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Molecularly Imprinted Polypyrrole for the selective detection of Dopamine and Serotonin

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Abstract. It is well known that dopamine (DA) and serotonin (5-HT) play a very significant role in biological systems and have a direct relationship with feelings, mental state, and physiological functions. The measurement of these molecules is of interest as a tool for diagnosis and monitoring of illnesses such as Parkinson's and Alzheimer's diseases. Molecularly imprinted polymers (MIPs) have been used in a growing number of applications to increase selectivity and sensitivity. In this work, the MIP concept was used to develop selective electrochemical sensors for quantitative determination of dopamine and serotonin. The results showed that the sensors have a linear response ($R=0.99$) between 5.0-50.0 μM , with a limit of detection of 1.04 and 0.89 μM , % RSD of 1.4 and 2.7, and a recovery percentage of 95.1 and 96.4% for a 30.0 μM solutions of DA and 5-HT, respectively. The amperometric response of the sensors was reproducible up to 3 days and not influenced by the presence of several interferents. A sensor was also studied for the simultaneous detection of the two molecules, the results showed a higher response for and with a sensitivity of *ca.* 70% of individual sensors.

Key words: Amperometric sensor, MIP, polypyrrole, dopamine, serotonin

1. Introduction

One of the challenges of analytical chemistry is the development of methods for the rapid determination of analytes with high selectivity and temporal resolution. Selectivity (ability to detect a chemical species in the presence of other components) is the most important figure of merit. Selectivity relies on molecular recognition through electrostatic and non-covalent interactions. Enzymes and crown ethers are biological and artificial examples of high molecular recognition capacity [1].

Molecularly imprinted polymers (MIPs) are also capable of molecular recognition [2, 3]. In the synthesis of MIPs, the monomer is polymerized in the presence of a target, analyte molecule, resulting in a polymer with specific recognition sites for the analyte as shown in Figure 1. Then, the analyte is removed, leaving impressions or "cavities" in the polymer that are complementary to the target molecule (step 2), The impressions induce a "molecular memory" effect for the target molecule [4].



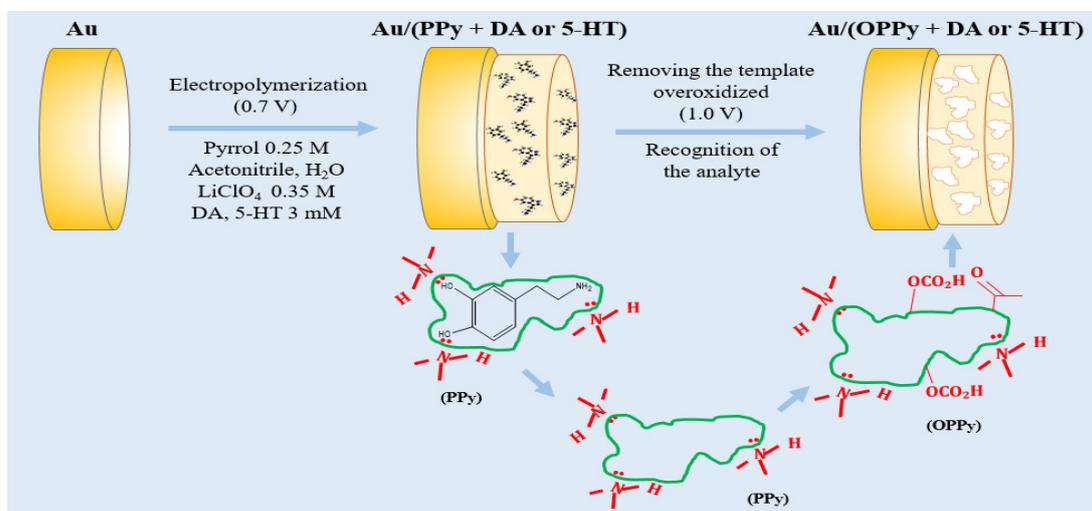


Figure 1. General scheme of the formation of polymers with the molecular imprinting method.

Dopamine is an important neurotransmitter in the central nervous system in mammals [5]. It plays a variety of functions in the renal and cardiovascular systems [6]. Deficiency of DA may lead to disorders such as Parkinson's disease [7].

Serotonin is important in physiological processes such as sleep, thermoregulation, and sexual activity. 5-HT deficiency is associated with anxiety, violent behavior and could play a role in schizophrenia [8]. The determination of DA and 5-HT in blood and plasma is of medical importance.

Conductive polymers have been used as MIPs for the detection of several individual neutral and ionic species [9]. Very relevant for the present study is the use of overoxidized polypyrrole (OPPy) for the simultaneous determination of DA and 5-HT.

The electroactivity of DA and 5-HT on metallic electrodes depends on the pH of the medium [10, 11]. DA and 5-HT have been detected in mixtures of such interferences as ascorbic acid [12], uric acid, and glucose. DA and 5-HT can interfere with each other's analysis due to the proximity between the oxidation peaks (220 and 380 mV vs. Ag/AgCl, respectively) [13, 14].

Conductive polymers have been used for analysis of DA and 5-HT individually. The sensors we developed here use the principle of MIPs for the simultaneous determination of DA and 5-HT. These sensors have potential applications in clinical studies and in point of care diagnostics and treatment.

2. Experimental

2.1. Equipment and Electrodes

Experiments were run in a 10 mL cell on a CHI 700 B electrochemical analyzer. A gold microelectrode (diameter = 25 μm ; area = $(6.72 \pm 0.11) \times 10^{-6} \text{ cm}^2$, a Pt wire, and Ag/AgCl/KCl (3.0 M) (all from CH Instruments) were used as the working, auxiliary, and reference electrodes, respectively.

2.2. Reagents and solutions

Water was class two (conductivity $\leq 0.06 \mu\text{S/cm}$. N_2 (AP 4.5, 99.98%) was supplied by Cryogas. CH_3CN , HNO_3 , phosphate salts were purchased from J.T. Baker. LiClO_4 and NaOH (99.5%) were from

Merck. DA (99%), 5-HT (98%), ascorbic acid (99%), and uric acid (99%), and pyrrole (98.0 %) were Sigma-Aldrich reagents.

2.3. Control experiments

Cyclic voltammetry (CV) was used to determine the electroactivity of the analytes. On Au microelectrodes, DA and 5-HT showed irreversible oxidation peaks at 0.25, and 0.40 V respectively.

2.4. Electrochemical synthesis of modified gold microelectrodes

MIPs were prepared by electropolymerization of pyrrole on the Au microelectrode at 0.70 V and a charge of 0.30 mC. The cell contained CH₃CN/water (98-2%), 0.25 M pyrrole, 0.35 M LiClO₄, 0.10 M PBS, and either 3.0 mM DA or 5-HT, at regulated pH and constant temperature. N₂ was bubbled for five minutes and then the flow was maintained above the solution during the experiment. The resulting PPy films containing the template were labelled Au/PPy-DA or Au/PPy-5-HT. The modified electrodes were subjected to 1.0 V (charge of 0.10 mC) in 0.10 M NaOH to form overoxidized PPy (OPPy) and cross-linking of the polymer chains. The sensors obtained by this procedure were labelled Au/OPPy-DA or Au/OPPy-5-HT.

2.5. Molecular recognition

The Au/OPPy-DA or Au/OPPy-5-HT microelectrodes were immersed in an aqueous solution of 10.0 μM DA or 5-HT in 0.10 M phosphate buffer solution (PBS), at a constant pH (5.0-9.0) under open circuit for three minutes. The current response was evaluated by CV and quantified by Square Wave Voltammetry (SWV). The best responses were obtained at pH 7.5 for DA and pH 7.0 for 5-HT.

2.6. Preparation of human plasma samples

The plasma samples were deproteinized by adding 50 mg of sulfosalicylic acid to 1.0 mL of plasma and centrifuged at 3000 rpm for 20 minutes to separate the free amino acids from the precipitated protein. The supernatant was then filtered and diluted in 5.0 mL of 0.10 M PBS to be submitted to the voltammetric analysis by CV and SWV, using the multiple standard addition method.

3. Results and Discussion

The response of the sensors was evaluated as a function of Py and analyte concentration and the storage conditions of the sensors. The SWV voltammograms of individual sensors and the corresponding calibration curves for each compound are shown in Figures 2 and 3, respectively.

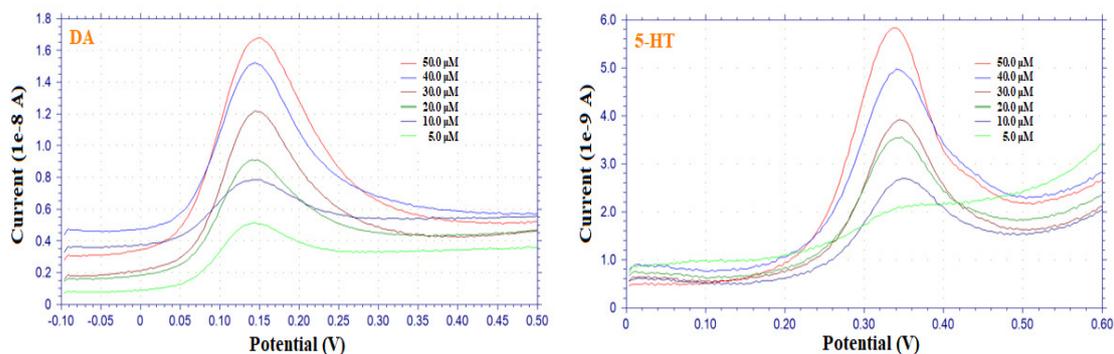


Figure 2. SWV voltammograms for different concentrations of DA and 5-HT in 0.1 M BPS, at pH 7.5 and 7.0 respectively.

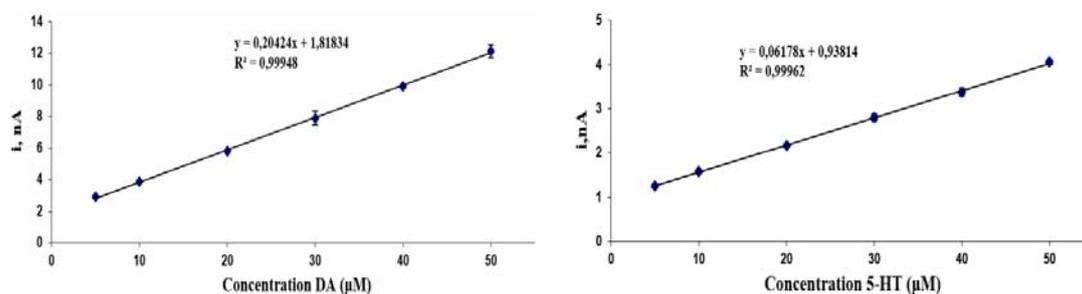


Figure 3. Calibration curve for DA and 5-HT (5.00 - 50.00) μM , in 0.1 M BPS pH 7.5 and 7.0 respectively.

The sensors showed linear response between 5.0-50.0 μM with detection limits (LOD) at 1.04 and 0.89 μM , and recovery percentages of 95.1 and 96.4% for 30.0 μM solutions of DA and 5-HT, respectively. The amperometric response of the sensors was reproducible up to 3 days.

3.1. Determination of dopamine and serotonin in human plasma

The solutions obtained from plasma samples were analyzed by SWV at pH 7.5 (DA) and 7.0 (5-HT), respectively, by the multiple standard addition method (1000 μM standard). The resulting voltammograms (25 mV/s) and the corresponding calibration curves are shown in Figures 4 and 5.

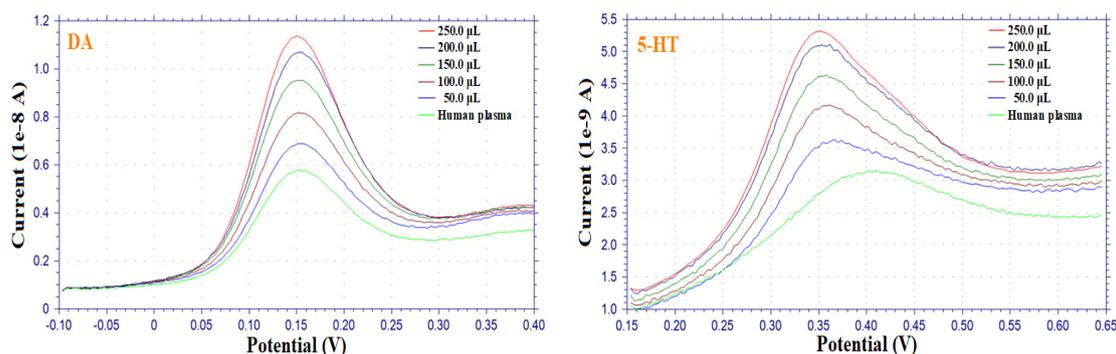


Figure 4. SWV voltammograms for plasma samples: Au/OPPy-DA, Au/OPPy-5-HT in 0.10 M BPS, pH 7.5 and 7.0 respectively.

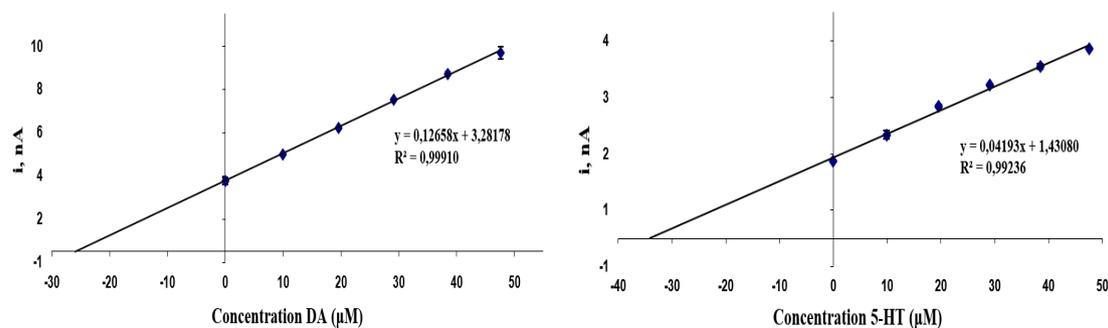


Figure 5. Calibration curves for plasma samples: Au/OPPy-DA, Au/OPPy-5-HT in 0.10 M BPS, at pH 7.5 and 7.0 respectively.

The sensors have a linear response ($R = 0.99$) between 1 and 50 μM , with LOD of 1.31 and 3.83 μM , % RSD of 1.4 and 2.7, and concentrations of DA and 5-HT in plasma samples of $129.65 \pm 0.83 \mu\text{M}$ and $174 \pm 2.68 \mu\text{M}$, respectively.

3.2. Simultaneous detection of for DA and 5-HT

A Au/OPPy-DA + 5-HT film was synthesized by using the best conditions described above. Figure 6 shows the SWV response of several concentrations of one analyte in the presence of a fixed concentration of the other. The signals for the two analytes are clearly observed, although they were only *ca.* 70% of those of the individual sensors. Thus, the individual calibration curves can't be used for the simultaneous determination of DA and 5-HT.

3.3. Effect of interfering substances in the determination of DA and 5-HT in human plasma

This effect was evaluated from the SWV voltammograms taken from the treated plasma in a solution containing ascorbic acid, tyrosine, uric acid, and tryptophan with a concentration 0.10 M of each one. The effect of interferents was negligible after several measurements indicating a very good selectivity of the sensors as shown in Figure 7.

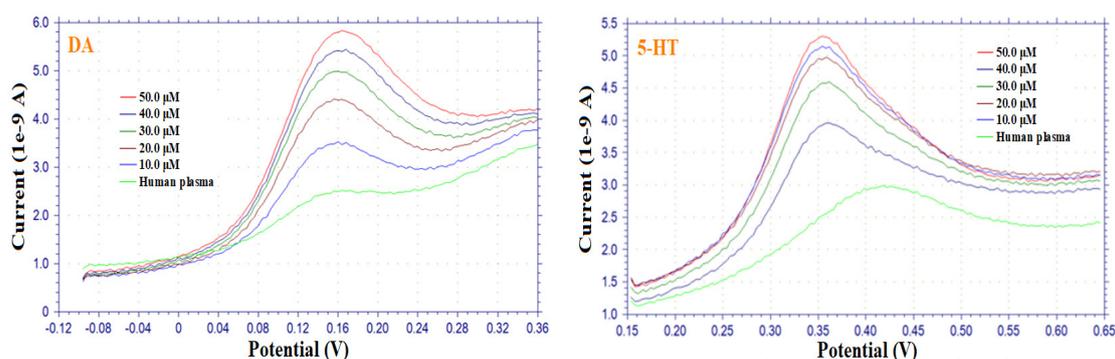


Figure 6. SWV voltammograms for human plasma samples doped with: A) DA (10.0-50.0 μM) and 5-HT (10 μM), in PBS, pH 7.5. B) 5-HT (10.0-50.0 μM) and DA (10 μM), in PBS, pH 7.5.

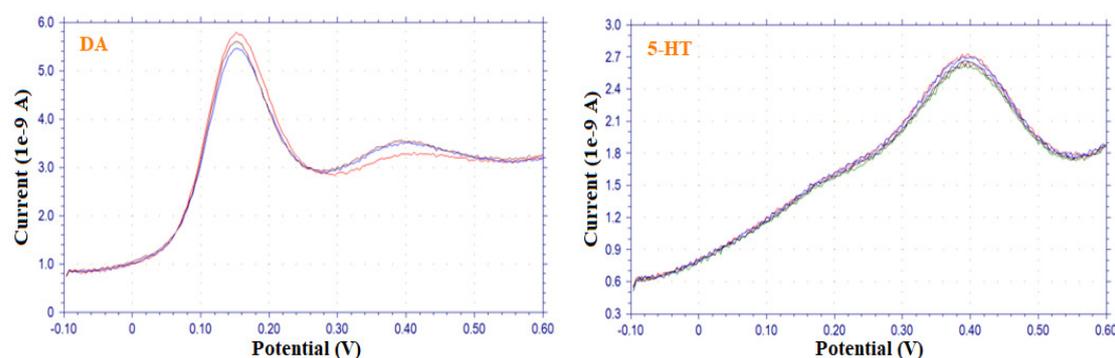


Figure 7. SWV voltammograms of plasma samples for DA and 5-HT in 0.10 M PBS at pH = 7.5 and 7.0 in a solution containing 0.1 M ascorbic acid, tyrosine, uric acid, and tryptophan.

4. Conclusions

MIPs based on PPy were made for the analysis of DA and 5-HT. The individual sensors showed good limits of detection and quantification that allow their use in samples with relatively low concentrations of these analytes. The analysis in human plasma suggests the use of these sensors for clinical analysis is viable. Preliminary results of the simultaneous detection of DA and 5-HT using the same microelectrode were not satisfactory.

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