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ELECTROANALYSIS OF SEROTONIN AT PLATINUM NANOPARTICLES MODIFIED ELECTRODE

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This work describes the detection of serotonin using a platinum nanoparticles-conductive polymer composite sensing nanomaterial deposited on to glassy carbon electrode. Electrode surface modification with the composite material has been achieved by sinusoidal currents preparation procedure. The sensor displayed a detection limit with a value of 1.9 μM serotonin. The sensor response was linear for concentrations ranging from 2 to 80 μM serotonin. The sensor was used in the voltammetric quantification of serotonin in real sample.

Keywords: serotonin, electrochemical sensor, sinusoidal currents, platinum nanoparticles, conducting polymers.

1. Introduction

The determination of neurotransmitters using chromatographic and spectrometric techniques represents a major tool in the control of neurodegenerative diseases. These analytical techniques ensure high accuracy and reliability of the measurements [1-3]. However, the need for pretreatment procedures and the use of expensive instruments have underpinned new research directions oriented to the development of low-cost, fast and sensitive analytical devices [4-7]. Electrochemical sensors prepared by judicious modification of electrode surfaces using sensitive nanocomposite materials have proved to fulfill the required analytical performance for neurotransmitters determination [8-10].

One of the most studied neurotransmitters is serotonin (ST) or 5-hydroxytryptamine. The monitoring of ST level is important in the management of related neurodegenerative diseases [11-13]. Several electrochemical sensors have been proposed for its quantification due to its physiological role in the human body.

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These sensors are using nanocomposite materials composed of conducting polymers (CP) and various inorganic fillers like metal and metal oxide nanoparticles (MeNP) [14, 15]. The synergy between the intrinsic properties of the organic polymers and those of the inorganic components enabled improved sensitivity and selectivity of the sought analytical measurements. The polymeric matrix ensures a suitable microenvironment for the incorporation of MeNP or even biological substances such as enzymes for biosensors fabrication [16]. The catalytic activity of MeNP is enhancing the analytical sensitivity of the measurements [17]. In this sense, galvanostatic, amperometric, potentiostatic and potentiodynamic methods are used [18, 19]. The polymer layer thickness and the size distribution of MeNPs could be controlled. Besides these methods, novel approaches based on alternating (sin wave) voltages (SV) and/or currents (SC) have been recently investigated [20, 21]. In these procedures, alternating voltages or alternating currents are applied over a constant voltage or current, respectively. Optimized electrochemical parameters of the excitation signal such as frequency and amplitude allow an increase in the electrochemical active area. This feature is of importance in the analytical applications by the observed increase in the sensitivity during the measurements of the analyte concentration.

Herein, the voltammetric quantification of serotonin using PtNP-poly(3,4-ethylenedioxythiophene) sensing material deposited on to a working electrode of glassy carbon (GC) is presented. The optimized SC procedure was used in the preparation of this sensor. The voltammetric quantification of serotonin in synthetic samples and real systems at the obtained sensor has been achieved.

2. Experimental

2.1. Reagents and materials

The reagents have been brought from Sigma. The Autolab potentiostat/galvanostat PGSTAT302N (from Metrohm AG-EcoChemie, Utrecht) has been used in the electrochemical experiments. The data were acquired using the software NOVA version 2.1.5 (Metrohm AG). A GCE (Metrohm AG, d = 3 mm) served as a working electrode and was modified by the composite material. The electrode potentials are measured against a silver-silver chloride electrode. The counter electrode was made of a rod of glassy carbon (Metrohm AG). All the measurements were carried out under Ar atmosphere. All cyclic voltammetry measurements have been carried out with 0.05 V/s potential scanning rate at room temperature. The SC approach has been developed by means of the software of the PGSTAT. A Tescan Vega 3 instrument has been used in the morphological characterization of the composite material.

2.2. Preparation of CPs-PtNPs nanocomposite material

The sensor has been prepared by applying a SC with selected amplitude (I_{sin}) and frequency (f) onto a constant current (I_{dc}). The SC method has been applied for an optimized electrodeposition time (t). The procedure is composed of two steps: firstly, the polymer layer is generated in the presence of the monomer at 10 mM concentration in aqueous solution of 0.1 M lithium perchlorate by applying these optimum parameters of the SC procedure: I_{sin} = 20 µA, f = 0.05 Hz, I_{dc} = 15 µA, t = 300s; secondly, the Pt nanoparticles are prepared from the metallic precursor solution of 5 mM K_2PtCl_6 in H_2SO_4 of 0.5 M concentration by applying the optimized parameters of the SC procedure: I_{sin} = 20 µA, f = 0.1 Hz, I_{dc} = -30 µA , t = 100 s.

2.3. Analytical applications

The voltammetric determination of serotonin at the PtNP-PEDOT sensing platform was achieved by means of cyclic voltammetric measurements with 0.05 V s^{-1} scanning rate. Analytical measurements were performed in buffered solution with optimum pH value of 7.0. The calibration curve for serotonin has been obtained by multiple standard addition protocol. The sensor's performance was also estimated. Real samples have been subjected to the analysis using the obtained sensor.

3. Results and discussion

3.1. Preparation of PtNP-PEDOT sensor

In Figure 1, the main signals recorded during the preparation of PEDOT are displayed. The sinusoidal voltage with values from 0.60 to 0.91 V represents the system's response upon the application of SC. The polymerization of the monomer (EDOT) is occurring above 0.80 V and up to 0.90 V. Potential values higher than 0.95 V are res electroANALYSIS of serotonin at platinum nanoparticles modified electrode ulting in the polymer over-oxidation. Bellow 0.80 V potential values, the polymerization process doesn't occur. Consequently, the applied SC allows for the deposition of PEDOT. The polymer deposition is occurring on the SC anodic cycle, while the process is discontinued on the cathodic cycle. These observations are well correlated with the shape of the system response. This special characteristic of the SC method is ensuring in this way the increase of the roughness of the prepared polymer matrix by the modulation of the polymerization process according to the frequency of the excitation current.

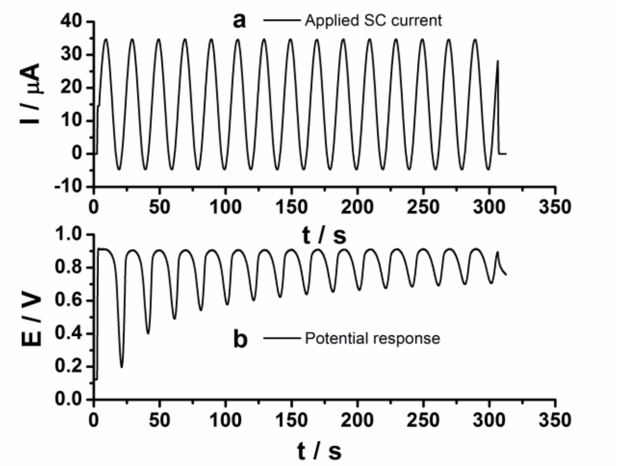


Fig. 1. (**a**) The applied SC and (**b**) the system response for PEDOT preparation.

The Pt nanoparticles have been prepared on the PEDOT matrix by means of the SC approach (see Figure 2). A sinusoidal voltage in the range (+0.5 V to -0.15 V) was obtained after the application of the SC. The applied signal ensures the electrochemical deposition of PtNP.

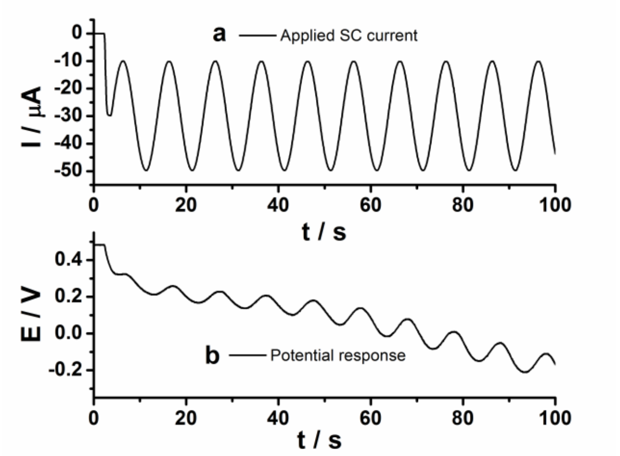


Fig. 2. (**a**) The applied SC and (**b**) the system response for PtNP preparation.

The successful deposition of PEDOT and PtNP-PEDOT materials has been checked by cyclic voltammetry (CV). Thus, the CV traces have been registered in solution of the redox specie, i.e. 5×10^{-3} M $K_4[Fe(CN)_6]$ (in 0.5 M KNO_3 aqueous solution), after each deposition step and the obtained data are presented in Figure 3.

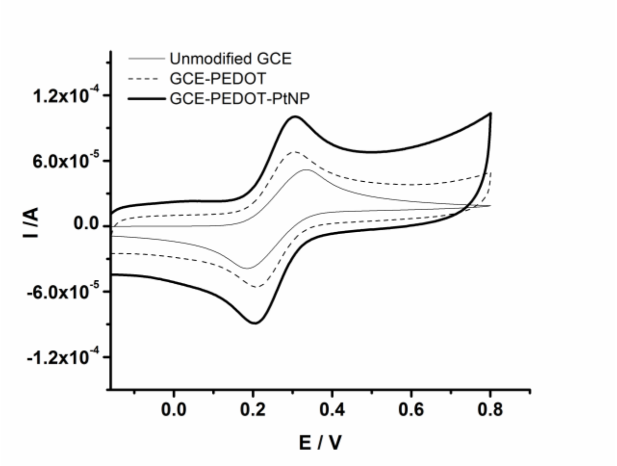


Fig. 3. CV traces registered for unmodified GCE, PEDOT, and PtNP-PEDOT modified electrodes.

The modification of the electrode with the nanocomposite material resulted in an enhanced anodic peak current versus the bare electrode (48% increase) and PEDOT modified electrode (32% increase). This behavior is determined by the electrocatalytic property of the sensing material that allows the improvement of electron transfer rate. This enhanced electron transfer could be exploited in the analytical measurements of the analyte by providing an increase of the sensitivity. The PtNP-PEDOT composite material has been also prepared by classical galvanostatic method in order to make a comparison with the SC procedure performance. In the galvanostatic method, the parameters for PEDOT matrix deposition were as follows: I_{dc} = 25 μA and t = 300 s. Subsequently, the following parameters were used for PtNP electrodeposition: I_{dc} = -25 μA and t = 100 s. The values of the deposition times were identical to those used in the SC method. The electrochemically active area has been determined for each case using the ferrocyanide redox species and the Randles-Sevcik formula [22]:

$$I_p = 2.69\times10^5\ n^{3/2}\ A\ D^{1/2}\ v^{1/2}\ C$$

In the case of ferrocyanide redox species, $D = 7.6 \times 10^{-6}$ cm^2 s^{-1} [23], and n = 1. Electrochemically active area values were of: 0.099 cm^2 for the galvanostatic method, and 0.103 cm^2 for the SC method, respectively. There was an increase of the electrochemically active area value for the composite material prepared by sinusoidal current. It should be noted that during the electrodeposition times of 300 s for PEDOT and 100 s for PtNP, respectively, the galvanostatic procedure ensures a continuous synthesis of the materials. In the case of the SC procedure, a half fraction from the mentioned electrodeposition times is actually spent in the anodic cycle of the sinusoidal signal and it is effectively used in the synthesis of the materials. The comparison is assuming a 100% efficiency of the electrodeposition processes. Thereby, these findings reveal the suitability of SC approach for the sensor fabrication.

The characterization of the sensor in sulfuric acid solution has pointed out the presence of Pt nanoparticles within the nanocomposite material (see Figure 4). The CVs registered in solution containing 0.5 M H_2SO_4 are characterized by a cathodic wave situated at 0.45 V. The cathodic wave is determined by the electrochemical reduction of Pt oxide that was produced on the potential cycling in the anodic direction and attests the successful deposition of Pt nanoparticles.

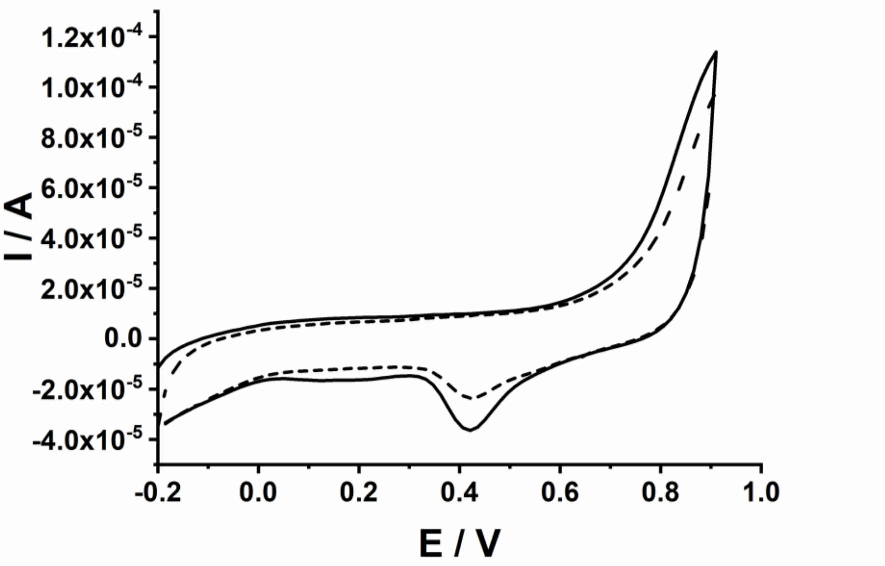
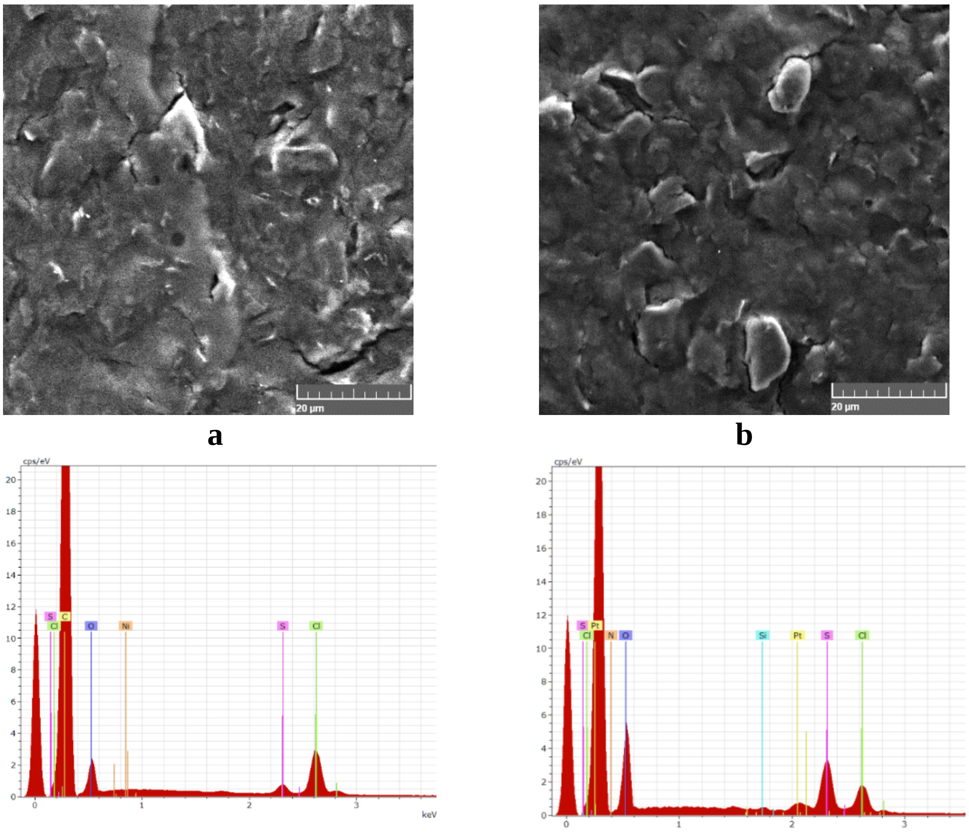


Fig. 4. The CVs obtained at the electrochemical sensor in sulfuric acid solution. The first two potential scans are displayed.

The nanocomposite material was also studied by scanning electron microscopy (SEM). Hence, SEM measurements have shown an increase in the roughness of PEDOT matrix (see Figure 5a) and have confirmed the preparation of PtNP (see Figure 5b). The EDX analysis supports the preparation of the PEDOT (see Figure 5c), respectively the deposition of PtNP (see Figure 5d). The SEM investigation is in agreement with the electrochemical characterization data presented above.



a b

c d

Fig. 5. SEM measurements for PEDOT (**a**), and PtNP-PEDOT (**b**). EDX spectra for PEDOT (**c**), and PtNP-PEDOT (**d**).

3.2. Analytical applications

The electrochemical sensor has been investigated for serotonin detection in synthetic samples as well in real systems. The optimization of the sensor's performance has included the pH influence. The pH study has been conducted in buffered systems with pH comprised between 4 and 8 containing an amount of serotonin of 80 μM. The corresponding CVs are presented in Figure 6. The change in pH from 4 to 8 produced the displacement of peak potential in cathodic direction. The slope was equal to (-0.049) V/pH unit. This slope value is close to that indicating same numbers of electrons and protons observed for the analyte reaction [23]. Furthermore, the current response was almost constant for pH range 6 to 8. Thus, the pH = 7.0 was used in the subsequent applications.

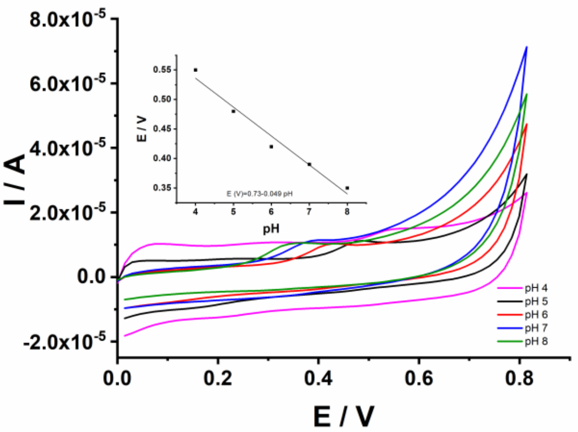


Fig. 6. The pH study in the range 4-8 in buffer systems containing 80 μM ST. Inset: anodic peak potential dependence on pH.

The sensor response for the analyte detection has been studied by CV in solution of pH=7.0 and various amounts of serotonin from 2 to 80 μM. The obtained CVs are displayed in Figure 7. The voltammetric sensor presented a linear response with the regression equation: I_{pa} (μA) = 12.2 + 0.057 [ST] (μM) and r = 0.9996. The sensitivity was equal to 0.057 μA/μM according to the above linear equation. The detection limit (DL) and quantification limit (QL) have been calculated with a formula described previously [21]. The obtained DL and QL values have been in the same order: 1.9 μM, and 6.3 μM. The DL and QL values are better than those for a AuNP based sensor [21]. The repeatability and the reproducibility have been calculated and displayed as RSD% values (n=3). The obtained repeatability was of 3.6% at the 40 μM analyte level. The reproducibility has been investigated by applying 3 sensors obtained in the same way for 40 μM ST detection. The reproducibility was equal to 4.7%. It could be noted that the voltammetric determination of target analyte was achieved with overall high analytical performance.

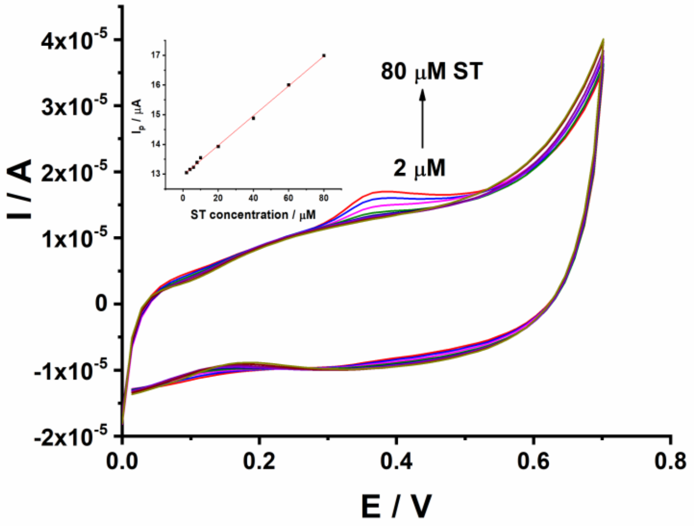
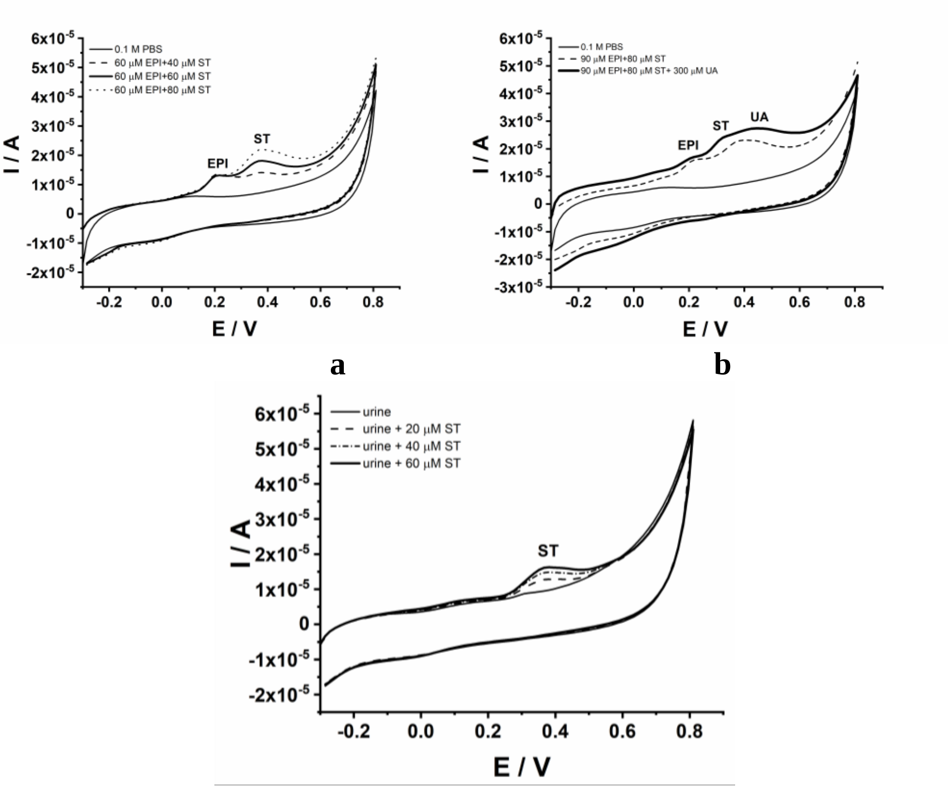


Fig. 7. The sensor response for various amounts of ST from 2 to 80 μM. The calibration plot is presented in the inset.

The selectivity study has included epinephrine (EPI) and uric acid (UA) that are interfering in the determination of serotonin because of the close values of the oxidation potentials. The determination of ST alongside with 60 μM EPI was performed (see Figure 8a). The obtained data show that each species can be individually determined with high accuracy. The sensor response toward ST increases with ST addition, meantime the signal for EPI remains unmodified. The influence of EPI and UA on the detection of ST has been also investigated (see Figure 8b). There were separated anodic waves for each species in the case of the contemporary presence of the investigated interfering species EPI and UA.

The proposed sensor has also been evaluated for its efficiency and applications in the electroanalysis of the ST analyte in real samples characterized by complex composition (see Figure 8c). Urine samples have been diluted with 0.1 M PBS at 1:40 ratio and afterwards were enriched by known standard ST amounts: 20, 40 and 60 μM, respectively. The ST was not detected in the real samples. The oxidation potential of ST is not affected by the matrix sample. The sensor response is increasing with the addition of ST and confirms its applicability in the analysis of real samples which are characterized by complex matrix. The recovery values were comprised between 98.2% and 114.9%. The results illustrate the suitability of the electrochemical sensor based on PtNP-PEDOT nanocomposite material for practical applications.



a **b**

c

Fig. 8. **(a)** The sensor response towards 60 µM EPI, and 40, 60, and 80 µM ST. **(b)** The sensor response for 90 µM EPI and 80 µM ST; 90 µM EPI, 80 µM ST, and 300 µM UA, respectively. **(c)** The sensor response in real sample and after addition of 20, 40, and 60 µM ST.

The performance of the sensor was compared taking into consideration the data previously published (see Table 1). It could be noted that the sensor performance is comparable to other sensors based on different sensing materials and obtained by other preparation methods.

Table 1

Performance in the serotonin detection of various electrochemical sensors

Electrochemical sensor	Detection limit (µM)	Linear range (µM)	Ref.
rGO-PEDOT:PSS/Nafion	0.16	0.05 - 50	[8]
Polylactic acid 3D printed	0.5	0 - 20	[10]
ITO/CuO-Cu_2O	0.12	2 - 100	[12]
AuNPs-PEDOT	5.7	10 - 320	[21]
GCE modified with carbon spheres	0.7	40 - 750	[24]
GCE-PEDOT-PtNP	1.9	2 - 80	This work

4. Conclusions

The voltammetric detection of serotonin at a sensor prepared by an innovative fabrication method has been presented. The SC method has allowed the rapid synthesis of the nanostructured sensing material onto electrode surface with enhanced electron transfer properties and good analytical performance. The morphological and electrochemical characterization of the composite material confirmed the deposition of Pt nanoparticles. The sensor displayed low values for the detection and quantification limits. The sensor response toward serotonin was linear on a large concentration range. The selectivity of the sensor was demonstrated in the electroanalysis of synthetic as well real samples with good performance.

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