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Research Article

Electrochemical Properties and Determination of Serotonin with Graphene/Coal Tar Pitch/Pencil Graphite Sensor Electrode using Square Wave Adsorptive Stripping Voltammetry Technique

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ABSTRACT

In the present work, electrochemical and spectroelectrochemical behaviors of Serotonin (5-HT) were studied by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. Cyclic voltammetric experiments of 5-HT on graphene/coal tar pitch/pencil graphite electrode (GR/CTP/PGE) were carried out between 0.0 V and 1.8 V potential range at a scan rate of 100 mV s^{-1} with 20 cycles in non-aqueous media. Surface characterizations were performed using CV, EIS and scanning electron microscope (SEM). Effect of different pH values was investigated by square wave voltammetry (SWV) for determination of 5-HT. Optimization of accumulation time was determined using square wave adsorptive stripping voltammetry (SWAdSV) within potential range of -0.2 to +0.6 V. 5-HT standard solutions changing from 75 µM to 1.0 µM were prepared and the corresponding peak currents were measured. From the obtained data calibration equation was derived $Ip = 0.0329C_{5\text{-HT}} + 0.1511$ with correlation coefficient (R^2) 0.9958. LOD was 3.51×10^{-7} M and LOQ was 1.05×10^{-6} M.

Araştırma Makalesi

Kare Dalga Adsorptif Sıyırma Voltametrisi Tekniği Kullanılarak Grafen/Katran/Kalem Grafit Sensör Elektrot ile Serotonin'in Tayini ve Elektrokimyasal Özellikleri

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Voltametrisi

ÖZ

Bu çalışmada Serotonin'in (5-HT) elektrokimyasal ve spektroelektrokimyasal davranışları dönüşümlü voltametri (CV) ve elektrokimyasal impedans spektroskopisi (EIS) teknikleriyle incelenmiştir. Grafen/katran/kalem grafit elektrot (GR/CTP/PGE) üzerinde 5-HT'nin CV deneyleri, 0,0 V ile 1,8 V potansiyel aralığında sulu olmayan ortamda Ag/Ag^+/(10 mM $AgNO_3$) referans elektrotu kullanılarak 20 döngü ile 100 mV s^{-1} tarama hızında gerçekleştirildi. Yüzey karakterizasyonları CV, EIS ve taramalı elektron mikroskobu (SEM) kullanılarak yapıldı. 5-HT'nin tayininde farklı pH değerlerinin etkisi kare dalga voltametri (SWV) tekniği ile araştırıldı. Biriktirme süresinin optimizasyonu, -0,2 ile +0,6 V potansiyel aralığında kare dalga adsorptif sıyırma voltametrisi (SWAdSV) kullanılarak belirlendi. 75 µM'den 1,0 µM'ye kadar değişen 5-HT standart çözeltileri hazırlandı ve karşılık gelen pik akımları ölçüldü. Elde edilen verilerden kalibrasyon denklemi $Ip = 0{,}0329C_{5\text{-HT}} + 0{,}1511$ ve korelasyon katsayısı (R^2) 0,9958 olarak hesaplandı. LOD $3{,}51 \times 10^{-7}$ M ve LOQ $1{,}05 \times 10^{-6}$ M olarak belirlendi.

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1. Introduction

Serotonin, or 5-hydroxytryptamine (5-HT), serves as a crucial signaling molecule within both animals and plants (Szeitz & Bandiera, 2018). Investigating neurotransmitters like 5-HT remain a highly dynamic area of research, particularly in the context of addressing chronic conditions such as Alzheimer's and Parkinson's diseases. These neurotransmitters hold pivotal roles in regulating the central nervous system, with deviations in their levels being implicated in various neurological disorders. The normal 5-HT level in humans is reported as 0.25-0.74 mM by the US Department of Health, Human Services, and NIH. Increase or decrease in the normal concentration of 5-HT can cause depression, migraines, anxiety, eating disorders, sudden infant death syndrome (SIDS) and blood clots, etc. (Koluaçık et al., 2018).

Electrode modification can be widely performed in aqueous and non-aqueous media (Islamoglu et al., 2023; Demir Mülazımoğlu & Mülazımoğlu, 2013; Demir Mülazımoğlu et al., 2012). Conducting polymers are widely employed as modifiers in fabrication of modified electrodes. In this context, modification of glassy carbon (GC) electrode with gold nanoclusters/overoxidized polypyrrole films has been carried out to prepare poly(pyrrole-3-carboxylic acid) films for electrochemical detection of 5-HT (Li & Lin, 2007). Surface deactivation due to polymerization or adsorption of oxidized molecules on electrode surface is crucial disadvantages of modified electrodes. Various electrodes such as GC electrode (Albayrak et al., 2017), carbon paste electrode (Mülazımoğlu & Yılmaz, 2010), PGE (Korkmaz et al., 2023) were used to determination of different species. For this reason, pencil can be regarded as a significant contender for disposable electrodes due to its cheapness and easy to use (Durmuş & Mülazımoğlu, 2023; Özcan & Ilkbas, 2015).

Development of a rapid, sensitive, and selective 5-HT determination method is very important and vital in terms of health and clinical treatment. Electrochemical methods stand out as the most promising techniques, thanks to their high selectivity and low detection limits in contrast to traditional chromatography and spectroscopy methods. Nevertheless, the efficacy of electrochemical methods hinges largely on the choice of working electrode employed in measurements. Conventional carbon and metal electrodes, typically, exhibit subpar performance when it comes to detecting 5-HT. For this reason, there is a trend of substituting pencils with alternative materials (Özcan & Ilkbas, 2015). For determination of 5-HT, different analytical methods have been used such as; differential pulse voltammetry (Kim et al., 2012), SWV (Motsaathebe & Fayemi, 2021), fluorometry (Mumtaz et al., 1982), high-performance liquid chromatography (Kema et al., 2000), chemi-luminescence (Barnett et al., 1998) and mass spectrometry (Yilmaz et al., 2019).

In this study, electrochemical and spectro electrochemical behaviors of 5-HT were investigated on modified GR/CTP/PGE surface using CV and EIS techniques. 5-HT modified GR/CTP/PGE was characterized by CV, EIS and SEM. The scanning speed of GR/CTP/PGE was investigated using LSV technique to achieve diffusion-controlled transfer to the electrode surface. The detection ability of 5-HT was investigated using SWAdSV technique.

2. Material and Method

2.1. Chemicals and reagents

All chemicals and reagents were obtained from sources of analytical purity, including Sigma-Aldrich, Alfa Aesar, and Merck. Phosphate buffer solution (PBS) of different pH levels was formulated by combining standard solutions of KH_2PO_4 and K_2HPO_4, with pH adjustments made using 0.1 M NaOH solution. Ultra-pure distilled water with resistance of 18.2 MΩ cm was utilized in the preparation of all aqueous solutions. (MP MINIPURE Purification System, DEST UP).

2.2. Electrodes and equipments

GR/CTP/PGE was prepared in accordance with the literature and was used as working electrodes (Üstündag & Erkal, 2017). While BAS MF-2063 model Ag/AgCl 3M KCl(sat) was used as aqueous media reference electrode, BAS MF- 2042 model Ag/Ag$^+$/(10 mM $AgNO_3$) was used as non-aqueous media reference electrode. Platinum wire electrode obtained from BAS model MW-1032 served as auxiliary electrode. Electrochemical experiments were performed using the traditional three-electrode system. CV, SWV, LSV, EIS and SWAdSV studies were performed on a reference GAMRY Reference 600+ potentiostat/galvanostat/ZRA. SEM studies were conducted using a Hitachi-SU 1510. The pH value in aqueous solutions was determined using a pH meter equipped with a glass composite pH electrode (VWR pH enomenal, UK). Room temperature (25 ± 1 °C) was chosen as the most appropriate parameter for all experiments.

3. Results and Discussion

3.1. Modification of GR/CTP/PGE with 5-HT

The modification process of GR/CTP/PGE was carried out using the 5-HT via alcohol oxidation method with the CV technique. CV experiments were executed 1 mM 5-HT solution prepared in a 100 mM NBu_4BF_4 solution dissolved in CH_3CN, in potential range of 0.0 V to 1.8 V at a scan rate of 100 mV s^{-1} with 20 cycles in non-aqueous media *vs.* Ag/Ag$^+$/(10 mM $AgNO_3$ in CH_3CN).

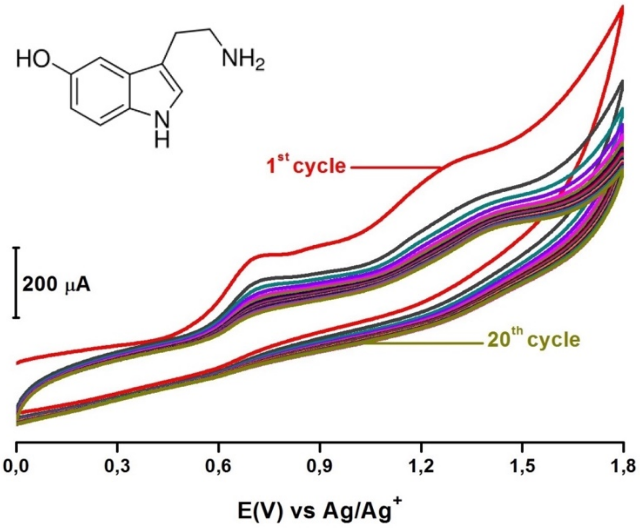

Figure 1. CV voltammogram of the modification of 5-HT on GR/CTP/PGE surface in the range from 0.0 to 1.8 for 20 cycles.

When the modification voltammogram given in Figure 1 was examined, peaks corresponding to oxidation of $–NH_2$ moiety at approximately 0.7 V and, subsequently, covalent bonding of C-O at around 1.2 V were observed. In the modification voltammogram, it was clearly seen that the peaks decrease starting from second cycle. The modification process was carried out in 20 cycles to prevent small gaps called pin holes from remaining.

3.2. Characterization of 5-HT/GR/CTP/PGE surface by CV, EIS and SEM

In non-aqueous media, a redox probe solution was prepared using a 1 mM ferrocene solution in a 100 mM NBu_4BF_4 supporting electrolyte solution prepared in CH_3CN. In the prepared solution, CV was performed on GR/CTP/PGE and 5-HT/GR/CTP/PGE surface in potential range of -0.1 V to 0.6 V at a scan rate of 100 mV s^{-1} with one cycle against an $Ag/Ag^+/(10$ mM $AgNO_3)$ reference electrode. Voltammograms were obtained for both bare GR/CTP/PGE and 5-HT-modified GR/CTP/PGE surface under the same conditions and these voltammograms were presented as overlayed in Figure 2. The higher peak currents observed for oxidation and reduction peaks on 5-HT/GR/CTP/PGE surface compared to GR/CTP/PGE surface indicate the increased activity of the modified electrode surface.

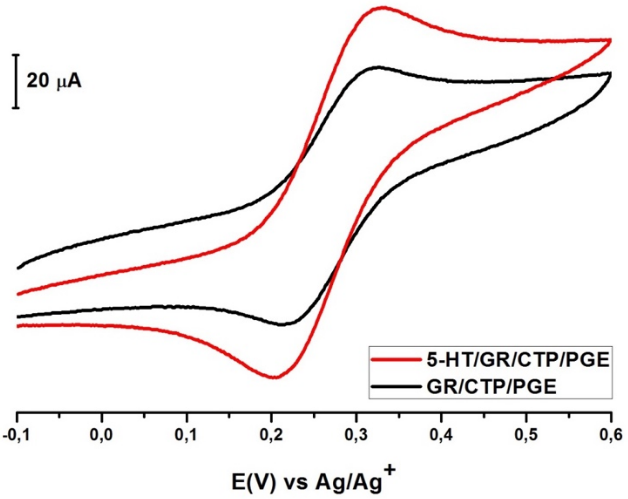

Figure 2. Cyclic voltammograms of 1.0 mM ferrocene redox probe, potential range from -0.1 to 0.6 V on bare GR/CTP/PGE and 5-HT/GR/CTP/PGE's surfaces.

To support the characterization study performed in non-aqueous media, 1 mM solution of HCF(III) redox probe was prepared by dissolving it in pH 2.00 BR buffer in the aqueous media. CV was conducted within potential range of 0.5 V to -0.2 V at a scan rate of 100 mV s^{-1}, comprising a single cycle against Ag/AgCl 3M KCl(sat) reference electrode.

Voltammograms were obtained for both bare GR/CTP/PGE and 5-HT/GR/CTP/PGE surface. These voltammograms were presented as overlayed in Figure 3. Similar to the characterization conducted in non-aqueous media, higher peak currents on the modified surface were observed in aqueous media using the CV technique.

Following the electrochemical characterization processes performed using CV technique, impedance measurements were carried out using another electrochemical technique, EIS with a 100 mM KCl solution including a $Fe(CN)_6^{3-}/^{4-}$ redox probe.

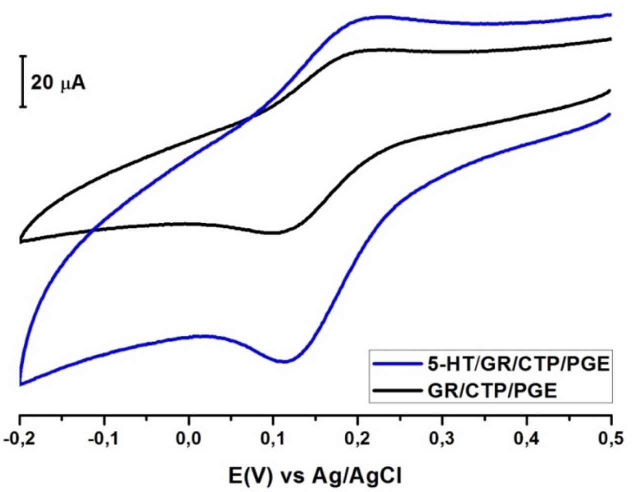

Figure 3. Cyclic voltammograms of 1 mM HCF(III) redox probe, potential range from 0.5 V to -0.2 V on GR/CTP/PGE and 5-HT/GR/CTP/PGE's surfaces.

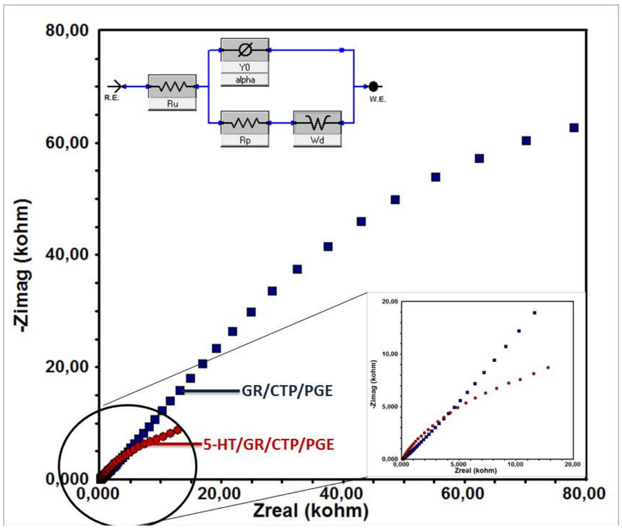

Figure 4. Nyquist plots for electrochemical impedance spectra of 1 mM $Fe(CN)_6^{3-/4-}$ redox couple solution in 100 mM KCl on GR/CTP/PGE and 5-HT/GR/CTP/PGE's surfaces at frequency range of 100 000–0.01 Hz at 10 mV wave amplitude.

The obtained Nyquist plots were compared between the modified electrode and GR/CTP/PGE. The graph representing this comparison is provided in Figure 4. When 5-HT/GR/CTP/PGE was compared with GR/CTP/PGE surface, it was shown 5-HT/GR/CTP/PGE surface was more active.

After the electrochemical characterization studies, microscopic images of the surfaces were obtained using SEM technique. The acquired SEM images were presented in Figure 5A, representing the image of GR/CTP/PGE surface, and Figure 5B, representing the image of 5-HT/GR/CTP/PGE surface. Morphologically, the difference between two images serves as sufficient evidence for modification of electrode surface. These images were obtained by scanning approximately 1 µm areas magnified 5000 times.

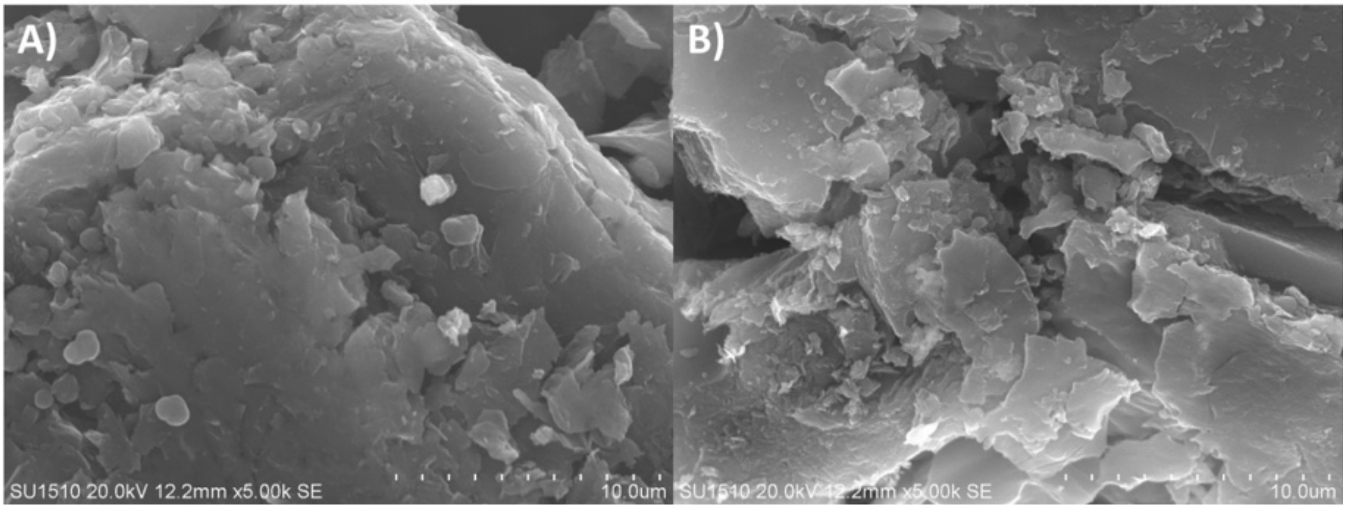

Figure 5. SEM images of A) GR/CTP/PGE and B) 5-HT/GR/CTP/PGE surfaces.

3.3. Effect of scan rate

The surfaces, resulting from characterization processes, were assessed, leading to the presumption the surface obtained following modification on GR/CTP/PGE was deemed suitable for intended application. To determine whether molecule reaches electrode surface electrochemically in a diffusion-controlled manner, scan rate studies were conducted. In these studies, 1 mM 5-HT solution prepared in a 100 mM NBu_4BF_4 support electrolyte in CH_3CN was used. The study was conducted using LSV and overlayed voltammograms were presented in Figure 6A. The voltammograms were obtained at scan rates of 10, 25, 50, 100, 150, 200, 250 and 300 mV s^{-1}. In line with Equation 3.1 of the Randles-Sevcik equation, the graph's linearity, depicting square root of scan rates against peak current, suggests that the molecule binds to electrode surface under diffusion-controlled conditions.

$$i_p = 2.69x10^5 n^{3/2} A D^{1/2} C v^{1/2} \qquad \text{(Equation 3.1)}$$

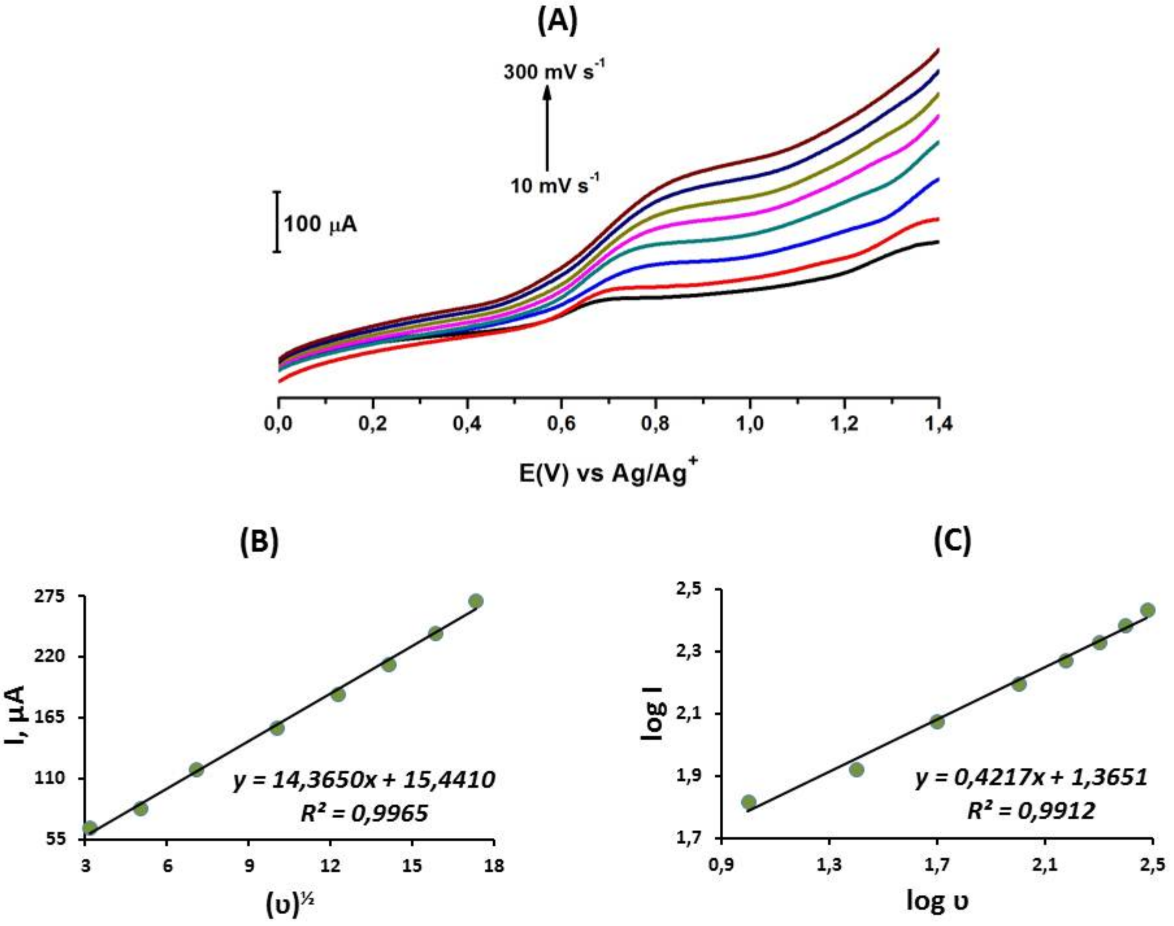

Figure 6. A) LSV oxidation voltammograms of 1 mM 5-HT on GR/CTP/PGE surface at various scan rates of 10, 25, 50, 100, 150, 200, 250 and 300 mV s^{-1} at the potential range from 0 to +1.4 V. B) Shows plot of peak current vs. square root of scan rate. C) A plot of log scan rate vs. log peak current.

The correlation coefficient, $R^2=0.9965$, for the obtained line in Figure 6B suggested a strong correlation for the linear fit. Additionally, slope of the plot of log Ip against log ν in Figure 6C was approximately 0.5, indicating molecule was transported to electrode surface in a diffusion-controlled.

3.4 Effect of pH

The impact of pH of the solution is important in determining 5-HT. As the physiological pH of the human body is pH 7.4, in this study, 0.1 M PBS was used in various pH ranges (pH 6.8, 7.2, 7.6 and 8.0) with SWV. SW voltammograms were given (Figure 7A) and optimum pH was determined. The response of GR/CTP/PGE to 5-HT (1 mM) at different pH values were shown in Figure 7B.

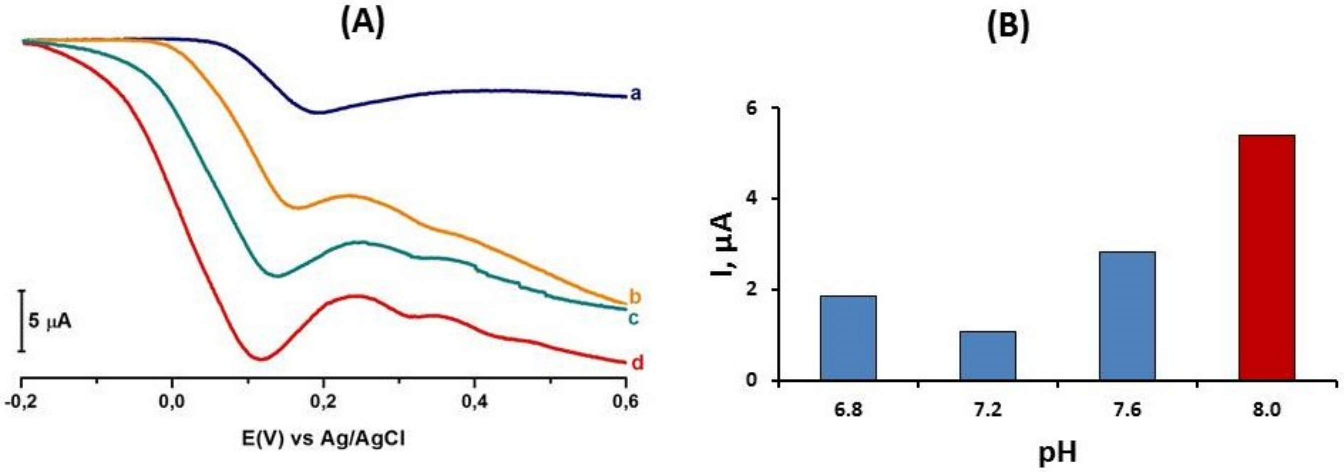

Figure 7. A) SW voltammograms obtained on GR/CTP/PGE surface of 1 mM 5-HT solution prepared at various pH of 0.1 M PBS (a) 6.8, b) 7.2, c) 7.6, d) 8.0). B) Peak current values for 5-HT in PBS with various pH on GR/CTP/PGE.

3.5. Optimization of accumulation time

Experimental conditions involved conducting current measurements using SWAdSV within potential range of -0.2 to +0.6 V. In Figure 8A, the voltammograms obtained from measurements taken within the 0 to 90 seconds range under optimal conditions were overlayed. Additionally, a graph (Figure 8B) depicting relationship between peak current and accumulation time was plotted. From this graph, it was determined that an accumulation time of 30 seconds appropriate for this study.

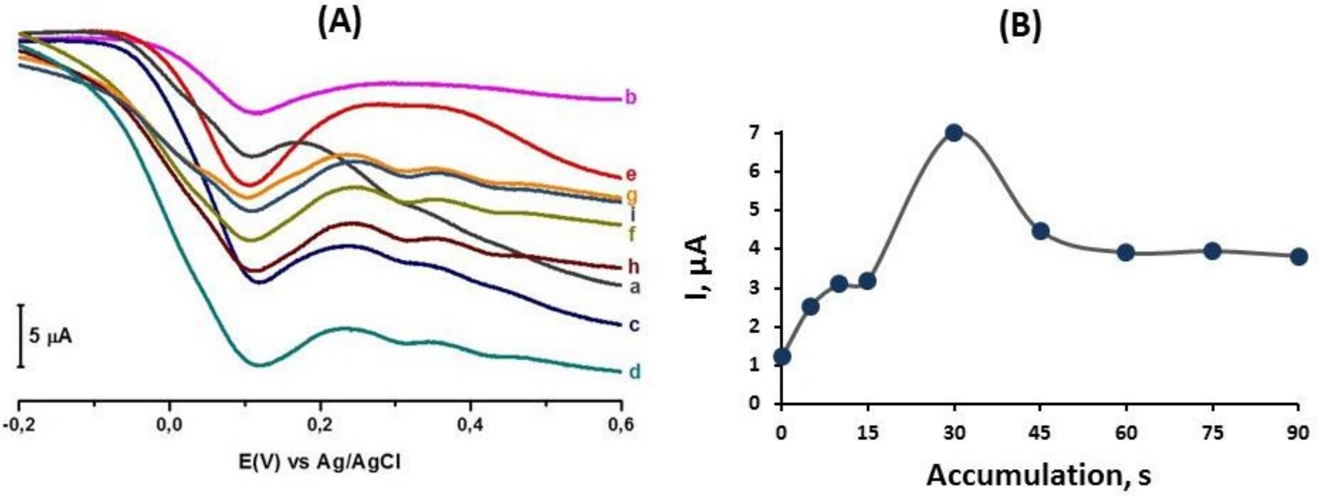

Figure 8. A) SWAdSV's obtained on GR/CTP/PGE surface of 1 mM 5-HT solution prepared in 0.1 M PBS pH 8.0 solution and potential range of -0.2/+0.6 V, for various accumulation times (0, 5, 15, 30, 35, 45, 60, 75, 90 s), B) Graph plotted with peak current values against accumulation time.

3.6. Analytical performance of 5-HT

The analytical performance of GR/CTP/PGE surface was evaluated by constructing a calibration curve at different concentrations of 5-HT (75, 50, 25, 10, 5, 1 µM) in PBS at pH 8.0 solution using SWAdSV. Voltammograms were taken at different concentrations and these voltammograms were superimposed in Figure 9A. Figure 9B was shown appearance of linear regression between peak current variation and 5-HT concentration. From the obtained data calibration equation was calculated $Ip = 0.0329C_{5\text{-HT}} + 0.1511$ with linear correlation coefficient (R^2) 0.9958. LOD was $3,51 \times 10^{-7}$ M and LOQ was $1,05 \times 10^{-6}$ M.

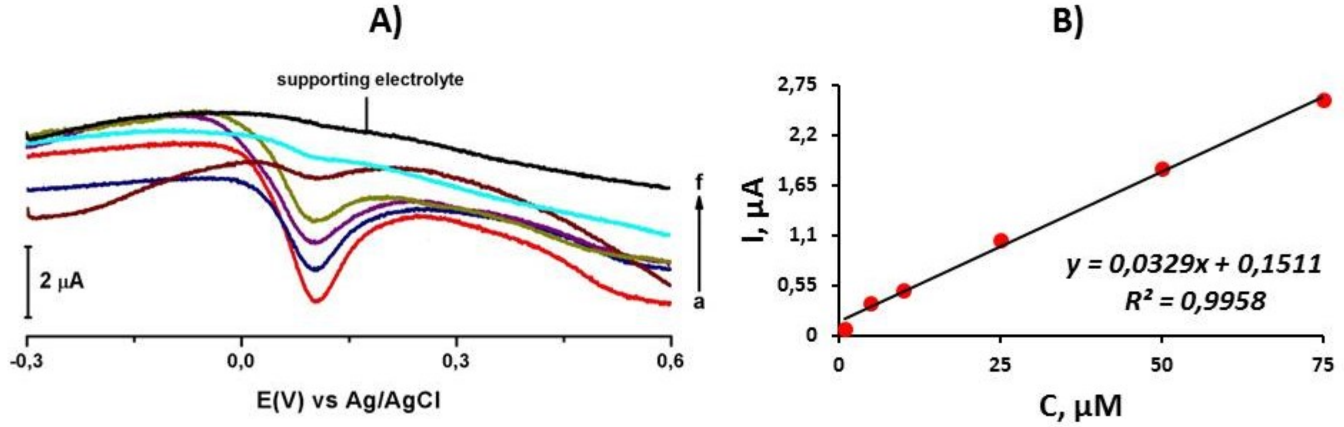

Figure 9. A) Overlaying SWAdSV's for solutions prepared various concentrations of 5-HT (a) 75, b) 50, c) 25, d) 10, e) 5, f) 1 µM) in the 0.1 M PBS at pH 8.0 on GR/CTP/PGE (Accumulation time 30 s and stirring rate 350 rpm) and B) Linear calibration curve of peak current versus 5-HT concentration in SWAdSV measurements.

4. Conclusions and Recommendations

In this study, GR/CTP/PGE synthesized in accordance with the literature, which has a low cost, renewable, broad, and electroactive surface, was applied as the working electrode for all analysis.

It can be clearly seen 5-HT binds on the surface after modification with the CV technique in non-aqueous media, through CV tests performed with ferrocene redox probe in non-aqueous media, with HCF(III) redox probe in aqueous media, and through impedance tests performed with EIS using $Fe(CN)_6^{3-/4-}$ redox probe in aqueous media.

In addition, surface characterization was obtained using SEM technique. With SEM technique, it can be seen bare GR/CTP/PGE is a bulk structure and 5-HT/GR/CTP/PGE is a layered structure. According to the results obtained from scanning rate studies, 5-HT is transported to electrode surface under diffusion control.

After determining the optimum parameters, concentration-peak current calibration graph drawn using 5-HT solutions prepared at different concentrations (75, 50, 25, 10, 5, 1 µM) in pH 8.0 PBS evidenced by a correlation coefficient of 0.9958.

Proposed method provides a simple, rapid and economical to determination of 5-HT using GR/CTP/PGE by SWAdSV technique. Overall, this research provides a comprehensive analysis of electrochemical behaviors of 5-HT, offering valuable insights into its detection and quantification, which could have significant implications in various fields such as neuroscience and pharmaceuticals. In future studies, trace amounts of 5-HT in natural or synthetic samples can be easily determined.

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CRedit author statement

ŞK: Experimental work and data analysis, drawing of figures in the article, carried out certain experiments data plotting and analysis.
HS: Experimental work, data curation and writing the introduction and experimental part of the article.
ADM: Facilitation in the experimental work, technical discussion, Writing – review & editing.

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