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Square-Wave Adsorptive Stripping Voltammetric Determination of Serotonin at Glassy Carbon Electrode Modified with Safranine O

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A poly(safranine O) modified glassy carbon electrode has been prepared by an electrooxidative polymerization of safranine O. The electrochemical behavior of serotonin (5-HT) at this modified electrode was investigated by cyclic voltammetry (CV) and square wave adsorptive stripping voltammetry. The modified electrode was employed as an electrochemical sensor for the detection of serotonin concentration and exhibited a typical enhance effect on the current response of serotonin and lower oxidation overpotential. Due to the relatively low currents and different potentials in the electrochemical responses to ascorbic acid and dopamine, the modified electrode is a useful and effective sensing device for the sensitive and selective serotonin determination in the presence of ascorbic acid and dopamine. At optimised conditions, the calibration curve for serotonin was showed two linear segments: the first linear segment increased from 3.0×10^{-8} to 1.0×10^{-6} mol L^{-1} and second linear segment increased up to 1.0×10^{-5} mol L^{-1}. The detection limit was determined as 5.0×10^{-9} mol L^{-1} using SW-AdSV. The proposed method was successfully applied to determine serotonin in human serum samples.

Keywords: Serotonin, biomolecules, safranine O, electrochemical sensing, determination, serum analysis.

1. INTRODUCTION

The bioactive indolamine serotonin (5-hydroxytryptamine, 5-HT), is an important neurotransmitter and vasoconstrictor. Serotonin is synthesized in the brain and body from tryptophan an amino acid. Tryptophan converts into 5- hydroxytryptophan then into serotonin (5-hydroxytryptamine), if all of the co-factors are present. However, widely variable "normal" concentrations of plasma 5-HT (0.5–44 μg/L) have been reported [1]. Low serotonin levels (or serotonin deficiency) are often attributed to anxiety, depression, panic attacks, insomnia, obesity,

fibromyalgia, eating disorders, chronic pain, migraines, and alcohol abuse [2,3]. Extremely high levels of 5-HT can manifest toxicity and potentially fatal effects known as serotonin syndrome [4]. Effective determination of 5-HT levels is of greater value because of their coexistence within biological systems.

To date, a variety of analytical methods such as spectrophotometry [5], fluorometry [6-8], enzyme immunoassay [9,10], radioimmunoassay [11], capillary electrophoresis (CE) [12] and GC–MS [13], have been developed for the determination of serotonin. Serotonin is usually assayed by HPLC with either electrochemical or fluorometric detection [14,15]. However, these reported techniques are expensive and require time-consuming derivatization step and also in some cases low sensitivity and selectivity makes them unsuitable for a routine analysis. The concentration of 5-HT is very low in biological systems. The other problem which must be solved in the electrochemical detection of 5-HT is the co-existence of many interfering compounds in biological systems. Among these interfering compounds, ascorbic acid (AA), and dopamine (DA) are particularly important because they can all be oxidized at similar potentials resulting in overlap of voltammetric responses. The oxidation potential of 5-HT (pH 7, 0.38 V) is close to that of DA (0.22 V) and AA (0.2 V) on the unmodified glassy carbon electrodes. To overcome these problems, one of the most common ways is using a modified electrode to improve the measuring sensitivity of 5-HT and remove the interference of AA and DA to 5-HT detection. Therefore, different electrochemical methods using various modified electrodes were also proposed for the determination of 5-HT. Most electrochemical methods rely on the modification of electrodes such as boron-doped diamond electrode [16], poly(phenosafranine) modified electrode [17], graphene modified GCE [18], *meso*-tetrakis(2-aminophenyl) porphyrin-SWCNT-modified GCE [19], 5,5-ditetradecyl-2-(2-trimethylammonioethyl)-1,3-dioxane bromide lipid film modified GCE [20], poly(3,4-ethylenedioxy pyrrole)-SWCNT-modified GCE [21], poly rutin (Ru) modified paraffin-impregnated graphite electrode (WGE) [22], calixarene-based voltammetric sensor [23], carbon nanofiber based biosensor [24], carbon ionic liquid electrode [25], poly(3,4-ethylene dioxythiophene) modified Pt electrode [26] and nanocluster/overoxidized-polypyrrole composite modified electrode[27].

Safranine O (SFO) is an electroactive polyaromatic cation. It is available under a variety of mixtures and purities. Safranine-O and Safranine-T are all mixtures of the methylated and unmethylated forms. At any rate, all of them behave as a single component when their electrochemical behaviour is of concern [28,29]. Most of the electrochemical studies on Safranine are focused on its electrodeposition on glassy carbon electrodes leading to stable redox-active films. Electropolymerization of safranine on the GCE has been constructed by cyclic voltammetry (CV) in phosphate buffer solution (PBS) [28-33]. The properties of the resulting polymer [poly(safranine)] has been characterized, its electrochemical properties investigated, and has been applied to sensors [28-33]. Recently, safraninee film modified glassy carbon electrode was used for electrocatalytic effect on the electrooxidation of 4-nitrophenol, epinephrine, uric acid and parathion [29-32]. The modified electrode showed good stability and reproducibility.

In stripping voltammetry a preconcentration step is combined with a stripping step, thereby enhancing sensitivity and selectivity. Wang et al. first presented square wave anodic stripping voltammetry (SW-ASV) using undecylcalix[4]resorcinarene modified glassy carbon electrode (CUCR/GCE) for detection 5-HT [23]. The modified electrode showed a linear voltammetric response

for the 5-HT within a concentration range of 1.0 $\times 10^{-7}$ to 1.0×10^{-5} mol L^{-1}, and a value of 3.0×10^{-8} mol L^{-1} was calculated for the detection limit. The goal of this study is to give a general overview of the reliability and possibility for using nonmercury type electrode for the square wave adsorptive stripping voltammetric determination of serotonin in human blood serum analysis. A poly(SFO) film on the glassy carbon electrode was prepared by the oxidative electropolymerization of safranine in phosphate buffer (pH 5.5), and the resulting poly(safranine) modified electrodes exhibited a good electrocatalytic activity for 5-HT oxidation and good stability.

2. EXPERIMENTAL

2.1. Apparatus

The voltammetric experiments were performed in an electrochemical assembly with a platinum wire as the counter electrode, a glassy carbon electrode (3 mm diameter) as working electrode and a Ag/AgCl reference electrode. Cyclic voltammetry (CV) experiments were carried out with a Gamry Reference 600 potentiostat (Gamry, USA). All experiments were performed at room temperature (25^{o}C). Before each experiment, the working electrode was polished with a slurry containing 0.3 μm and then with 0.05 μm sized aluminum oxide particles for 5 min. After each treatment, the electrode was washed and ultrasonicated in distilled water for 5 min to remove retained aluminum oxide particles on the electrode surface. The pH values of the solutions were measured by a Hanna HI 221 pH-meter using the full range of 0-14. Supporting electrolyte of phosphate (NaH_2PO_4 - Na_2HPO_4) and acetate buffers were prepared in distilled water.

2.2. Reagents and materials

All chemicals used were of analytical-reagent grade, and distilled water was used throughout. Serotonin, ascorbic acid and dopamine were obtained from Sigma (St. Louis, MO, USA), and they were all used as received. The stock solutions of serotonin, dopamine (1.0×10^{-3} mol L^{-1}) and ascorbic acid (1.0×10^{-2} mol L^{-1}) were prepared daily by dissolving serotonin, dopamine hydrochloride and ascorbic acid in distilled water. The solutions were protected from light and stored at 4°C. Before use, all sample solutions were prepared by appropriate dilutions to the desired concentration with distilled water. Safraninee O was purchased from Merck and it was used as received. All potentials reported were versus the Ag/AgCl. All electrochemical measurements were performed at ambient temperature.

2.3. Electrode Preparation

Before each electrochemical modification, the glassy carbon electrode was first polished with 0.05-μm alumina in a water slurry using a polishing cloth and rinsed with 1:1 HNO_3, acetone and water, respectively. Poly(safranine O) films were obtained by electrochemical polymerisation, cycling the applied potential from −0.8 to 1.2 V vs. Ag/AgCl at scan rate 50 mV s^{-1} in the polymerisation

solution containing 0.5 mM safranine O [33]. The fastest polymerisation occurred in solutions at pH 5.5 (*i.e.* pH 5.5+100 mM KCl). Then, a poly(SFO)-modified film was formed on the GCE surface. The electrodes thus prepared were treated in an ultrasonic bath for a few minutes to dissolve safranine monomer adsorbed on the electrode surface or trapped in the polymer matrix. All the resulting modified electrodes were washed with phosphate buffer solution before electrochemical measurements. The poly(SFO)-modified GCE was stored in phosphate buffer solution (PBS) at pH 7.0. Fig.1 gives a possible structure of poly(SFO) [31,33].

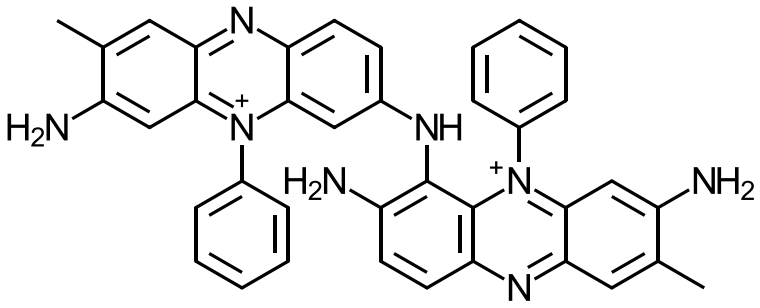

Figure 1. Poly(SFO).

2.4. Analytical Procedure

A three-electrode system was used, including a poly(SFO)-modified GCE as the working electrode, a platinum electrode as a counter electrode, and Ag/AgCl (3.0 M KCl) as a reference electrode. The electrodes were dipped in the acetate buffer solutions. After recording the square-wave adsorption stripping voltammogram (SW-AdSV) of the blank solution, an accurate concentration of the 5-HT solution was added and measured. The optimized experimental conditions for the square wave adsorptive stripping voltammetric determination of 5-HT were: 0.1 acetate buffer solution (pH=4.0) as supporting electrolyte, step size = 8 mV, frequency (f) = 25 Hz, pulse size = 100 mV, accumulation time = 60 s, and accumulation potential E = 0.0 V.

3. RESULTS AND DISCUSSION

3.1. Characteristics of the poly(SFO) film

Electropolymerization process of safranine O was carried out using multiple scan (15 cycles) cyclic voltammetry [17,29,30-33]. The CVs of the poly(SFO)-modified GCE at various scan rates in phosphate buffer (pH 7.0) in the potential range of -0,6 to 0,1 V (vs. Ag/AgCl) are shown in Fig. 2. The peak currents were directly proportional to the applied potential scan rates. The linear regression equations are I_{pa} (μA)= 0.0418 v (mV s^{-1}) + 0,8397 (R = 0.9989) and I_{pc} (μA) = -0.0472 v (mV s^{-1}) + 0,1535 (R = 0.9980). This results demonstrated that the polymer film was formed on the GC electrode surface.

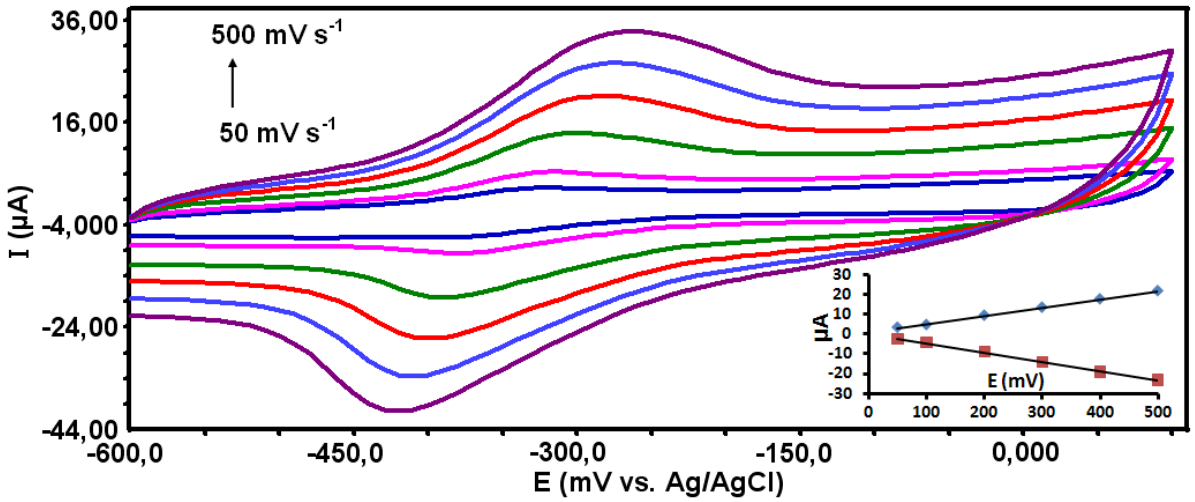

Figure 2. Cyclic voltammograms at the composite polymer modified electrode in phosphate buffer solution (pH 4.0) at the scan rate of 50, 100, 200, 300, 400, and 500 mV s^{-1}, respectively.

3.2. The electrochemical behavior of 5-HT

The voltammetric response of 5-HT on the modified electrode was determined via CV. Fig. 3 presents cyclic voltammograms (CVs) of 0.1 mM 5-HT in 0.1 M acetate buffer recorded at bare GCE (c), and poly(SFO)-modified GCE (d). At the bare GCE, 5-HT shows an irreversible behavior with relatively weak current response at about 585 mV. The CVs of 5-HT at the poly(SFO)-modified electrodes exhibited oxidation peaks with a higher magnitude of oxidation current than that of bare GCE.

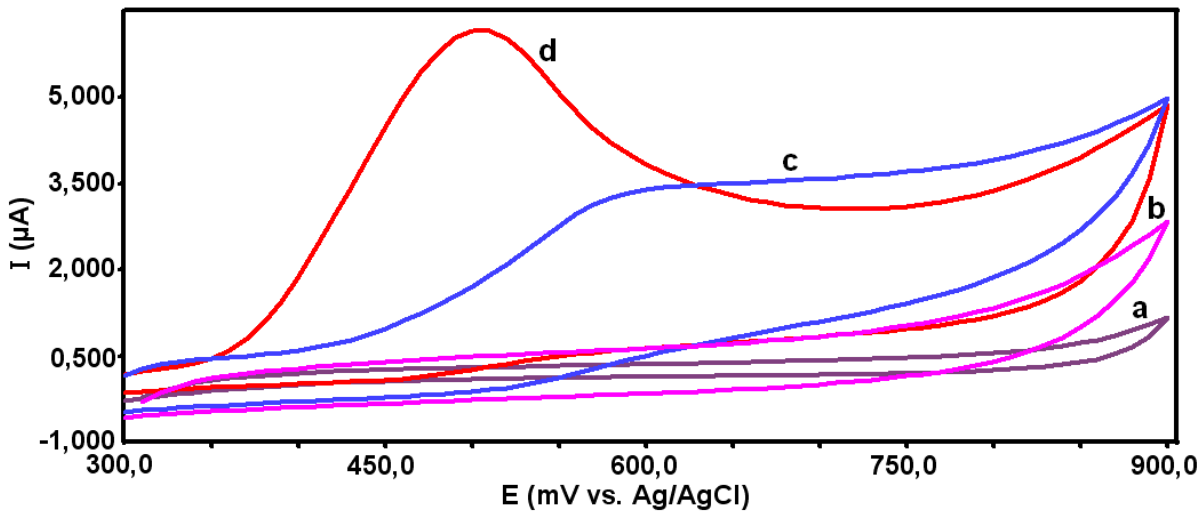

Figure 3. CVs of 5-HT on the bare GCE and poly(SFO)-modified GCE in 0.1 M acetate buffer (pH 4.0) at scan rate of 50 mV s^{-1} (a) and (b) represent the absence of 0.1 mM 5-HT, (c) and (d) represent the presence of 0.1 mM 5-HT.

As can be seen from Fig. 3 (d), 5-HT exhibits a well-defined oxidation peak on the poly(SFO)-modified GCE with E_{pa} = 506 mV, and the over-potential of 5-HT becomes lower (79 mV) than that on the bare GCE. The background current of poly(SFO) modified GCE (curve b in Fig.3) is higher than that of the bare GCE (curve a in Fig. 3), which is ascribed to the large surface area of the poly(SFO) film. The poly(SFO)-modified electrodes exhibited excellent electrocatalysis for 5-HT oxidation. The results demonstrated that the poly(SFO)-modified electrode possessed a strong electrocatalytic activity for the oxidation of 5-HT.

3.3. The effect of pH

The effect of solution pH on the redox response of 5-HT was investigated. Cyclic voltammograms of 0.1 mM 5-HT in 0.1 M acetate buffer solution at the poly(SFO)-modified GCE with different solution pH values are shown in Fig. 4. As can seen in Fig. 4, the peak current increased with solution pH ranging from 3.6 to 4.0, and then the decrease of the roxidation peak current was observed when the solution pH was higher than 4.0. Increasing the solution pH value beyond 3, gave the sensor with a reduced response. Considering the detection sensitivity, pH 4.0 was chosen in the following investigation. The oxidation peak potential (E_{pa}) of 5-HT varied linearly in the range of pH values from 3.6 to 8 with the slope of −51 mV pH^{-1}, which was near to the theoretical value of −59 mV pH^{-1}. This result was agreement with the Nernst equation for a two-electron and two-proton transfer reaction. Fig. 4 (inset) shows E_{pa} as a function of pH with the linear equation as: E_{pa} (vs. Ag/AgCl)= 0.7248 -0.051 pH (R = 0.9988). Thus, at the poly(SFO)-modified electrode, the electrochemical reaction of 5-HT is a two-proton coupled two-electron process.

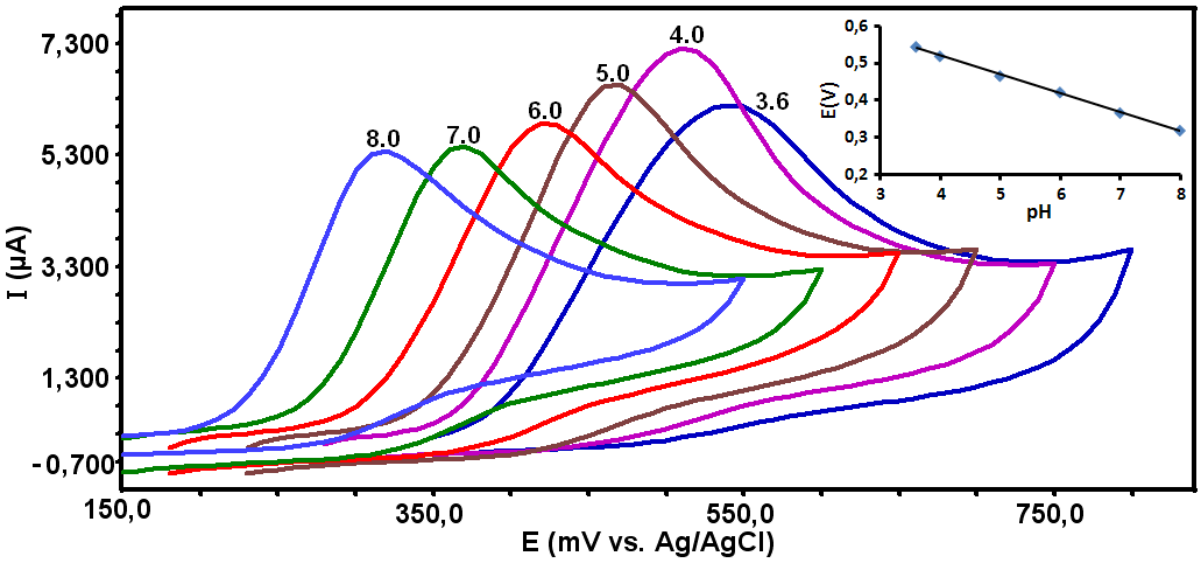

Figure 4. Effects of pH on peak potential and peak current of the electrochemical oxidation of 5-HT at the poly(SFO)-modified electrode. Inset: The plot of potential vs. pH value. Scan rate:50 mV s^{-1}.

3.4. The effect of scan rate

The effect of the potential scan rate on the current response of 5-HT was tested. Fig. 5 shows the CVs of the poly(SFO)-modified GCE at various scan rates (v) obtained in 0.1 M acetate buffer (pH 4.0) containing 0.1 mM 5-HT. As shown in Fig. 5, the peak currents increased with increasing the potential scan rate. As can be seen (Fig.5 inset), the oxidation peak currents were found linearly proportional to the scan rate ranging from 25 to 500 mV s^{-1} and the linear regression equation is I_{pa} (μA)= 0.0682 v (mV s^{-1}) + 1.9747 (R = 0.9989). In the scan rates ranging from 25 to 500 mV s^{-1}, the linear regression equations of the E_{pa} vs. logarithm of the scan rates are expressed as E_{pa} (vs. Ag/AgCl) = 0.0754 log v (mV s^{-1}) + 0.3865 (R=0.9938). This experimental results indicated that the poly(SFO)-modified electrode reaction of 5-HT is a typical adsorption-controlled process. In addition, with the increase the potential scan rate, the oxidation peak potential shifted positively, indicating that the electron-transfer rate decreased.

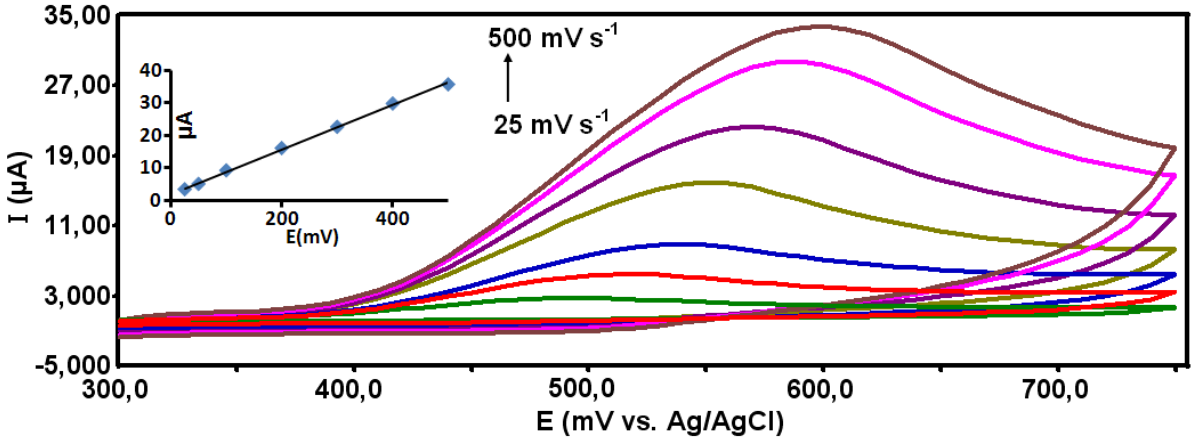

Figure 5. CVs of 0.1 mM 5-HT on poly(SFO)-modified electrode at different scan rates from 25 to 500 mV s^{-1} (25, 50, 100, 200, 300, 400 and 500), respectively. Inset: is the plot of the peak current of 5-HT versus scan rate.

3.5. SW-AdSV parameters

The peak currents depend on the SW-AdS voltammetric parameters. The effect of the accumulation time was studied for a 5-HT concentration of 10 μM. To achieve the maximum sensitivity of SW-AdS peak, step size, pulse size, frequency (f), accumulation potential and time parameters have to be carefully optimized for a 5-HT concentration of 10 μM. The adsorptive stripping peak currents were then obtained under different pHs, pulse sizes, frequencies, accumulation potentials, and times. The highest stripping peak current was obtained at pH 4.0 similar to those found in cyclic voltammetric experiments. Stripping current responses were increased with pulse sizes up to 100 mV, but the base-line current also increased. Varying the value of square wave frequency also plays an important role for the measured signal of SW-AdSV approach. The adsorptive stripping peak intensity initially increased with frequency from 5 to 25 Hz, and then became distorted and ill-defined.

The frequency value of the method was selected as 25 Hz. The step size from 5 to 10 were investigated. The stripping peak current increased linearly with the step size up to 8 mV. Accumulation by the adsorption of 5-HT on the electrode surface, could enhance the current response and improve the detection sensitivity. Therefore, the influence of accumulation time and accumulation potential on the electrode response was investigated by SW-AdSV. The accumulation potentials from -0.4 V to + 0.4 V were applied under stirring at 400 rpm. The stirring was stopped, and after waiting for 5 s equilibrium period, the stripping voltammogram of 5-HT was recorded. The experimental results indicated that the highest efficiency of the accumulation of 5-HT was obtained when the accumulation potential was equal to 0.00 V, so for further electrochemical measurements this potential was adopted. When the accumulation potential was more positive than 0.0 V, the adsorptive stripping peak current changed only very slightly while the background current increased greatly. The accumulation time of the analyte was investigated in the range from 30 to 180 s. The stripping peak current of 5-HT increased significantly with the increase of the accumulation time, and reached a maximum at 60 s, suggesting that poly(SFO) thin film can very rapidly accumulate 5-HT. Then, the current increased slightly when the time exceeded 60 s. Therefore, 60 s was adopted as the accumulation time further experiments.

3.6. Analytical parameters

Table 1. Comparison of the analytical performance of the different modified electrodes for the determination of 5-HT.

Modified Electrode	Linear range (μM)	Detection limit (μM)	Reference
Boron-doped diamond electrode	0.01-100	0.01	[16]
Poly(phenosafranine)	NR	NR	[17]
Graphene modified GCE	1–36	0.005	[18]
meso-Tetrakis (2-aminophenyl) porphyrin-SWCNT	0.2-10	0.001	[19]
5,5-ditetradecyl-2-(2-trimethylammonioethyl)-1,3-dioxane	0.2-10	NR	[20]
Poly(3,4-ethylenedioxy pyrrole)-SWCNT	0.1-10	0.005	[21]
Poly rutin (Ru) modified paraffin-impregnated graphite electrode (WGE)	3.0-90	0.8	[22]
Calixarene-based voltammetric sensor	0.1-10	0.03	[23]
Carbon nanofiber based biosensor	1-10	0.1	[24]
Carbon ionic liquid electrode	0.75–90.35	0.36	[25]
Poly(3,4-ethylene dioxythiophene) modified Pt electrode	20-100	71	[26]
Nanocluster/overoxidized-polypyrrole composite	0.007-2.2	0.001	[27]
Poly(safranine O)	0.03-10	0.005	This work

NR: not reported

In order to obtain a much more sensitive peak current, the SW-AdSV was used for the determination of 5-HT. Fig. 6 depicted the SW-AdSV curves of different concentration of 5-HT at

poly(SFO)-modified GCE. Using the SW-AdSV method, the peak current increased linearly with 5-HT concentration with very good correlation coefficients. The calibration curve for 5-HT shows two linear segments: the first linear segment increases from 0,03 to 1.0 µM with linear regression equation of I_p (µA)= 6.9913 C + 0.2301 (R = 0.9993), and second linear segment increases up to 10 µM with linear regression equation of I_p (µA)= 0.7160 C + 9.1798 (R^2 = 0.9979). The limit of detection of the method, defined as C_L=3Sy/x/b [36] (where Sy/x is the standard deviation of y-residuals and b is the slope of the calibration plot). The detection limit was 0.005 µM, which is lower than that at CUCR/GCE (0.03 µM) [23]. Under the optimized conditions, the repeatability of the proposed method expressed as relative standard deviations (RSDs, n =5) were found to be 1.5 and 1.3 % for the concentration of 0.5 and 5.0 µM, respectively.

Table 1 shows the comparison between the analytical performance of the present method and previous literature methods for the determination of 5-HT. These results reveal that the proposed poly(SFO)-GCE has a large advantage over other reported methods in terms of linear working range [16-26] and limit of detection [16, 22-26].

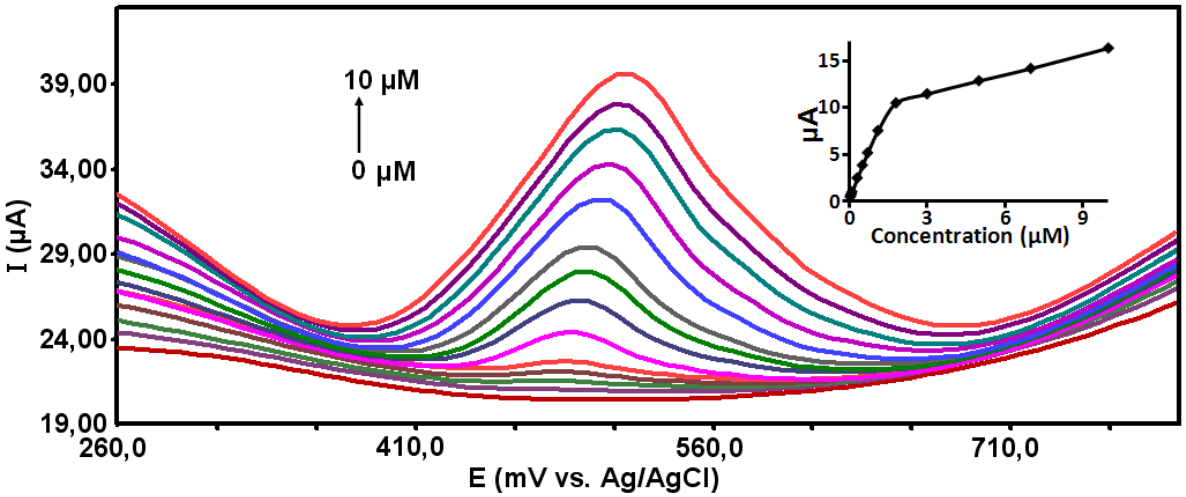

Figure 6. SW-AdSV on poly(SFO)-modified electrode for different 5-HT concentrations (from 0 to 10) : 0.0, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5, 0.7, 1.0, 2.0, 3.0, 5.0, 7.0 and 10 µM in 0.1 M acetate buffer. Inset is the relationship of current responses to 5-HT concentration.

3.7. Repeatability and stability of the modified electrode

The measurement repeatability of the poly(SFO)-modified GCE was tested with the same 5-HT concentration. The RSDs of 0.5 and 5.0 µM 5-HT were 1.5 % and 1.3 % for five repetitive assays, respectively. This result alone indicated that this method had good reproducibility. The storage stability of the proposed sensor was also studied. When not in use, the poly(SFO) modified electrode was suspended above phosphat buffer solution at 4°C in a refrigerator. The response to 5-HT were investigated. After 5 and 10 days, the sensor retained 96% and 91% of its initial response current, respectively. The results indicate that the poly(SFO) film has a good stability.

3.8. Interference studies

In biological environments, the main interference of catecholamines compounds is the presence of high concentration of AA and also the presence of dopamin. The oxidation potential of each interferent ascorbic acid (E_{pa}= 220 mV), dopamin (E_{pa}=308 mV) at the poly(SFO) film-coated electrode was found to be more negative than that of 5-HT (E_{pa}= 491 mV). Therefore, it could be possible for selective detection of 5-HT in the presence of AA and dopamine but also selective for the simultaneous determination of these three species present in a mixture. Separation of anodic peaks of 88, 183, and 271 mV between DA-AA, DA-5-HT, and AA-5-HT, respectively, allows us to determine AA and DA simultaneously by using SW-AdSV. The result showed that 100 fold of bilirubin and tocopherol, 10 fold of uric acid did not interfere with the oxidation signal of 0.5 µM 5-HT (signal change below 5%). On the other hand, no interference has been found when including up to 1000 µM of glucose.

Table 2. Application of the SW-AdSV method to the determination of 5-HT in spiked blood serum samples (n=3).

Sample	Added (µM)	Found (µM)	R.S.D. (%)	Recovery (%)
A	0.05	0.048	4.5	96
B	1.0	1.065	3.9	106.5
C	5.0	5.49	3.5	109.8

3.9. Applications

To demonstrate the applicability of the proposed poly(SFO)/GCE as an electrochemical sensor, determinations 5-HT in human blood serum were performed by spiking diluted samples with known amounts of 5-HT. Human blood samples were obtained from healthy volunteers. SW-AdSVs of unspiked and spiked samples were obtained under optimum conditions. The results for the determination of 5-HT in real sample are summarized in Table 2. Recovery values were calculated based on this method and the values are between 96 % and 109.8 %. The good recoveries confirm that the detection of 5-HT using poly(SFO) modified electrode is a reliable method for direct determination of 5-HT in human blood samples.

4. CONCLUSIONS

A poly (SFO) modified glassy carbon electrode was fabricated by electrochemical polymerisation***(CV)***. This modified electrode was evaluated satisfactorily in the detection of 5-HT in human blood serum without the necessity of sample pretreatments or time-consuming extraction. The poly(SFO)-modified electrode showed good electrocatalytic activity for the oxidation of 5-HT. A

better separation of oxidation peaks of 5-HT, DA and AA, indicate that the poly(SFO)/GCE facilitates the simultaneous determination of 5-HT, DA and AA with good stability, sensitivity and selectivity.

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