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MIP-based biomimetic sensor for the electronic detection of serotonin in human blood plasma

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ABSTRACT

Serotonin is an important signaling molecule in the human body. The detection of serotonin is commonly performed by high performance liquid chromatography (HPLC), which is costly and time consuming due to extensive sample preparation. We will show that these problems can be overcome by using molecularly imprinted polymers (MIPs) as synthetic receptors in combination with impedance spectroscopy as readout technique. The MIPs were prepared with several blends of the underlying monomers and the best performing MIP material was selected by optical batch-rebinding experiments. MIP microparticles were then integrated in an impedimetric sensor cell and dose–response curves were measured in PBS buffer and in non-diluted blood plasma. The sensor provides reliable data in the physiologically relevant concentration regime as an independent validation by HPLC measurements demonstrates. Finally, we show that the impedimetric response upon serotonin binding can be attributed to a capacitive effect at the interface between the MIP particles and the plasma.

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1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a metabolite of the essential amino acid tryptophan and its role in smooth muscle contraction was already reported in 1951 [1]. The structure of serotonin, its natural metabolite 5-hydroxyindoleacetic acid (5-HIAA), and its oxidation product are shown in Fig. 1. Today, it is known that the serotoninergic system is steering numerous behavioral and physiological functions including emotions, sleep, and appetite [2]. Abnormalities of serotonin-related processes in the central nervous system can lead to severe mental disorders including anorexia, depression, and schizophrenia [2,3]. Also, anomalous serotonin levels are found in patients with hypertension [4], migraine, fibrotic syndrome, and carcinoid tumors [5,6]. Recently, it was recognized that serotonin is also involved in the regulation of the gastrointestinal functions and this insight has led to novel treatments for gastrointestinal disorders [7–9]. For diagnostic purposes, serotonin concentrations are usually analyzed in portal blood and in the systemic circulation, where plasma levels of 5–20 nM define the typical range for healthy individuals [6,10].

Serotonin belongs to the class of indoles and this group of aromatic, heterocyclic molecules is sensitive to light, oxygen and changes in pH. Therefore, special precautions must be taken to prevent oxidation during preparation and handling of patients' samples [11,12]. Established detection techniques such as solvent extraction and ion exchange chromatography require extensive sample preparation and are aspecific with respect to other indole species [13]. This can be overcome by high pressure liquid chromatography (HPLC), which can distinguish between different indoles. Therefore, it is currently the most common technique in the field [11,14], but it is especially costly and requires sophisticated equipment, making it unsuitable for routine tests [2].

Other options include electrochemical techniques: For instance Sarada et al. were able to measure serotonin in the 10–1000 nM range in aqueous media using amperometric detection [15]. Wu et al. developed carbon-nanotube coated glass electrodes suitable for cyclic voltammetry measurements in spiked human blood serum [16]. Using the same technique, Kumara Swamy and Venton attempted a first in vivo measurement in the striatum of an anesthetized rat [17]. Upon administration of the serotonin precursor 5-hydroxytryptophan, a change in the oxidation current was observed, however it was not possible to determine the exact concentration. Summarizing, with electrochemical techniques it is not possible so far to measure serotonin selectively in the relevant concentration range of 'real' biological samples.

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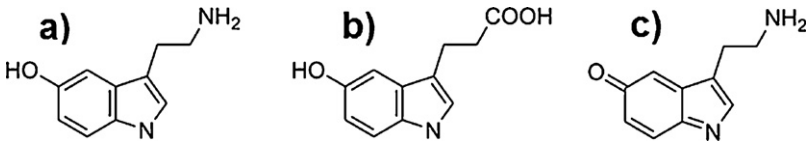

Fig. 1. The structure of serotonin (a), its metabolite 5-HIAA (b) and the chemical oxidation product (c).

An alternative route to serotonin detection can be found in molecular imprinting. Using molecularly imprinted polymers (MIPs) as synthetic receptors offers, in addition to specific recognition, several benefits: First, MIPs can be synthesized at a rather low cost via established polymer-chemical routes [18–20]. Second, MIPs can be stored for long time scales and maintain their receptor properties in wide ranges of temperature, pH, and ionic strength [21–23]. Third, the recognition of target molecules is reversible for non-covalent imprinting, allowing MIP-based sensors to be used repetitively [24].

Literature reports on the synthesis of MIPs for serotonin recognition are sparse and very recent: Kitade et al. developed a MIP with methacrylic acid (MAA) as functional monomer [25]. Analysis with optical spectometry allowed detecting serotonin in water with a detection limit of 1 μM. Okutucu and Telefoncu also used methacrylic acid and packed the MIPs obtained by bulkpolymerization into separation columns. The analysis of plasma platelet samples spiked with 100 nM of serotonin was performed with HPLC [26]. After optimization, Okutucu et al. detected spiked serotonin concentrations in the μM range also in human blood [27]. Khurshid et al. combined the charged monomer methacrylic acid with the neutral monomer methacrylamide to detect 1 mM of serotonin in the dimethylformamide with a microplate reader [28].

While the synthesis of the MIPs is low-cost, the aforementioned detection techniques are still not suited for point-of-care applications. This can be solved by combining MIP receptors with electronic read-out strategies. In previous work, first examples were demonstrated with MIP-based impedimetric sensors for nicotine [29] and for histamine [30,31]. These sensors have detection limits of 2 nM in buffer solutions, but the functioning in biological matrices was not yet demonstrated. Other electronic readout techniques for MIP-based sensors, such as voltammetry and quartz crystal microbalances, have also been reported, but the detectable concentration range is generally at the millimolar scale [31,32]. Since the serotonin level in human blood is typically 5–20 nM, impedance spectroscopy is therefore the method of choice. To our knowledge, there are no previous reports in literature combining the molecular imprinting of serotonin with impedimetric read-out.

The goal of this study was threefold: first, we aimed to optimize the monomer blends and MIP synthesis with respect to affinity and selectivity. Especially, the MIPs should allow discriminating between serotonin, its oxidized form and its metabolite 5-HIAA. Next, the goal was to develop an impedimetric sensor that is able to cope with the fast oxidization of serotonin and special care needs to be taken to circumvent non-specific responses from competing molecules in the complex matrix of blood plasma. To this end, non-imprinted polymers (NIPs) will be used as a reference channel. Finally, we aimed to set up equivalent-circuit modeling to assist in understanding the physical origin of the impedance increase, which is observed upon the molecular recognition of serotonin at the MIP-coated sensor electrodes.

2. Materials and methods

2.1. Chemical reagents

Ethylene glycol dimethacrylate (EGDM), methacrylic acid (MAA), acrylamide (AM) and dimethylsulfoxide (DMSO) were purchased from Acros (Geel, Belgium). Prior to polymerization, the stabilizers in the MAA and EGDM were removed by filtration over alumina. Azobusisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland). The target molecule serotonin and its competitor, the metabolite 5-HIAA, were obtained from Acros (Geel, Belgium). All solvents were of analytical grade (Acros, Geel, Belgium) and used without further purification. The PPV derivative, OC_1C_{10}-PPV, which served as the immobilization layer on the impedimetric sensor electrodes, was synthesized via the sulfinyl precursor route [33]. All chemical and physical properties of this conjugated polymer were in agreement with previously reported data. For the impedance measurements a home-made 1 × phosphate buffered saline (PBS) solution was used.

2.2. MIP synthesis

The MIP synthesis was optimized to achieve a high affinity and selectivity for serotonin. The combination of two functional monomers, methacrylic acid (MAA) and acrylamide (AM), was studied by varying the monomer ratios. Hereby, the following MAA:AM ratios were considered: 1:0, 1:1, 1:3, and 0:1. As a measure of the specificity, the imprint factor was selected, which corresponds to the amount of target molecules bound per gram of the MIP divided by that of the NIP. The imprint factors were determined by optical batch rebinding experiments at a free target concentration of 0.3 mM using a Varian Cary 500 UV–vis–NIR spectrophotometer (Leuven, Belgium). Briefly summarized, MIPs composed solely of MAA or AM did not show specific binding properties. Upon mixing MAA and AM in a 1:1 ratio, an improved imprint factor of 1.4 was achieved. The highest imprint factor (3.64) was obtained with MIPs synthesized from the 1:3 blend. This MIP was prepared as follows: first, a mixture of MAA (2.84 mmol), AM (8.50 mmol), EGDM (22.72 mmol) and AIBN (0.61 mmol) was dissolved in 7 ml DMSO together with the template molecule serotonin (5.67 mmol). This solution was degassed with N_2 and polymerized in a UV oven for 12 h. After polymerization, the bulk polymer was ground and sieved to obtain microparticles with a size smaller than 25 μm. This was verified by SEM-images, which showed a similar particle size distribution for the MIP and NIP-particles. Finally, the serotonin was removed from the MIP powders by Soxhlet extraction with methanol (48 h), a mixture of acetic acid/acetonitrile (1/1) (48 h) and again methanol (12 h). The extracted powders were dried in vacuum for 12 h at room temperature. A non-imprinted polymer (NIP) was synthesized accordingly, but without the presence of the target molecule. These MIP and NIP powders were used in all further batch-rebinding and impedimetric measurements.

2.3. Preparation of blood-plasma samples and HPLC characterization

Blood samples were obtained from three healthy volunteers, person A, B and C, and divided over 4 ml K_2EDTA tubes in order to prevent coagulation. The blood collection tubes also contained 0.1 ml of ascorbic acid (1.4 g ascorbic acid/100 ml distilled water). This addition is necessary to prevent the conversion of serotonin to 5-HIAA by the enzyme monoamine oxidase, which normally occurs within several seconds. It was proven earlier that this

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