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Voltammetric Determination of 5-Hydroxyindoleacetic Acid at Poly (p-amino benzene sulfonic acid) Modified Sensor

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Abstract- In this study, the quantitative amount of 5-hydroxyindoleacetic acid (5-HIAA) was determined at poly (*p*-amino benzene sulfonic acid) (*p*-ABSA) modified glassy carbon electrode. The redox property of 5-HIAA was investigated by cyclic voltammetry. The cyclic voltammograms showed that the redox reaction of 5-HIAA was irreversible. Scan rate study showed that the redox reaction of 5-HIAA was controlled by both diffusion and adsorption on the poly (*p*-ABSA) modified sensor. Differential pulse voltammetry technique was used for the quantitative analysis of 5-HIAA in phosphate buffer solution at pH 7.00. The linear working range of the calibration graphs was determined as 1×10^{-5}–9×10^{-5} M (R^2, 0.9912), and the detection limit was determined as 5.3×10^{-7} M. Recovery values in the analysis of urine samples were between 99.4% and 103.0%. The results showed that the modified sensor can be applied to the determination of 5-HIAA in the presence of ascorbic acid. The proposed sensor is promising for routine analysis due to its high selectivity, reproducibility, long-term stability, and high recovery values in biological samples.

Keywords- 5-Hydroxyindolacetic acid; Serotonin; *p*-Amino benzene sulfonic acid; Differential pulse voltammetry; Cyclic voltammetry

1. INTRODUCTION

5-Hydroxyindolacetic acid is the main metabolite of serotonin [1]. The amount of this neurotransmitter may vary in some pathological conditions, such as carcinoid tumours of enterochromaffin cells in the small intestine [2–4]. Therefore, it secretes excessive amounts of serotonin and the amount of 5-HIAA increases in the urine. The considered normal range is 2–8 mg/24 hours (approximately 2–8 mg L^{-1}) for 5-HIAA in urine. Levels above 25 mg/24 hours (25 mg L^{-1}) are considered positive for carcinoid tumour. However, patients with these tumours can produce 5-HIAA in amounts 50 times higher than normal levels [1]. However, urinary 5-HIAA concentrations are diet dependent, and even minor changes above the abnormal limits may be associated with a non-tumour stomach condition. Therefore, it requires frequent repetition of measurements for accurate diagnosis.

In recent years, liquid chromatography with mass spectrometry [5–8], fluorescence spectrometry [9] and electrochemical determination methods have been used for the quantitative determination of 5-HIAA [10,11]. However, although the chromatographic method is popular over other methods, the presence of interferences is a major disadvantage for this method. In addition, the determination of these substances by the chromatographic method is possible if the electrochemical detector is used. Alternatively, the electrochemical method has recently attracted more attention due to its advantages such as more selective, more sensitive, less expensive and shorter preparation time compared to other methods. It is possible to analyse drug active ingredients from biological fluids in a fast, sensitive and economical way without the need for any separation method with voltammetric methods [12–19]. One of the advantages of voltammetric methods is that they are used to explain the pharmacological action mechanisms of many compounds that are of physiological importance in the field of molecular biology due to the role of drug active substances in redox reactions.

When a bare electrode is used as a working electrode in electroanalytical applications, electroactive interferences such as ascorbic acid show electro activity on the conductive electrode surface, contributing to the amperometric response of the desired species, significantly changing the characteristic peak of the relevant species. As a result, satisfactory potential differences cannot be achieved in the peak separations. This disadvantage of the bare electrode necessitated the use of modified sensors. In addition, electrode modification offers many other advantages such as reducing over potential, increasing reaction rate and improving sensitivity [20–22].

In this study, glassy carbon electrode (GCE) surface was modified with p-ABSA using the cyclic voltammetry method at a certain film thickness. Poly (p-ABSA) selective membrane sensors were prepared on the GCE surface for the determination of 5-HIAA in the presence of ascorbic acid (AA). In the resulting poly (p-ABSA) electrode, the oxidation currents of 5-HIAA and AA were significantly increased compared to bare GCE using differential pulse voltammetry (DPV) technique. Moreover, although the peak potentials of 5-HIAA and AA

overlapped at the bare electrode, they were separated by a potential difference of about 310 mV at the poly (p-ABSA) modified sensor. The results showed that the modified sensor can be applied in the detection of 5-HIAA in the presence of AA. Voltammetric determination of 5-HIAA in real samples was performed in biological samples such as human urine to demonstrate the analytical performance and electro catalytic property of the modified sensor. Recovery values showed that the results were in agreement with the 5-HIAA value in the human urine samples. The results showed that the proposed method can be easily used to identify some catechol amines in the presence of ascorbic acid in clinical analyses with high selectivity, reproducibility, reproducibility and long-term stability properties.

2. EXPERIMENTAL SECTION

2.1. Materials

All chemicals used during the experiments were of analytical grade and were Merck or Sigma Aldrich. Aqueous solutions were prepared in ultrapure water. The stock solution of 5-HIAA was prepared daily with ultrapure water. The solution of *p*-ABSA monomer was prepared with a 0.1 M KCl solution at a concentration of 150 mM. Phosphate buffer solution (PBS) was prepared by dissolving a pH 7.4 tablet (BioShop, Canada Inc. Burlington) in 100 mL of ultrapure water. The pH of PBS was adjusted with 5 M NaOH and 1 M H_3PO_4 solutions. The analyser's own urine sample was used as the urine sample. In the analysis step, urine samples were diluted tenfold with PBS pH 7.0.

2.2. Devices

All electrochemical analyses were performed with Gamry Interface 1010B (Gamry, USA) potentiostat and triple electrode system (BASi C3 Cell Stand). Cyclic voltammetry (CV) was used to fabricate the modified sensor, CV and electrochemical impedance spectroscopy (EIS) were used to characterize the surface of the modified sensor, and DPV was used for the determination of 5-HIAA. As the triple electrode system, a glassy carbon working electrode (GCE), a platinum wire auxiliary electrode and a Ag/AgCl reference electrode were used. The pH of the solutions was measured with the 620 Lab pH Meter. Ultrapure water was used in the preparation of all solutions (Milli Q, Millipore, 18.2 MΩ.cm, Merck). In addition, shapes were drawn in the Origin 2019b program with the help of data from the software.

2.3. Preparation of poly (*p*-ABSA) modified sensors

Before electrochemical measurements, GCE was polished on cleaning pads with 1.0, 0.3 and 0.05 µm alumina powders. Then, it was electrochemically cleaned with CV technique by applying 10 cycles at a scanning rate of 100 mV s^{-1} in the potential range of -0.7 – 1.7 V in 0.5 M H_2SO_4 solution. While preparing the modified sensors, a solution of 150 mM *p*-ABSA monomer in 0.1 M KCl was prepared. And then, poly (*p*-ABSA) was grown on the GCE surface

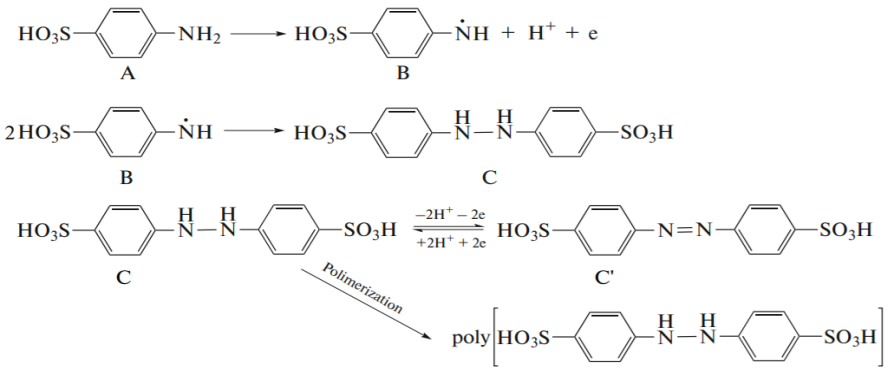
by electrochemical polymerization by applying the CV technique with 12 cycles at a scanning rate of 50 mV s^{-1} in the potential range of -1.5–2.5 V. Then, the modified sensor was washed with ultrapure water and electro activated by applying DPV technique in the potential range of -0.4 –1.0 V in PBS pH 7.0 until no monomer residues remained on its surface.

3. RESULTS AND DISCUSSION

3.1. Electro polymerization and electrochemical behaviour of *p*-ABSA

The aim of this study was to selectively detect 5-HIAA in the presence of interfering species such as AA. For this purpose, firstly, a poly (*p*-ABSA) modified sensor, which is a stable and fast sensor design that can selectively detect 5-HIAA, was designed.

Electro-polymerization of poly (*p*-ABSA) (Scheme 1) was carried out on the GCE surface with CV technique (12 cycles) at 50 mV s^{-1} scanning rate in the potential range of -1.5-2.5 V in 0.1 M KCl solution containing 150 mM *p*-ABSA.



Scheme 1. Electro-polymerization of poly (*p*-ABSA) on the GCE surface

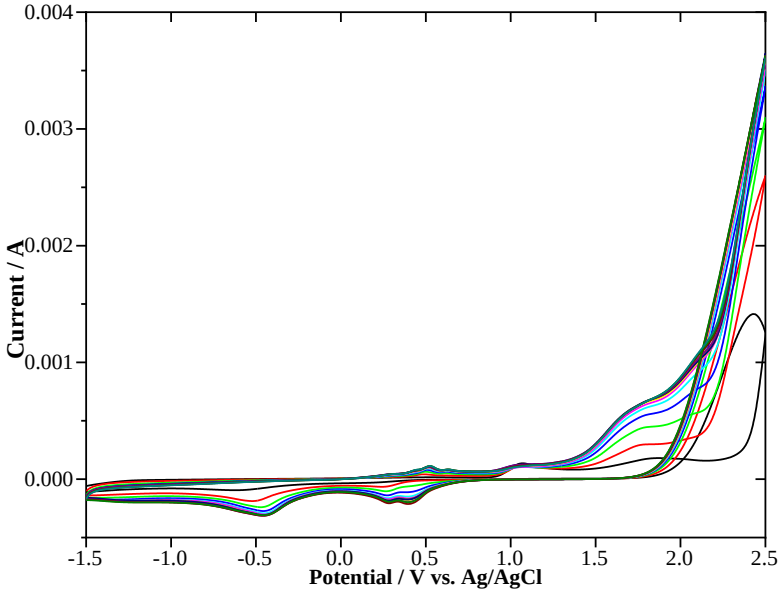


Figure 1. CV response of 150 mM *p*-ABSA in 0.1 M KCl, 12 cycles, 50 mV s^{-1}

When the CV responses in Figure 1 are examined, the fact that it gives two reduction and two oxidation peaks at 0.5, -0.5 V, supports the mechanism suggested in Scheme 1 [23,24] and explains that $2e^-$ transfer occurs.

3.2. Electrochemical characterization of poly (*p*-ABSA) modified sensor

The modified sensor was electrochemically characterized by CV and EIS techniques in 0.1 M KCl containing 5.0 mM $Fe(CN)_6^{3-/4-}$. The potentiostatic EIS technique was applied as a sinusoidal signal in the frequency range of 0.01 to 10,000 Hz. Figure 2 shows the EIS Nyquist plots for the modified sensor and GCE. The impedance data were examined in order to better compare the surface properties of the modified sensor. The charge transfers resistors (CTR) calculated using semicircular diameters gave an idea about the surface properties, and the EIS data were found to be consistent with the CV results. The CTR values for the modified sensor and the GCE electrode were 27.50 Ω and -9.91 Ω, respectively. This proves that the electron transfers rate of the poly (*p*-ABSA) modified sensor is faster than that of GCE.

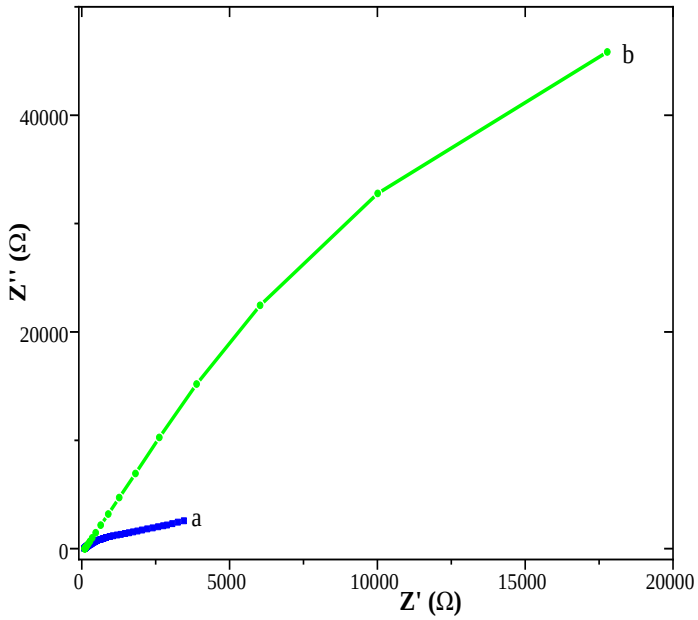


Figure 2. EIS responses at **a.** the GCE and **b.** the poly (*p*-ABSA) electrode 0.1 M KCl solution of 5 mM $K_4Fe(CN)_6$

CV responses at increasing scan rates were examined at the GCE (Figure 3A) and poly (*p*-ABSA) modified sensor (Figure 3B) in 0.1 M KCl containing 5.0 mM $Fe(CN)_6^{3-/4-}$ in order to explain the electron transfer rate of the poly (*p*-ABSA) modified sensor. The oxidation peaks current increased as the scanning speed increased, while the cathodic reduction peak current remained almost constant at the poly (*p*-ABSA) modified sensor. Results obtained with the CV

technique (Figure 3A) showed well-defined redox peaks corresponding to $Fe(CN)_6^{3-/4-}$ for GCE. The peak-to-peak separation (AEp) value was calculated as 200 mV for the GCE. This indicated a slow generation of electron transfer kinetics on the GCE surface. However, with electro polymerization, ΔEp between anodic and cathodic peaks was found to be 90 mV, and this difference decreased compared to GCE. Electro catalytic properties of the sensor also increased significantly by modifying the GCE with poly (*p*-ABSA) (Figure 3B).

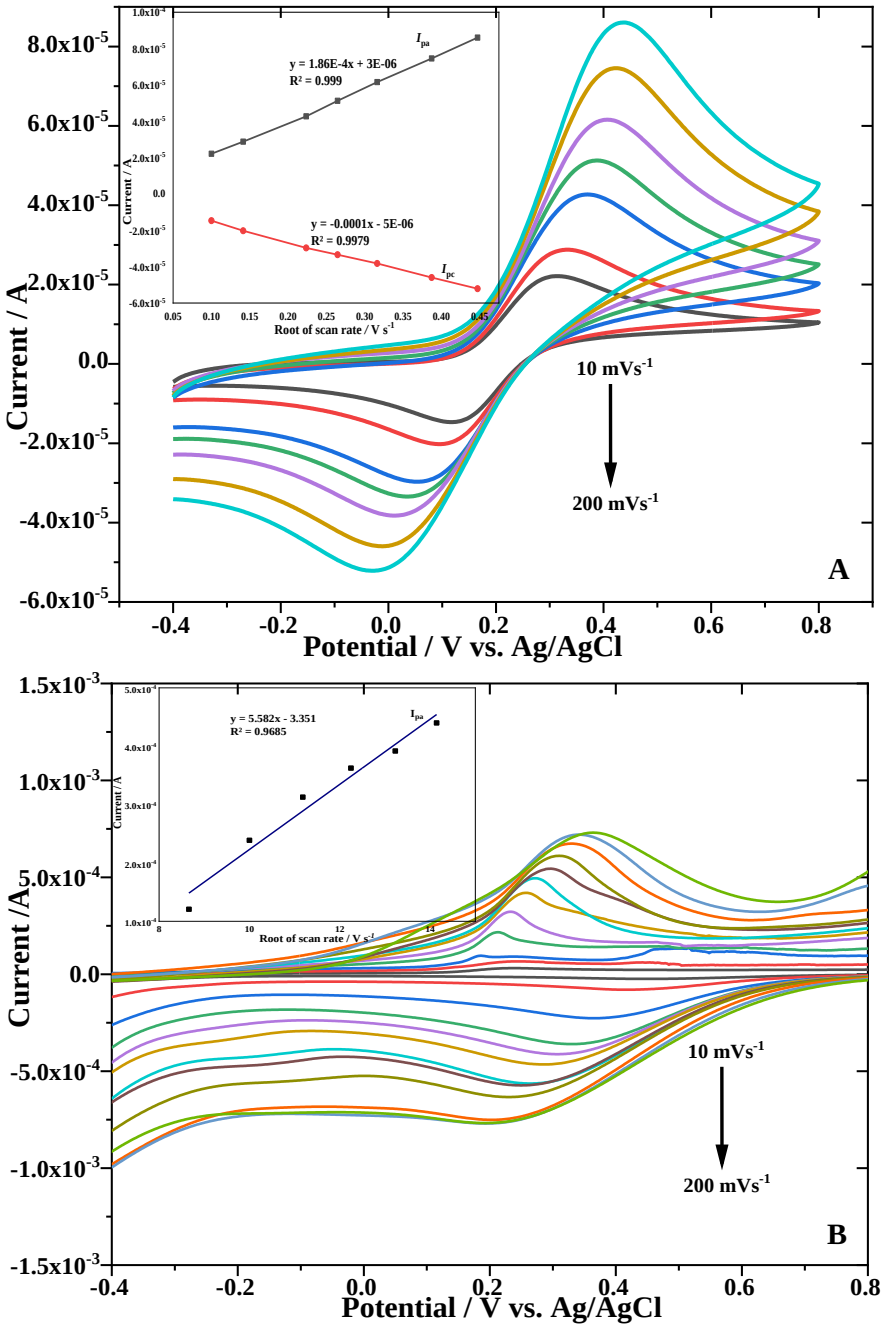


Figure 3. CV responses at **A.** GCE and **B.** poly (*p*-ABSA) modified sensor in 0.1 M KCl containing 5.0 mM $K_4Fe(CN)_6$ at increasing scanning rates. Insets: Display linear curves corresponding to the square root of scan rate and current values

The charge transfer resistance is related to the electroactive surface of the electrode. The CV responses shown in Figure 3 were used to evaluate the electroactive surface areas of the GCE and the modified sensor. Based on the Randles Sevcik equation (Eq. 1), the square root ($v^{1/2}$) of the increasing scan rates versus the I_P values was plotted, and the active surface area was calculated using the slope values.

$$I_P = 2.69\ x\ 10^5 n^{3/2} A D^{1/2} v^{1/2} C \quad (1)$$

In the equation, I_p is the anodic peak current (Ampere), n is the number of electrons given and received, A is the surface area of the electrode (cm^2), D is the diffusion coefficient ($cm^2\ s^{-1}$, 7.6×10^{-6} cm^2s^{-1}), C is the redox probe as the concentration of $K_4Fe(CN)_6$ in the KCl electrolyte (mol/cm^3) and v represents the scanning rate (V/s). As can be seen from Figure 3, electrochemical activation caused an increase in the oxidation peak. The oxidation peaks current increased as the scanning rate increased, while the cathodic reduction peak current remained almost constant at the poly (*p*-ABSA) modified sensor. With the increase in scanning rate, cathodic peak currents and peak potential shifted to negative, while anodic peak currents and peak potentials shifted to positive. This indicates a decrease in the rate constant of electron transfer and the active surface area of the electrode. Surface areas for the GCE and poly (*p*-ABSA) modified sensor were calculated as 0.0177 cm^2 and 0.169 cm^2, respectively. It was observed that the active surface area increased with the modification of GCE.

3.3. Film thickness and pH effect

The polymer film thickness and electrolyte environment, where the best resolution and highest peak current response can be obtained, were investigated for the electrochemical oxidation of 5-HIAA at the poly (*p*-ABSA) modified sensor. Modified sensors of different thicknesses were produced by electro polymerization of 150 mM *p*-ABSA monomer in 0.1 M KCl at a scanning rate of 50 mV s^{-1} for 2 to 18 cycles in the potential range of (-1.5) - (2.5) V on the GCE surface by CV technique. DPV responses were investigated for 0.1 mM HIAA in PBS pH 7.0 at poly (*p*-ABSA) modified sensors prepared in different thicknesses. The results obtained were summarized in Figure S1. The highest peak current for the electrochemical oxidation of HIAA was obtained at the poly (*p*-ABSA)/GCE modified sensor produced with 12 cycles. Thus, the (*p*-ABSA/GCE) modified sensor to be used in future studies was prepared by coating with 12 cycles of thin film.

Solutions of 0.1 mM HIAA in KCl, NaCl, $NaNO_3$, Na_2SO_4, $LiClO_4$ and pH 7.0 PBS electrolytes were prepared to decide the electrolyte environment in which voltammetric analyzes took place, and DPV responses were investigated at *p*-ABSA/GCE modified sensor. The highest peak current was obtained in PBS electrolyte solution at pH 7.0 (Figure S2). Thus, in the next step, different pH solutions of PBS were prepared, and DPV analyzes were performed for 0.1 mM HIAA in these solutions. According to the DPV results, the most

suitable electrolyte medium for HIAA at the *p*-ABSA/GCE modified sensor was obtained in PBS electrolyte solution at a pH of 7.0 (Figure S3). As a result of all these optimization parameters, PBS at pH 7.0 electrolyte solution was used in all further studies.

3.4. Voltammetric behavior of 5-HIAA

The CV behavior of 5-HIAA under optimum conditions was investigated to explain the electrochemical behavior of 5-HIAA. The cyclic voltammetric behavior of 0.1 mM 5-HIAA in PBS pH 7.0 at *p*-ABSA/GCE modified sensor at increasing scanning rates (30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150 mV s^{-1}) was investigated to understand whether the redox reaction of 5-HIAA was adsorption or diffusion controlled. The peak currents increased as the scanning rates increased. An anodic oxidation peak was observed at approximately 0.3 V (Figure S4).

Also, the peak potentials remained stable with increasing scan rates. Based on these data, 5-HIAA prepared in PBS pH 7.0 was found to have irreversible reaction kinetics at the p-ABSA/GCE modified sensor. Moreover, no linear relationship was found between the oxidation peak current and the square root of the scan rate (Figure S5A). However, a linear relationship was detected between the oxidation peak current and the scan rate (Figure S5B). This indicates that 5-HIAA is diffusion-controlled current on the *p*-ABSA/GCE modified sensor. To understand the process of 5-HIAA on the *p*-ABSA/GCE modified sensor and GCE, the linear relationship between log *Ip* and log *v* was examined. The slope of the linear curve for diffusion-controlled systems is 0.5, and the slope of the curve for adsorption-controlled systems is 1.0 [25,26]. Since the slope of the linear curve between log *Ip* and log *v* is close to 1.0, it can be stated that the process of 5-HIAA on the *p*-ABSA/GCE modified sensor is adsorption-controlled (Figure S5C). Based on all these current-dependent linear curves of the scanning rate, it was concluded that 5-HIAA is both adsorption and diffusion controlled on the *p*-ABSA/GCE modified sensor.

3.5. Analytical parameters

Limit of detection (LOD) and limit of quantification (LOQ) values were calculated using DPV technique at *p*-ABSA/GCE modified sensor in PBS pH 7.0 under optimized conditions to determine the analytical parameters of 5-HIAA. The LOD was calculated from the equations of 3 s/m and LOQ of *10 s/m*. "*m*" was the slope of the calibration graph plotted against the oxidation peak currents of increasing concentrations of 5-HIAA. "*s*" was the standard deviation of the voltammetric oxidation peak currents obtained by measuring ten times the lowest concentration of 5-HIAA. Obtained voltammograms and calibration graphs were given in Figure 4. In addition, the validation parameters were summarized in Table 1. The DPV measurements in solutions of 5-HIAA at concentrations of 8.0, 10.0, 12.0, 19.8, 29.4, 38.8, 48.0, 65.9, 74.6, 83.1, 91.5 µM prepared in PBS pH 7.0 were recorded at the *p*-ABSA/GCE

modified sensor. It was observed that the peak current increased with increasing 5-HIAA concentration, with a linear relationship in the range of 10 µM -92 µM, with the regression equation I (µA) = 0.1347C (µM) + 6.5522. The LOD and LOQ values for 5-HIAA were calculated as 0.53 µM and 1.77 µM, respectively. The sensitivity of the modified sensor was calculated by dividing the slope of the calibration curve by the active surface area of the modified sensor. The sensitivity of the modified sensor was calculated as 0.80 0.80 $\mu A.cm^{-2}/\mu M$ for the linear range of 10 µM - 92 µM. These results proved that the modified sensor produced was quite sensitive even for very low concentrations. Furthermore, the obtained results at the *p*-ABSA/GCE modified sensor was sensitive, and the previously reported methods were summarized in Table 2.

Table 1. Validation parameters

Equation of the calibration curve	I (µA) = 0.1347C(µM) + 6.5522
Measured Potential (mV)	293
Linear Range (µM)	10.0 – 91.5
Slope ($\mu A.\mu M^{-1}$)	0.1347
Intercept (µA)	6.5522
Correlation Coefficient	0.9912
Standard Deviation of Slope	0.0048
Standard Deviation of Intercept	0.279
Limit of Detection (LOD)	0.531
Limit of Quantification (LOQ)	1.768

Table 2. Comparison of the electroanalytical determination of 5-hydroxyindolacetic acid using various electrode materials

Electrode materials	Method of detection	Linear range (µM)	LOD (µM)	Ref
MMIC/SPE		18264.00	1.4	[14]
AuNPs/SPCs	SWV	0.5–200	0.022	[15]
NGITO	OSWV	0.1 - 100	0.027	[16]
poly(sulfosalicylic acid) microsensor	DPV	0.5 - 10	0.25	[17]
ITO	DPV	100 - 50	7.5	[27]
p(P3CA)/Pt/f-CNF/GCE	SSWV	0.01 - 100	0.02	[28]
ABSA/GCE-*p*	DPV	92 – 10.0	0.53	Present work

In addition, ten consecutive DPV responses were investigated to determine the reproducibility of the proposed modified sensor. Peak current values for 10^{-5} M 5-HIAA were examined in ten separate sensors produced (Figure S6). No significant reduction in repeated DPV responses was observed for 5-HIAA. This shows the stability of the interface. By

comparing the oxidation peak currents, the coefficient of variation (% RSD) value was found to be 1.75%. Since this value is less than two (<2), it can be stated that the newly produced modified sensor showed excellent stability. With the modified sensor used in this study, high peak currents and peak resolutions were obtained, with a very low detection limit. This makes the produced modified sensor very attractive.

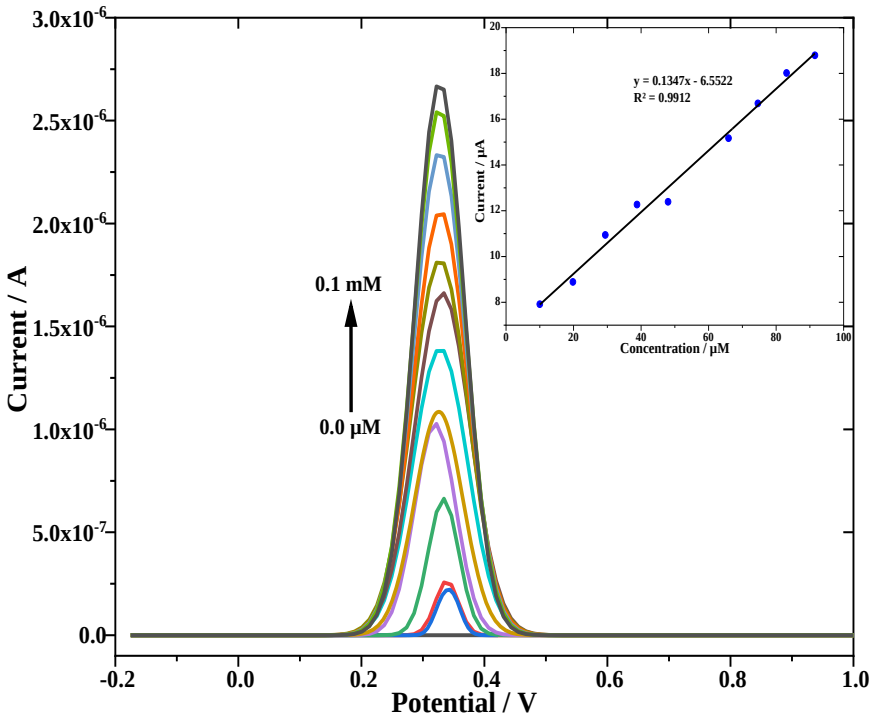


Figure 4. DPV results of increasing concentrations of HIAA in PBS pH 7.0 (8.0, 10.0, 12.0, 19.8, 29.4, 38.8, 48.0, 65.9, 74.6, 83.1, 91.5 µM) at the *p*-ABSA/GCE modified sensor. Inset: Calibration curve of HIAA in the linear range of 10.0 – 91.5 µM (10.0, 19.8, 29.4, 38.8, 48.0, 65.9, 74.6, 83.1, 91.5 µM) in PBS pH 7.0

3.6. Interference study

The DPV responses of 5-HIAA and AA at the modified sensor were shown in Figure 5. Curve "a" represents the response received with the modified sensor in PBS pH 7.0. Other voltammograms correspond to DPV responses at the *p*-ABSA/GCE modified sensor in solutions containing concentrations of 100, 198, 294, 388, 480 µM AA in the presence of 0.48 µM 5-HIAA. The anodic peak potentials for AA and 5-HIAA at the *p*-ABSA/GCE modified sensor were observed at +0.023 and +0.333 V, respectively. AA and 5-HIAA were analyzed simultaneously with a potential difference (ΔEp) of 310 mV. These results showed that there was no interference in the electrochemical determination of 5-HIAA in the presence of AA. In

the presence of AA at the *p*-ABSA/GCE modified sensor, 5-HIAA could be detected sensitively and stably.

Figure S7 shows DPV responses at various concentrations of 5-HIAA at the modified sensor in the presence of AA. Differential pulse voltammograms in Figure S7 correspond to oxidation of 0.10, 0.20, 0.29, 0.39, 0.48 µM 5-HIAA in the presence of 100 µM AA. Signals of both 5-HIAA and AA could be obtained with good resolution. The anodic peaks of AA and 5-HIAA were observed with good separation at potentials of 47 mV and 317 mV. In addition, the peak current of 5-HIAA increased with the increase in the concentration of 5-HIAA, while the peak current of AA remained constant. Based on this, it can be said that it is possible to simultaneously detect 5-HIAA and AA at the *p*-ABSA/GCE modified sensor.

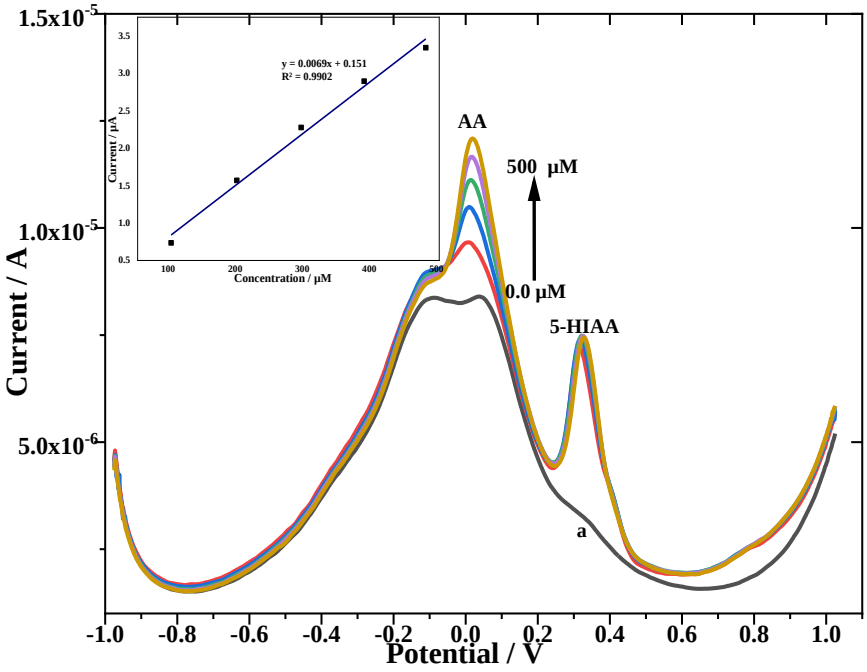


Figure 5. DPV responses (a, PBS pH 7.0 background) obtained as a result of increasing AA concentrations (100, 198, 294, 388, 480 µM) by keeping 0.48 µM 5-HIAA concentration constant at the *p*-ABSA/GCE modified sensor. Inset: It shows the relationship between the peak currents received in response to increasing AA concentrations

3.7. Applicability of the *p*-ABSA/GCE modified sensor in urine samples

The applicability of the *p*-ABSA/GCE modified sensor was tested by adding a standard 5-HIAA solution to the urine sample using the DPV technique. Urine samples diluted tenfold with pH 7.0 PBS were spiked from 5-HIAA standard solution at final concentrations of 9.9 µM, 19.6 µM, 29.1 µM, 38.4 µM, and 47.5 µM. And after each addition, electroanalytical measurements were carried out at the *p*-ABSA/GCE modified sensor (Figure S8). After

determining the oxidation peak currents of the voltammograms, the concentrations were calculated from the correct equation of the relevant calibration curve. Based on these results, recovery values were calculated. These values were summarized in Table 3. In the light of these results, the applicability of the modified sensor in real samples was found to be quite dramatic.

Table 3. Data obtained as a result of adding the standard 5-HIAA solution to the urine sample

Added 5-HIAA (μM)	Measured 5-HIAA (μM)	Recovery (%)
9.9	10.2	102.6
19.6	19.5	99.4
29.1	29.4	100.9
38.4	39.6	103.0
47.5	47.2	99.4

4. CONCLUSION

In this study, the electrochemical oxidation property of 5-HIAA was investigated by DPV and CV techniques using a *p*-ABSA/GCE modified sensor. The oxidation peak was determined in voltammograms at different film thicknesses in PBS and at different pH (3.0-9.0) between -0.2 V and +1.0 V potential. It was observed that the prepared modified sensor was conductive and responded to the oxidation of the 5-HIAA.

The measured oxidation peaks current for 0.1 mM 5-HIAA was determined as 0.20 μA and 19.34 μA at the GCE and the modified sensor, respectively. When the peak currents of 5-HIAA at the GCE and the modified sensor were compared, it was observed that the peak current was approximately 130 times higher at the modified sensor. Electro catalytic effect of the GCE surface covered with polymeric film facilitated electron transfer. This showed that the modified sensor was more sensitive. In addition, the validation parameters (such as LOD, LOQ) were evaluated by determining the amount of 5-HIAA in the presence of ascorbic acid and in biological samples such as urine using the voltammetric method. In addition, recovery studies were carried out to determine the accuracy of the applied method. With the results in this study, it was concluded that the interfering substances did not affect the applied methods.

The applied voltammetric techniques were found to be competitive with spectroscopic methods such as HPLC and UV, due to their advantages such as being economical, fast and sensitive, working with a small amount of sample and making determinations without the need for time-consuming processes such as separation. In addition, the results showed that the modified sensor can be easily used for the determination of 5-HIAA in routine analysis of biological fluids.

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Declarations of interest

The authors declare no conflict of interest in this reported work.

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