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Artificial enzyme-based catalytic sensor for the electrochemical detection of 5-hydroxyindole-3-acetic acid tumor marker in urine

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ABSTRACT

A novel approach towards the development of biomimetic electrochemical sensors is presented. It is based on magnetic-core/porous-shell molecularly imprinted composites as recognition phase. The selected substrate is 5-hydroxyindole-3-acetic acid (5-HIAA), an urinary metabolite used as carcinoid tumor maker. Using 5-HIAA as template molecule, hemin, 4-vinylpyridine and ethylenglycoldimethacrylate crosslinker are polymerized onto the magnetic core to obtain an artificial enzyme with peroxidase activity. An aliquot of a suspension of the resulting material is deposited onto a carbon screen printed electrode, and the solid material is fixed with a permanent magnet to prepare the sensor. The product of the catalytic oxidation of 5-HIAA at the sensing phase is monitored by differential pulse voltammetry. This sensor allows the detection of 5-HIAA with good sensitivity (0.72 ± 0.01 nA μM^{-1}) and linear response over a concentration range covering the whole window of normal and pathologic urinary levels of 5-HIAA 1–50 μM (0.2–9.6 ppm) with a detection limit of 1.4 μM (0.27 ppm). Urine samples of healthy individuals, spiked with known concentrations of 5-HIAA, yield good recovery values and reproducibility for normal and pathological concentrations (90–100% recovery values, with RSD below 10%).

1. Introduction

5-hydroxyindole-3-acetic acid (5-HIAA) is the main metabolite of serotonin. The levels of this neurotransmitter can be altered in some pathological conditions, such as carcinoid tumors of the enterochromaffin cells of the small intestine, which release large amounts of serotonin, leading to a substantial increase of urinary excretion of 5-HIAA. The normal range for urinary 5-HIAA is 2–8 mg/24 h (roughly ~2–8 ppm). Excretion levels above 25 mg/24 h (~25 ppm) are considered positive for carcinoid tumor, although patients with these tumors can produce even 50 times higher amounts of 5-HIAA than normal levels [1]. Unfortunately, concentration levels of 5-HIAA found in urine are diet-dependent and small changes even above abnormal limits may be related to a non-tumor gastric disorder, which implies that consecutive measurements for diagnosis are required in order to prevent false positives.

Since the early 80's, 5-HIAA has been routinely determined in urine by liquid chromatography with electrochemical detection (HPLC-ED) [2] and this is today the reference analytical assay for this tumor marker. Despite its high sensitivity and excellent selectivity, HPLC-ED methods are time consuming and require relatively expensive instruments, skilled workers and waste large amounts of solvents. Direct electrochemical determination of 5-HIAA has proven to be feasible and is a cost effective alternative. However, direct examination of biological samples is expected to cause severe electrode fouling and interferences. Hence, the design of modified electrodes for 5-HIAA analysis has received considerable attention [3]. A strategy, not previously explored for this analyte, which can provide a significant increase in selectivity and avoid a large amount of unspecific matrix effects, is the use of electrodes modified with molecularly imprinted polymers, MIPs, as specific receptors [4–6].

Successful integration of these receptors with electrochemical transducers has proven to be difficult due to the non-conducting character of most of developed MIPs, although there have been

Abbreviations: 4-VPY, 4-vinylpyridine; 5-HIAA, 5-hydroxyindole-3-acetic acid; DMSO, dimethyl sulfoxide; DPV, differential pulse voltammetry; HPLC, high-performance liquid chromatography; MIC, molecularly imprinted catalytic polymer; MIP, molecularly imprinted polymer; MMIC, magnetic molecularly imprinted catalytic polymer; MMIP, magnetic molecularly imprinted polymer; MPs, magnetic particles; PDA, photodiode array detector; SPE, screen printed electrode; TEOS, tetraethyl orthosilicate; TPM, 3-(trichlorosilyl)propyl methacrylate.

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significant advances using electrogenerated MIPs [7–10]. Electrochemical sensors based on affinity MIPs rely on the measurement of changes in the electrical properties that occur when the template molecule binds to the recognition site in the sensor surface, most often far from the conductive surface of the transducer, resulting in limited sensitivity. To overcome this limitation, catalytic imprinted polymers mimicking redox enzymes have been proposed [11–14]. These new biomimetic receptors are able not only to select but also catalyze the transformation of the target into soluble and electrochemically active products, diffusing towards the electrode surface, and repeat the process many times, amplifying the response. Using these materials as recognition elements, electrochemical detection can be achieved onto the surface of the transducer in a suitable potential window where no interference appears [13,14]. Among this kind of electrode modifiers, catalytic imprinted microgels offer a good alternative for the construction of a new family of catalytic biomimetic sensors [14]. The challenge for the design of these sensors however, is to find an easy way to couple these materials with the transducer.

Herein, we demonstrate how this can be done by using a magnetic molecularly imprinted polymer which combines the easy handling of a magnetic material with the substrate specificity and longtime stability of molecularly imprinted catalytic polymers (MICs). Despite magnetic molecularly imprinted polymers (MMIPs) have been obtained by different polymerization techniques [15–17] and applied in several fields such as separations and sensors [18–21], their use in combination with catalytic materials is a recent exception [22]. In this work a synthetic catalyst, which incorporates 4-vinylpyridine (4-VPY) as functional monomer and hemin as catalytic center, is prepared as a thin microgel layer onto the surface of magnetic microparticles, given rise to a magnetic molecularly imprinted catalytic polymer (MMIC) which can be considered an artificial enzyme [22]. This receptor displays peroxidase-like activity and is easily immobilized onto a carbon screen-printed transducer by means of an external magnetic field. Detection is performed by measuring products of 5-HIAA catalytic oxidation through its electrochemical reduction processes monitored by differential pulse voltammetry (DPV). This voltammetric biomimetic sensor is used to measure the concentration of 5-HIAA in urine.

2. Materials and methods

2.1. Reagents and chemicals

5-hydroxyindole-3-acetic acid (5-HIAA) (≥98%), 1-heptanesulphonic acid and hydrogen peroxide (30%) were purchased from Sigma–Aldrich (USA). Potassium hydroxide (85%), potassium dihydrogenphosphate (99%) and acetonitrile (HPLC grade) were purchased from Merck. Orthophosphoric acid (85%) was purchased from Fluka. All solutions were prepared with high purity water produced by a Direct-Q 5 purification system (Millipore). Magnetic attachment to the SPE was carried out with the help of a neodymium-iron-boron (NdFeB) permanent magnet of 4 mm diameter × 1.5 mm height with an axial magnetization of 13,200–13,700 Gauss (350 g in each magnet face) (Supermagnete, Germany).

2.2. Apparatus

Electrochemical measurements were conducted using a potentiostat/galvanostat Eco Chemie μAutolab II. It was controlled by a computer using GPES 4.9.007 software. Disposable screen-printed electrodes (SPEs) were purchased from DropSens (Oviedo, Spain), connected to the potentiostat through a specific connector (DropSens, Oviedo, Spain). High-performance liquid chromatography (HPLC) was performed using a Shimadzu LC-20AD series HPLC, (Kyoto, Japan) equipped with a SPD-M20A UV–vis photodiode array detector (PDA). A reverse phase Pinnacle DB C18 column (150 mm × 4.6 mm i.d., 5 μm) coupled to a Rheodyne 7725i rotating valve with a 20 μL loop, supplied by Teknokroma (Barcelona, Spain) was used for separation purposes.

2.3. Preparation of the electrochemical sensor

The catalytic sensor was fabricated by depositing the imprinted polymer composite onto screen printed carbon electrodes (4.0 mm diameter) as is schematically depicted in Fig. 1. Catalytic molecular imprinted polymers were synthesized following a method we previously developed [22]. It involves four successive steps in a layer-by-layer approach: magnetic core synthesis, protective

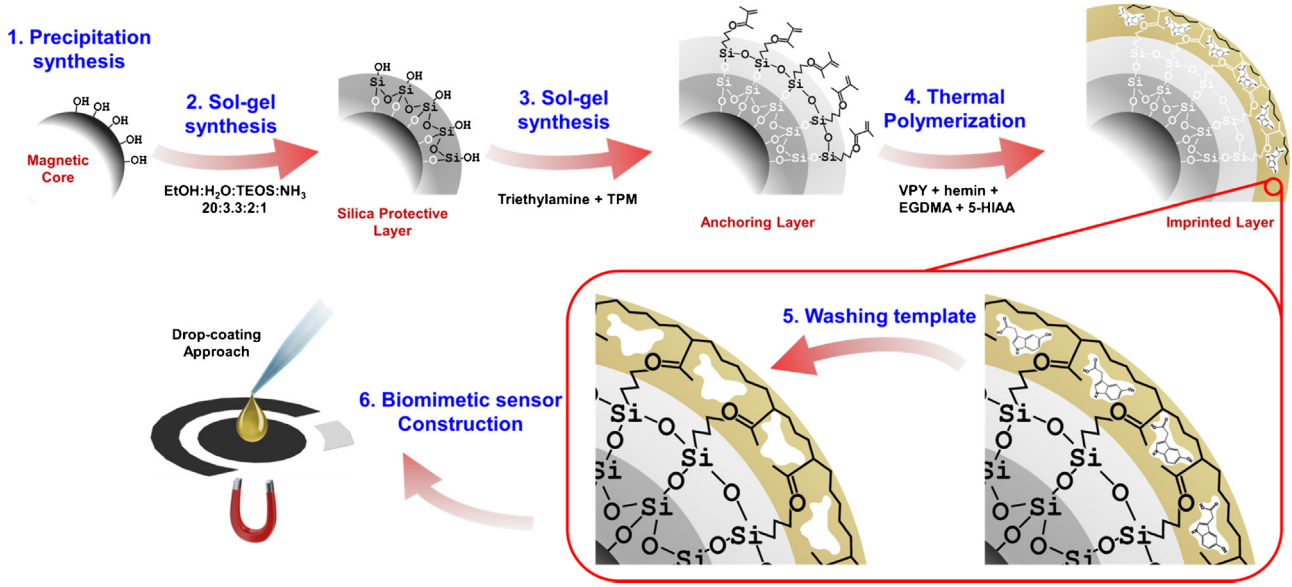

Fig. 1. Steps involved in the preparation of the catalytic sensor. TEOS: tetraethyl orthosilicate, TPM: 3-(trichlorosilyl)propyl methacrylate, 4-VPY: 4-vinylpyridine; 5-HIAA: 5-hydroxyindole-3-acetic acid; EGDMA: ethylene glycol dimethylacrylate.