold electrode, gold/LA, gold/LA/EDC-NHS and gold/LA/EDC-NHS/MIN electrodes are presented in Fig 1a. As depicted the monolayer assembly on the electrode surface led to an increase of  $R_{CT}$ . The surface of the gold electrode covered with SAM blocks almost all faradaic current from penetrating to the surface. The Randles circuit (included in Figure 1a) was used as equivalent circuit for the modelling of the electric properties of the modified electrodes where  $R_{\Omega}$  is the dynamic solution resistance or cell resistance which describes the resistance measured between the working and reference electrodes,  $R_{CT}$  is the charge transfer resistance, which describes the insulation of the electrode, CPE the constant phase element, modelling non-ideal capacitors and W the finite Warburg element describing the time dependence of mass transport [9].

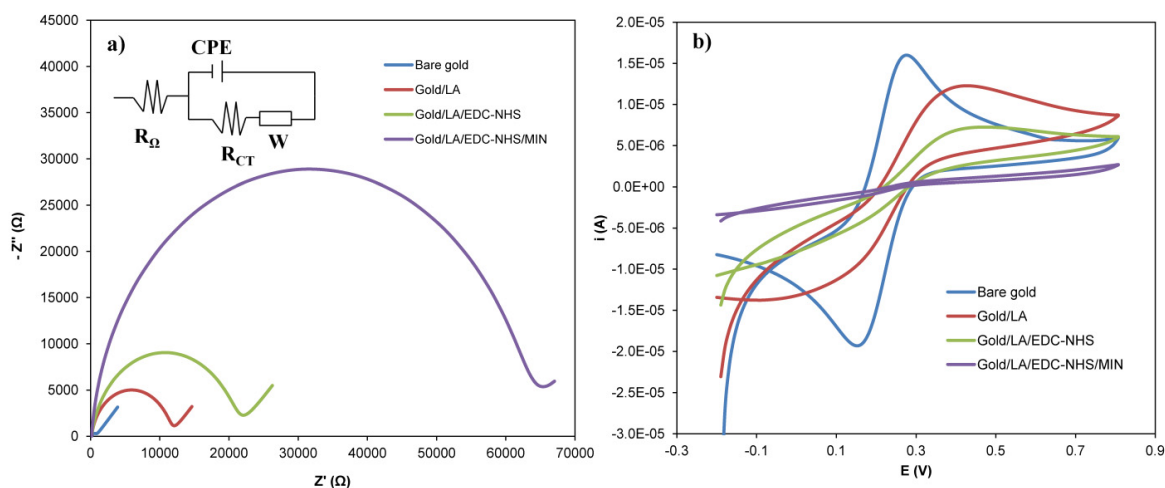


Fig. 1. Nyquist plots (a) and cyclic voltammograms (b) measured after each electrode modification step. Measurements recorded using 2 mM [Fe(CN)<sub>6</sub><sup>4-</sup>]/[Fe(CN)<sub>6</sub><sup>3-</sup>] prepared in KCl 0.1 M as supporting electrolyte.

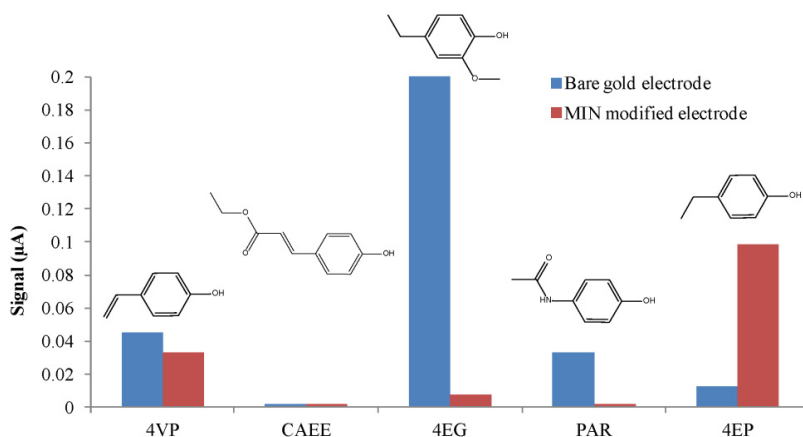


Fig. 2. DPV signals ( $\mu\text{A}$ ) corresponding to the bare gold electrode and MIN modified electrode in  $1 \text{ mg L}^{-1}$  of 4EP and different analogues: 4-vinylphenol (4VP), coumaric acid ethyl ester (CAEE), 4-ethylguaiacol (4EG), and paracetamol (PAR).

EIS observations were confirmed by CV following the ferricyanide/ferrocyanide redox pair. Fig. 1b shows the cyclic voltammograms before and after modification of the gold electrode with the LA, EDC/NHS and finally nanoparticle immobilisation. It can be deduced from the figure that the peak current of  $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$  decreased after each modification step, what clearly denotes the electrical insulating property in every coating.

### 3.2. 4EP measurements

4EP was quantified by DPV using the bare gold and the MIN modified sensor as working electrodes. As it can be observed, the linearity of the MIN modified electrode was noticeably higher than the one obtained with the bare gold electrode, results are summarised in table 1. The detection limit (LOD) of the developed sensor was around 10 times lower than the limit of the non modified electrode. It can be therefore concluded that modifying the gold electrode with high affinity MIP nanoparticles represents a promising approach to increase sensitivity of metallic electrodes, and it may constitute an alternative to other existing commercial nanomaterials.

Table 1. Results obtained after measuring 4EP with different electrodes.

Electrode	LOD ( $\text{mg L}^{-1}$ )	Linear range ( $\text{mg L}^{-1}$ )	$R^2$
Bare gold electrode	0.29	0.96-5	0.9951
MIN modified electrode	0.07	0.24-5	0.9987

To determine the selectivity of the MIN modified electrode, four analogues were measured and compared to the results obtained with the bare gold electrode under the same experimental conditions (Fig. 2). In all cases, the signals corresponding to the structural analogues were lower than the 4EP signal using the MIN modified electrode. Besides, the MIN sensor provided lower responses to analogues but higher for the target compound, thereby demonstrating sensor selectivity towards 4EP.

### Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (project CTQ2013-47921-P) and the University of The Basque Country UPV/EHU (project US13/10).

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