Modification of Carbon Paste Electrode with Molecularly Imprinted Polyaniline for Amitriptyline Analysis by Voltammetry

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ABSTRACT

Modification of carbon paste electrodes with molecularly imprinted polyaniline (MI-PANi) for voltammetric analysis of amitriptyline (AMT) has been studied. The polymer was synthesized from aniline as monomer, ammonium peroxodisulfate as initiator, and AMT as template with a mole ratio of 2:1:0.1 using the previously developed method. AMT was then extracted from the polymer pores using water at 60°C in order to leave a selective bonding site for AMT. The MI-PANi modified electrode was fabricated with a mass ratio of activated carbon, MIP, and paraffin of 9:4:7. The modified electrode was applied to analyze AMT standard solution using differential pulse voltammetry (DPV) technique under conditions of deposition potential of 0.6 V, deposition time of 60 s, scan rate of 25 mV/s, and pH 8. The method validity was expressed by a linearity value of 0. 9904 in the concentration range of 20 – 100 ppb, lower detection limit of 15.33 ppb, precision of 94.25 – 99.74%, accuracy value of 75.08 – 103.31%, sensitivity of 0.1222 nA/ppb. The electrode showed good selectivity to AMT in the presence of glucose equal to one-tenth of the AMT concentration, as indicated by a current deviation of 4.68%. The application of the modified electrode for AMT analysis by voltammetry showed a recovery of 99.08%.

Keywords: *amitriptyline, carbon paste electrode, imprinted polymer, polyaniline, voltammetry*

INTRODUCTION

AMT is a type of tricyclic antidepressant classified as a potent drug, which works by increasing adrenaline and serotonin levels in the central nervous system. The effective dosage of AMT ranges from 12.5–25 mg once to three times daily, equivalent to 75 mg per day (Nurfahanum, 2022). The use of this drug becomes dangerous in overdose and if consumed by healthy individuals (without psychiatric disorders), as it may cause psychic hypofunction. AMT is known as a drug that is often misused; therefore, the concentration of AMT in pharmaceutical preparations needs to be monitored. The determination of AMT and other TCA compounds in pharmaceutical formulations has been extensively studied, including by gas chromatography (Santos et al., 2014). Potentiometry using an imprinted polymer-modified carbon paste electrode has also been developed as a method for AMT detection (Shofiyyah, 2019).

The analysis of AMT concentrations can be performed using voltammetric methods because AMT is an electroactive compound. The advantages of voltammetric methods include rapid analysis time, simple sample preparation, high sensitivity and selectivity, detection limits down to 10^{-10} M, the ability to determine multiple elements/compounds simultaneously, and relatively low instrument operational costs (Wang, 2006). The performance of voltammetric methods depends in part on the type of working electrode used; therefore, modification of the working electrode is often carried out. One of the most attractive electrode modification

techniques in recent years involves the use of molecularly imprinted polymers (MIPs), either by coating the electrode surface or by mixing MIP materials into the electrode components. Molecularly imprinted polymers are materials used to modify electrodes, where the synthesized polymer possesses the ability to recognize target molecules specifically (Tom and Foster, 2010). A MI-PANi-modified carbon paste electrode has been developed for the potentiometric analysis of AMT (Shofiyyah, 2019). The results showed a wider measurement range and a considerably high detection limit.

In this study, an MI-PANi-modified carbon paste electrode was developed for the voltammetric analysis of AMT. PANi was chosen as the electrode modifier due to its high electrical conductivity and stability, as well as its straightforward synthesis procedure. The parameters studied in this research include the optimum deposition potential, deposition time, scan rate, and solution pH. Method validation was conducted to demonstrate the feasibility of the proposed method, including linearity, detection limit, precision, accuracy, recovery, sensitivity, and selectivity.

METHOD

Materials

The materials used in this study include amitriptyline–HCl ($C_{20}H_{23}N.HCl$; (98%); Sigma Aldrich, St. Louis, MO, USA), aniline ($C_6H_5NH_2$); Sigma Aldrich, St. Louis, MO, USA), hydrochloric acid (HCl); Sigma Aldrich, St. Louis, MO, USA), phosphoric acid (H_3PO_4); Sigma Aldrich, St. Louis, MO, USA), ammonium peroxodisulfate ($(NH_4)_2S_2O_8$); Sigma Aldrich, St. Louis, MO, USA), glacial acetic acid (CH_3COOH); (100%); Merck, Rahay, NJ, USA), sodium acetate trihydrate ($CH_3COONa.3H_2O$ (99.5%); Merck, Rahay, NJ, USA), sodium dihydrogenphosphatedihydrate ($NaH_2PO_4.2H_2O$ (98%); Merck, Rahay, NJ, USA), sodium hydrogenphosphate dihydrate ($Na_2HPO_4.2H_2O$ (99%); Merck, Rahay, NJ, USA). Paraffin pellets (Sigma Aldrich, St. Louis, MO, USA) and activated carbon (Sigma Aldrich, St. Louis, MO, USA) as materials for fabricating electrodes. All chemicals are analytical grade. Distilled water was used as a solvent.

Instrumentation

The instruments used were a ionmeter (Cyberscan 510, Frankfurt, Germany), a digital potentiostat (eDAQ ER 461 Echem), the proposed modified carbon paste electrode IZ-A (CPE-IZA) as the working electrode, Ag/AgCl as reference electrode, and platinum wire as auxiliary electrode. Zeolite characterization was carried out using X-ray diffractometer (Shimadzu, Kyoto, Japan) and Fourier transform infrared spectrophotometer (Shimadzu Kyoto Japan in the range of 4000-400 cm^{-1}. A pH-meter (Cyberscan Eutech Instrument pH 510, Frankfurt, Germany) was used to measure the pH. Centrifuge (HITECH EBA 20, Westphalia, Germany), vacum oven (Model 5851, Amityville, NY, USA), agate mortar, polypropylene bottle and glassware commonly used in chemical laboratories.

Procedure

1. Synthesis of imprinted PANi

Aniline (0.4080 g) was dissolved in 7.5 mL of 1 M HCl and stirred at 50 °C for 30 minutes. Then, 0.5008 g of ammonium peroxodisulfate dissolved in 2.5 mL of distilled water was added dropwise with slower stirring. The mixture was left for 12 hours at room temperature to form a dark green polymer. The product was washed with 1 M HCl until the filtrate became colorless and then dried in air for 12 hours. Non-imprinted PANi (NI-PANi) was prepared similarly by adding 0.1216 g of amitriptyline to 0.8 g of aniline in 15 mL of 1 M HCl. MI-PANi was obtained by extracting AMT from the polymer using hot water (40–60 °C), followed by centrifugation three times at 50 °C for 20 minutes each. Extraction was

stopped when no AMT was detected in the filtrate. PANi, NI-PANi, and MI-PANi were characterized by FTIR.

2. *Sensor Fabrication*

The modified carbon paste electrode was prepared by mixing activated carbon, MI-PANi powder, and paraffin in a mass ratio of 9:4:7 (total mass 0.3 g). The mixture was heated until a paste formed, then immediately packed into a micropipette tip with a silver wire embedded inside. The remaining part of the tip was filled with melted paraffin. The tip end was cut to obtain a specific electrode surface area, then smoothed with paper. The electrode was conditioned by soaking in 100 ppb AMT solution for 24 hours.

3. *Parameter Optimization*

Optimization of deposition potential was performed on 100 ppb AMT solution at pH 7 using the MI-PANi-modified carbon paste electrode by varying the potential from +0.1 V to +1.0 V (interval 0.1 V) with a deposition time of 60 s and scan rate of 50 mV/s. The potential giving the highest peak current was selected as optimum. Next, deposition time was optimized from 30 to 180 s (interval 30 s) at the optimum potential, and the time with the highest peak current was chosen. Scan rate was then optimized from 25 to 100 mV/s (interval 25 mV/s) under the optimum potential and time, and the rate yielding the highest peak current was selected. Finally, pH optimization was conducted using 100 ppb AMT solutions at pH 4, 5, 6, 7, and 8, measured under the previously determined optimum conditions, observing both peak current and peak potential shifts.

4. *Voltammetric Measurement*

A standard curve of AMT was constructed from measurements of 20–100 ppb AMT solutions (20 ppb intervals) under optimized conditions using the MI-PANi-modified carbon paste electrode. The current responses were used to determine linearity (correlation coefficient, R), precision (RSD), accuracy, detection limit (LoD), sensitivity, and % recovery. LoD was calculated using the standard deviation of the regression line (Sy/x) and the intercept (Yb): $LoD = Yb + 3Sy/x$. Accuracy was calculated as (measured concentration / actual concentration) × 100%. Recovery was determined by spiking pharmaceutical tablet samples with AMT standard.

5. *Selectivity Test*

Selectivity was evaluated by comparing the current response of 100 ppb AMT solutions with and without glucose as an interfering substance, at AMT:interferent ratios of 1:0.1, 1:0.5, and 1:1. The MI-PANi electrode was considered selective if no peak overlap or significant current difference was observed.

RESULT AND DISCUSSION

Results of Synthesis and Characterization of Polyaniline, NI-PANi, and MI-PANi

Polyaniline (PANi) was synthesized by mixing aniline, ammonium peroxodisulfate, and 0.1 M hydrochloric acid (HCl). The polymerization mechanism of aniline is an addition polymerization based on the breakage of double bonds in aniline by an initiator. The molecular weight of the obtained PANi was determined using an Ostwald viscometer, yielding a relative molecular weight of 1,330,836.3 g/mol, indicating that the PANi synthesis in this study produced long polymer chains. Non-imprinted polyaniline (NI-PANi) was synthesized using the same procedure as PANi, except that AMT was added as a template during the synthesis. The molar ratio of monomer, initiator, and template (analyte) used was 2:1:0.1 (Sreenivasan, 2007). The synthesis of MI-PANi is a subsequent step from NI-PANi synthesis, aimed at removing the analyte template to form imprints or active sites for the analyte molecules. This synthesis was performed by extracting the AMT trapped within the polymer network using hot water.

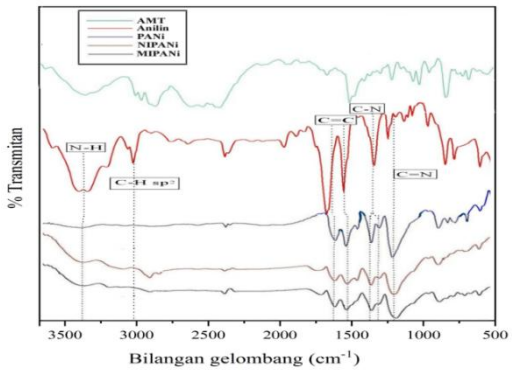

Figure 1. FTIR spectra of AMT, Aniline, PANi, NI-PANi, and MI-PANi

FTIR characterization was carried out to determine the functional groups in aniline, PANi, NI-PANi, and MI-PANi. The FTIR characterization results shown in Figure 3.1 indicate that PANi was successfully synthesized in this study. In the wavenumber range of approximately 3300–3400 cm^{-1}, which corresponds to the N–H band observed in the aniline spectrum, this band was no longer present in the PANi spectrum. Furthermore, the formation of polyaniline was confirmed by the peak at around 1149 cm^{-1}, which corresponds to the C=N stretching of the protonated quinoid ring, a characteristic peak of conductive polyaniline (Maddu et al., 2008). At wavenumbers around 1500 cm^{-1} and 1400 cm^{-1}, C=C bands from the benzene ring were observed in the spectra of PANi, NI-PANi, and MI-PANi. The wavenumber above 3000 cm^{-1} indicates the presence of N–H bands in all three spectra.

Deposition Potential Optimization

Voltammograms of 100 ppb AMT at pH 7 with various deposition potentials are shown in Figure 2. The highest anodic peak current was observed at a deposition potential of 0.6 V. In DPV stripping analysis, AMT is oxidized during electrodeposition and reduced during stripping at a potential more negative than the deposition potential, consistent with the observed stripping peak at 0.574 V.

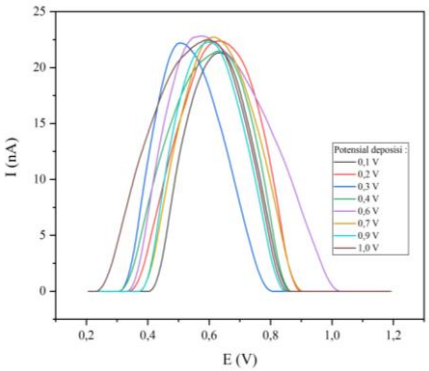

Figure 2. Voltammogram of 100 ppb amitriptyline solution at pH 7 with various potentials.

Deposition Time Optimization

The DPV results for 100 ppb AMT at various deposition times shown in Figure 3. The peak current increased up to 60 s and then stabilized from 90–180 s due to electrode surface saturation. Thus, 60 s was selected as the optimum deposition time.

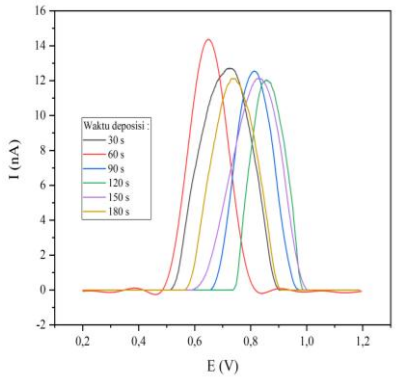

Figure 3. DPV voltammogram of 100 ppb AMT solution with various potentials.

Scan Rate Optimization

DPV voltammograms at various scan rates shown in Figure 4. The highest peak current was obtained at 25 mV/s, decreasing at 50 mV/s. Higher scan rates generate thermal effects that can degrade PANi conductivity (Wibawanto et al., 2012).

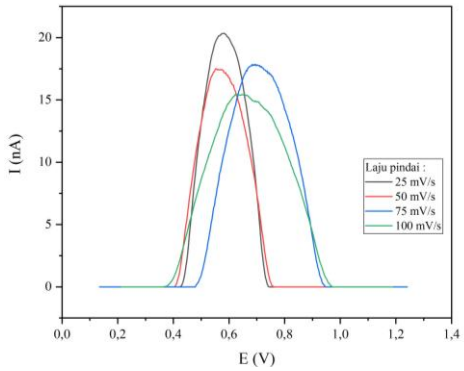

Figure 4. DPV voltammogram of 100 ppb AMT solution with various scan rates.

pH Optimization

Optimization was performed using AMT 100 ppb in pH 4–8 buffers Table 1. The highest current (19.98 nA) was observed at pH 8. AMT has lone pair electrons on its nitrogen atom, making it a Lewis base and stable under basic conditions, similar to methamphetamine as reported by Irdhawati (2009).

Table 1. Measurement results of peak potential and peak current of amitriptyline in various pH solutions using MI-PANi/CPE (Molecularly Imprinted Polyaniline/Carbon Paste Electrode).

pH	Peak Potential (V)	Current (nA)
4	0,558	14,6021
5	0,496	14,4262
6	0,524	19,2701
7	0,528	16,1562
8	0,534	19,9839

Validation of the Analytical Metho

A standard curve was constructed from AMT solutions at concentrations of 20, 40, 60, 80, and 100 ppb (pH 8) measured by voltammetry using the MI-PANi/CPE, with three replicates per concentration Figure 5. The correlation coefficient (R) was 0.9905, indicating good linearity. The slope of 0.1222 nA/ppb represents the sensitivity, meaning each 1 ppb

increase in AMT concentration produces a current change of 0.1222 nA. The detection limit (LoD) calculated using equations (1) and (2) was 15.33 ppb, which is lower than the 2.98 × 10^3 ppb reported for potentiometric AMT determination using MI-PANi/CPE (Shofiyyah, 2019).

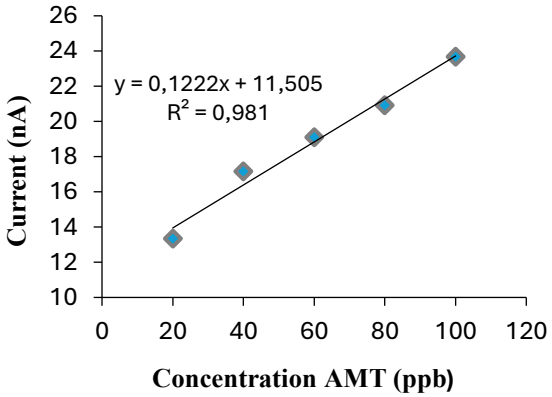

Figure 5. Amitriptyline Standard Curve

Precision, expressed as RSD from repeated measurements, ranged from 0.26% to 5.75%, well within the AOAC limit of 15% for 100 ppb analyte concentration (Taverniers et al., 2004). Accuracy ranged from 75% to 115%. The % recovery was determined by spiking a sample with 50 ppb AMT standard, giving a value of 99.08%, which falls within the acceptable range of 80–110% (Taverniers et al., 2004).

Selectivity Test Results

Electrode selectivity describes the ability of a method to measure an analyte selectively in the presence of other compounds in solution. In this study, selectivity was evaluated by examining the effect of glucose. The deviation in amitriptyline current at various AMT-to-glucose concentration ratios is shown in Table 2.

Table 2. Current deviation of amitriptyline (AMT) at various AMT-to-glucose (GLU) concentration ratios

AMT : Glucose	Current (nA)		Current Deviation (%)	
	CPE MI-PANi	CPE	CPE MI-PANi	CPE
1:0	19,00	18,92	-	-
1 : 0,1	18,11	24,27	4,68	28,27
1 : 0,5	22,01	30,51	15,83	61,26
1 : 1	26,72	36,74	40,63	94,28

Data in Table 3.2 show the current response for AMT analysis with the addition of glucose. The presence of glucose significantly affects the anodic peak current of AMT. At a 1:0.1 ratio (AMT:glucose), the current deviation was insignificant at 4.7%. As the glucose concentration increased, interference became more pronounced, with current deviations exceeding the tolerance limit (5%). The addition of glucose at a concentration ten times lower than that of AMT did not interfere with AMT measurement using the MI-PANi/CPE. Voltammetric analysis of AMT using the MI-PANi-modified CPE showed better selectivity compared to the unmodified CPE. This indicates that the imprinting technique used in this study successfully enhanced electrode selectivity by providing selective imprints for AMT.

CONCLUSIONS

Based on the results of this study, it can be concluded that the optimum conditions for the voltammetric analysis of amitriptyline using the MI-PANi-modified carbon paste electrode were a deposition potential of 0.6 V, a deposition time of 60 s, a scan rate of 25 mV/s, and a solution pH of 8. The method validation yielded a linearity (correlation coefficient) of 0.9905 for the amitriptyline standard curve, a detection limit of 15.33 ppb, precision values ranging from 94.25% to 99.74%, and accuracy values between 75.08% and 103.31%. The sensitivity of the MI-PANi/CPE was 0.1222 nA/ppb, with a recovery (% recovery) of 99.08%. The selectivity of the MI-PANi-modified carbon paste electrode for the voltammetric analysis of AMT was poor in solutions containing glucose at concentrations equal to or greater than the concentration of AMT.

AUTHOR CONTRIBUTIONS

In this study, JAH performed sample preparation, sensor fabrication, and analysis, as well as compiled the report. NWU carried out sensor fabrication and article writing, while MRA and AAW conducted the research and prepared the report.

CONFLICT OF INTEREST

In this study, the authors declare no conflict of interest

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