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Application of Carbon Paste Electrodes Modified with Imprinted Zeolite X as Potentiometric Sensors for Amitriptyline Analysis in Pharmaceuticals

Miratul Khasanah*, Alfa Akustia Widati, Ade Sukma Wahyuni and Dedy Samuel Bureni

Chemistry Department, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia

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Carbon paste electrodes have been developed to improve the performance and selectivity of electrodes for the potentiometric analysis of amitriptyline (AMT). Carbon paste electrodes modified with imprinted zeolites (IZ) were developed as potentiometric sensors for AMT analysis. In this study, zeolite X was synthesized using Na₂O, Al₂O₃, SiO₂, and H₂O at a mole ratio of 4.5:1:3:315. The IZ was synthesized by adding AMT to the mixture at an AMT/Si molar ratio of 0.0306 as a template. Subsequently, AMT was extracted from the zeolite framework to form active and selective AMT recognition sites. The optimum performance was achieved by the electrode composed of activated carbon, paraffin, and IZ at a composition of 12:7:1, which was expressed by a Nernst factor of 28.38 mV decade⁻¹, measurement range of 10⁻⁵-10⁻² M with a correlation coefficient of 0.9994, and a low detection limit of 1.15 × 10⁻⁵ M. The detection limit can accommodate the lower dose of AMT in pharmaceuticals. The modified electrode had a precision of 98.3-99.7% and an accuracy of 91.2-107%, with a response time of 24-211 s. Moreover, it was stable within four weeks after 70 uses. Application of the modified electrode in the determination of AMT in three pharmaceutical samples resulted in a 96-102% recovery. The presence of glucose, lactose, mannitol, and ZnSO₄ had no effect on the potentiometric analysis of AMT using the modified electrode. The excellent analytical performance of the modified carbon paste electrode suggests its potential applications in the analysis of AMT, an affordable medicine.

Keywords: Amitriptyline analysis, Potentiometry, Carbon paste electrode, Imprinted zeolite X, Amitriptyline selectivity, Affordable medicine

INTRODUCTION

Amitriptyline (AMT) (Fig. 1) is one of the most frequently prescribed antidepressants. The use of these drugs at unnecessarily high doses causes severe mental and physical problems, such as seizures, hyper reflexes, tachycardia, hypotension, anticholinergic symptoms, depression, and even drug addiction [1]. Moreover, AMT can inhibit the membrane-pumping mechanism responsible for the uptake of norepinephrine and serotonin by adrenergic and serotonergic neurons [2]. The maximum allowable daily intake of AMT is 50-200 mg [3]. Notably, AMT blood levels higher than 0.50 mg l⁻¹ are toxic; an overdose can affect the

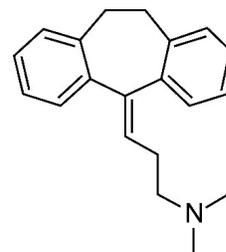


Fig. 1. Chemical structure of amitriptyline.

cardiovascular and parasympathetic nervous systems [4]. However, during the compounding of drug tablets, composition errors often occur owing to the quality of the preparation and the lack of uniformity in the levels of each tablet product. This is because of a lack of supervision by the authorities [5,6]; therefore, monitoring is essential.

*Corresponding author. E-mail: miratul-k@fst.unair.ac.id

Various analytical methods have been applied for AMT determination, such as ultraviolet-visible spectrophotometry [7], high-performance liquid chromatography (HPLC) [8], high-performance thin-layer chromatography [9], reversed-phase high-performance liquid chromatography (RP-HPLC) [10], gas chromatography [11], ultrasound-assisted ionic liquid-based dispersive liquid-liquid microextraction [12], and optical and electrochemical sensors [3]. Several limitations of these methods, including narrow measurement ranges, unacceptable detection limits, and high time consumption, render them unsuitable for the analysis of large amounts of samples.

Additionally, AMT detection was performed using electrochemical methods using a screen-printed CuO nanoparticle sensor with a measurement range of 1-200 M, a detection limit of 0.4 M, and a recovery of 83.7% [13]. Nafion-modified Au electrodes have been developed to serve as voltammetric sensors for AMT analysis with a detection limit of 3.4×10^{-8} M and a measurement range of 1×10^{-7} - 7×10^{-4} M [14]. Plasticized polyvinyl chloride (PVC) membrane-coated glassy carbon [15] and SiO₂/Al₂O₃/Nb₂O₅/DNA-modified carbon paste electrodes have been fabricated for voltammetric AMT analysis [16]. Both modified electrodes exhibited wide measurement ranges, high sensitivity, low detection limits, and good accuracy.

AMT may also be analyzed using potentiometric methods, which offer high accuracy and precision [17]. Potentiometric methods using PVC ion-selective electrodes (ISEs) have also been developed for AMT detection [18]. The PVC-modified electrode exhibited selectivity in the range of 10 nM-1.0 mM, a detection limit of 5.0 nM, and a Nernst factor of 58.4 mV decade⁻¹. Furthermore, other researchers have reported the use of molybdovanadate and molybdotungstate-modified electrodes [19] for potentiometric AMT analysis. The measurement range produced by this ISE was 1×10^{-4} - 7×10^{-2} M.

In previous studies, zeolites TS-1 and LTA have been developed as carbon paste electrode modifier materials for the analysis of uric acid [20,21], creatinine [22], creatine [23], and glucose [24] in human serum samples. The imprinted zeolite (IZ)-modified electrodes showed good performance for the potentiometric analysis of these compounds.

An X-type IZ-modified carbon paste electrode was designed in this study for the potentiometric measurement of AMT. The X-type IZ was fabricated from zeolite X. Various types of zeolites exhibit different sensor performances due to the unique ring and pore sizes and different Si and Al amounts. Therefore, investigations on the effect of the zeolite type are still of immense importance. In this study, the electrodes were constructed from a paste formed by combining different mixtures of activated carbon, paraffin granules, and IZ. Zeolites were synthesized using the hydrothermal sol-gel method with a Na₂O:Al₂O₃:SiO₂:H₂O molar ratio of 4.5:1:3:315 [25]. The optimal ratio of activated carbon, paraffin, and IZ as electrode materials, as well as the optimum pH of the AMT solution, were investigated in this study. The measuring range (linear dynamic range), Nernst factor, detection limit, selectivity, accuracy, response time, and electrode lifetime were used to evaluate the electrode performance (stability). Selectivity was studied by adding glucose, lactose, mannitol, and ZnSO₄ to analyte samples. Furthermore, the sensor was used to analyze AMT-HCl levels in pharmaceuticals.

MATERIALS AND METHODS

Materials

The chemicals used in this study were AMT-HCl (C₂₀H₂₃N-HCl (98%); Sigma Aldrich, St. Louis, MO, USA), glucose (C₆H₁₂O₆ (99.5%) Merck, Rahway, NJ, USA), lactose (C₁₂H₁₄O₁₂ (99%), Merck, Rahway, NJ, USA), mannitol (C₆H₁₄O₆ (99%), Merck, Rahway, NJ, USA), acetic acid (CH₃COOH (100%), Merck, Rahway, NJ, USA), disodium hydrogen phosphate dihydrate (Na₂HPO₄·2H₂O (99%), Merck, Rahway, NJ, USA), sodium acetate trihydrate (CH₃COONa·3H₂O (99.5%), Merck, Rahway, NJ, USA), tetraethyl orthosilicate (TEOS (99%); Merck, Rahway, NJ, USA), sodium dihydrogen phosphate dihydrate (NaH₂PO₄·2H₂O (98%) Merck, Rahway, NJ, USA), sodium aluminate (NaAlO₂ (50%), Sigma Aldrich, St. Louis, MO, USA), sodium hydroxide (NaOH (99%), Merck, Rahway, NJ, USA), silver wire (Ag (≥ 99.9%); Sigma Aldrich, St. Louis, MO, USA), n-hexane (C₆H₁₄; Merck, Rahway, NJ, USA), paraffin pellets (Sigma Aldrich, St. Louis, MO, USA), and activated carbon (Sigma Aldrich, St. Louis, MO, USA). Distilled water was used as a solvent.

Instrumentation

The instruments used in this study were an X-ray diffractometer (Shimadzu, Kyoto, Japan), Fourier transform infrared spectrophotometer (Shimadzu Pharmaspec UV-1800), potentiometers (Cyberscan 510, Frankfurt, Germany) with Ag/AgCl as the reference electrode, hotplate (Termolyne S46410-2, Rockland, MA, USA), pH meter (Cyberscan Eutech Instruments pH 510, Frankfurt, Germany), centrifuge (HITECH EBA 20, Westphalia, Germany), vacuum oven (Model 5851, Amityville, NY, USA), agate mortar, polypropylene bottle, 1000 μ l micropipette tips, and glassware.

Materials and Methods

Synthesis of IZ X. The IZ was synthesized by mixing NaAlO₂, NaOH, TEOS, and distilled water at a molar ratio of Na₂O, Al₂O₃, SiO₂, and H₂O of 4.5:1:3:315 [25]. The mixture was placed in a polypropylene bottle that was hydrothermally heated at 100 °C in an oven for 24 h. AMT solution was added to the mixture at a mole ratio of AMT/Si = 0.0306 under stirring for 30 min. Subsequently, the mixture was allowed to stand for 3 h to facilitate the incorporation of AMT into the zeolite framework. Furthermore, the mixture was repeatedly extracted using hot water (80 °C) by centrifugation for \pm 20 min until the filtrate attained a neutral pH. The obtained solid was then dried in an oven at 80 °C.

Fabrication of carbon paste electrodes modified with IZ X. The IZ X-modified carbon paste electrode was prepared by filling three-quarters of a micropipette tip embedded with silver wire (Ag) with melted paraffin. The remaining quarter of the micropipette tip was filled with a paste prepared by mixing activated carbon, solid paraffin, and IZ in varying compositions. The paste was filled into the micropipette tip under pressure until the tip was completely filled. Furthermore, the surface of the electrode was rubbed on white paper. For conditioning, the electrodes were submerged in a 10⁻³ M AMT solution for 24 h. The compositions of the activated carbon, paraffin, and IZ mixtures used for the preparation of the electrodes are listed in Table 1.

Optimization of pH solution. The influence of pH on the electrode potential was investigated by adjusting the pH of the AMT solution. A buffer was added to regulate the pH of the 10⁻⁵-10⁻² M AMT solution to 5, 6, 7, and 8. Each solution

Table 1. The Composition of Activated Carbon, Paraffin, and Imprinted Zeolite (IZ) for Electrode Preparation

Electrode	Composition (wt%)		
	Activated carbon	Paraffin	IZ
E1	65	35	0
E2	60	35	5
E3	55	35	10
E4	50	35	15
E5	45	35	20
E6	40	35	25

was analyzed potentiometrically using a carbon-IZ X-paste electrode under optimal working conditions, with Ag/AgCl as the reference electrode. The optimum pH was determined based on the measurement results using an electrode with an optimum composition that produced a Nernstian curve with a Nernst factor of $[\frac{59.2}{n} \pm 2]$ mV.

Electrode performance and method validation. In this study, the parameters used to evaluate the performance of the electrode and the validity of the method were the Nernst factor, measurement range, linearity, detection limit, precision, accuracy, response time, lifetime, selectivity, and electrode wearability. The electrode performance and validity of the analytical method were tested to determine the feasibility of the analytical method.

The Nernst Factor was determined using Nernst equation $E = E^0 + \frac{59.2}{n} \log[\text{OX}]$. This equation is analogue to a linear regression equation of the relationship curve between log [AMT] and the electrode potential (mV). The value of the Nernst factor was obtained from the slope of the equation. The measurement range was determined based on the concentration range of the 10⁻⁸-10⁻² M AMT solution, which gave linear and Nernstian lines. Linearity was expressed using the correlation coefficient (R²) derived from the linear regression equation. The detection limit was calculated by intersecting the linear and non-linear lines on the log[AMT] relationship curve with the electrode potential and extrapolating it to the abscissa to yield the log[AMT] value that corresponds to the detection limit of the electrode. Precision was determined by calculating the coefficient of variation (CV) and standard deviation (SD) of the potential

value of the 10^{-5} - 10^{-2} M AMT solution, which was measured three times. The SD and CV values were calculated using Eqs. (1) and (2), respectively.

$$SD = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}} \quad (1)$$

$$CV = \frac{SD}{\bar{x}} \times 100\% \quad (2)$$

Here, x_i is the result of each i^{th} measurement, \bar{x} is the average value of the measurement results, and n is the number of measurements. The accuracy values were obtained by measuring the amount of AMT solution in the linear concentration range. The accuracy was calculated using Eq. (3).

$$\text{Accuracy} = \frac{C_{sp}}{K_s} \times 100\% \quad (3)$$

Here, C_{sp} is the measured concentration and K_s is the true concentration.

The electrode response time was investigated by measuring the potential of a 10^{-5} - 10^{-2} M AMT solution using a carbon-IZ paste electrode. The electrode response time refers to the length of time required by the electrode to respond to the analyte and is calculated from the duration for which the electrode is immersed into the solution until a constant potential value is attained. The lifetime of the electrode was determined based on the total time the electrode was used for measurement and provided good performance until the electrode experienced a decrease in performance, which is indicated by a deviation in the Nernst factor value in a certain measurement range.

The recovery value shows the agreement between the value obtained by the analytical method and the actual value. The greater the recovery value obtained in the analysis of an analyte, the better the accuracy of the method used. The recovery value can be determined using Eq. (4).

$$R = \frac{C_1 - C_2}{C_3} \times 100\% \quad (4)$$

Here, R is the recovery, C_1 is the concentration of AMT in the standard and analyte solutions, C_2 is the concentration

of the AMT analyte, and C_3 is the concentration of the AMT standard.

The selectivity coefficient was determined using the matched potential method [26]. The electrode selectivity test for AMT in a matrix of glucose, lactose, mannitol, and $ZnSO_4$ was performed by measuring the potential of each matrix solution within the AMT measurement range. The potential data obtained were substituted into the linear regression equation of the AMT standard curve to obtain the AMT concentration. This value was used to determine the selectivity coefficient using Eq. (5),

$$K_{i, pot} = \frac{\Delta a_i}{a_j} \quad (5)$$

where Δa_i is the difference in analyte activity and a_j is the activity of the matrix. If $K_{ij} = 0$, the matrix does not interfere with the analysis. If $K_{ij} < 1$, the electrode is more selective towards the analyte than the matrix, but if $K_{ij} > 1$, the electrode is more selective towards the matrix than the analyte.

RESULTS AND DISCUSSION

Synthesis and Characterization of Zeolite-modified Carbon Paste Electrodes

Firstly, zeolite X was synthesized using TEOS as a silica source, $NaAlO_2$ and $NaOH$ as sources of aluminate and sodium, and distilled water (H_2O) as the solvent. The addition of an AMT-HCl solution to the synthesis of non-imprinted zeolite (NIZ) and IZ served as a template. An illustration of the IZ synthesis process is shown in Fig. 2.

X-ray diffraction was used to analyze the dense and regular crystalline structure of the synthesized zeolite, in particular, to verify the formation of zeolite X. The diffraction pattern of synthesized zeolite X is shown in Fig. 3 [27]. The diffractogram in Fig. 3 shows the low crystallinity of synthesized zeolite X, which is evident from the broad peak and absence of sharp diffraction peaks. However, for synthesized zeolite X, several peaks were formed that were close to the peaks of standard zeolite X, namely 12.12° , 19.32° , 23.70° , 27.79° , 30.62° , 40.61° , and 48.02° (Table 2).

Zeolite X synthesized from natural materials had the highest intensity for the 2θ value of 27.9112° . Therefore, the

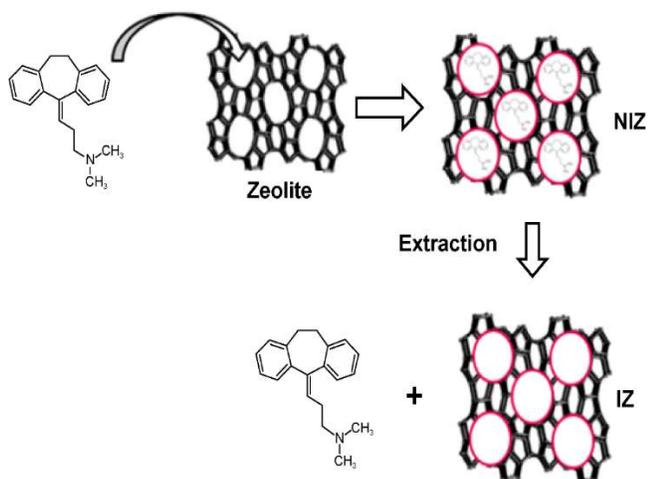


Fig. 2. Schematic of imprinted zeolite synthesis.

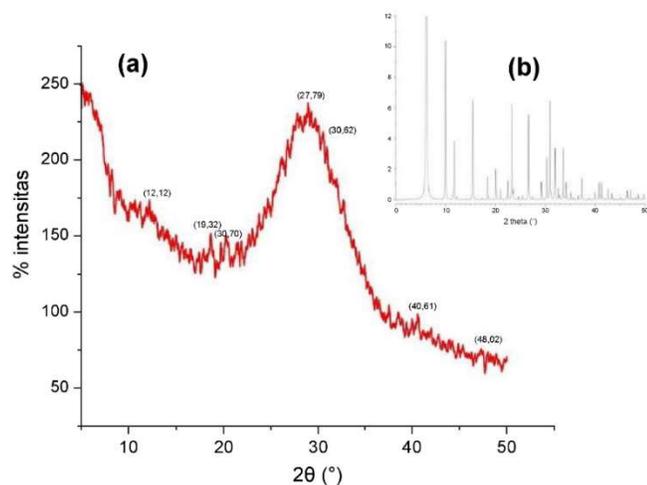


Fig. 3. Diffractogram pattern of (a) synthesized zeolite X and (b) standard zeolite X [27].

Table 2. Diffraction Data (2θ) of Synthesized and Standard Zeolite X Samples

Synthesized zeolite X (2θ , °)	Standard zeolite X (2θ , °)
12.12	12.25
19.32	20.07
23.70	23.58
27.79	27.37
30.62	30.30
40.61	40.79
48.02	48.24

zeolite X synthesized in this study is the most dominant phase, which is characterized by the appearance of the main peak at a 2θ value of 27.79° .

After zeolite was successfully synthesized, the IZ was prepared with the additional stage after the hydrothermal process. A number of AMT molecules were added to the zeolite to accommodate the formation of a selective template for AMT molecules. The Zeolite-modified electrode was studied by comparing the characteristic of carbon paste-zeolite, carbon paste-NIZ, and carbon paste-IZ electrodes. Figure 4 displays the SEM images of modified zeolite and carbon paste-modified zeolite and the composition of each sample is depicted in Table 3.

Zeolite had the main component of Si, Al, and O with low impurities from C and N. The presence of Na on the zeolite was predicted from the precursor of NaAlO_2 . After AMT was added to the zeolite to become NIZ material, the percentage of C and N was increased. Furthermore, the percentage of C and N was decreased on the IZ sample. It means that the extraction process of AMT successfully releases the AMT on the zeolite framework.

The zeolite-modified carbon paste electrode was prepared by mixing activated carbon, solid paraffin, and zeolite. The activated carbon is a conductive material that responds and delivers a signal therefore can be detected by a potentiometer. Paraffin acts as an adhesive between active carbon and imprinted zeolite. Imprinted zeolite serves as a specific template for AMT molecules, therefore can increase the selectivity of the electrode.

The concentration of C of carbon paste-zeolite, carbon paste-NIZ, and carbon paste-IZ was significantly increased because of the use of carbon paste and paraffin on the samples. The presence of Ca, Mg, and K in the samples was predicted as impurities from active carbon. Ca, Mg, and K are alkaline-earth elements that are abundant elements in the earth's crust. The highest concentration of C and N were also found on the carbon paste-NIZ because the AMT molecules existed on the framework.

Optimization of Electrode Composition

A mixture of activated carbon, paraffin, and IZ was used to prepare the carbon-IZ paste electrodes. The presence of activated carbon prolongs the response of the analyte to the Ag wire, which results in a detectable value. The presence of

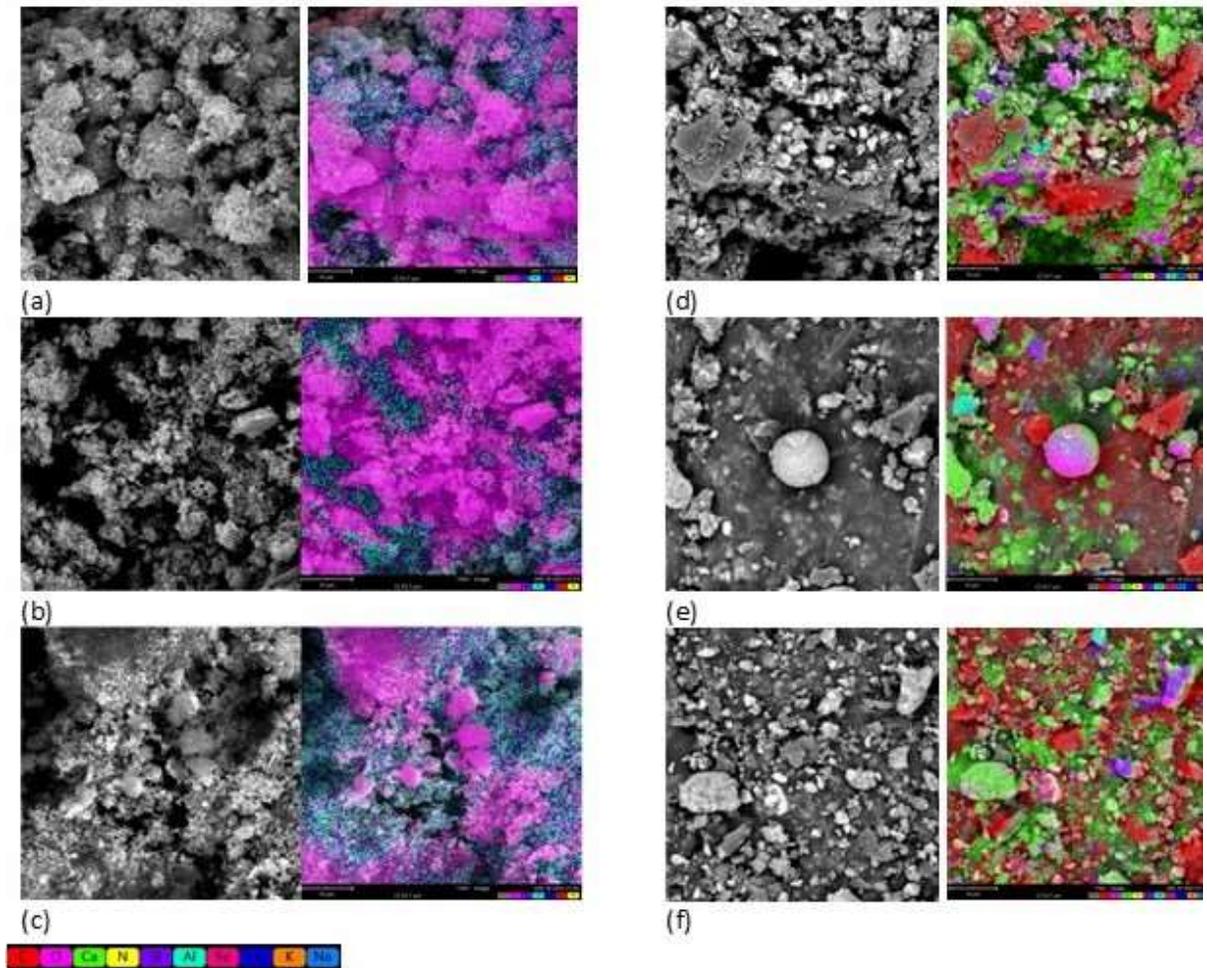


Fig. 4. SEM images of (a) zeolite, (b) non-imprinted zeolite, (c) imprinted zeolite; electrodes with (d) carbon paste-zeolite, (e) carbon paste-non imprinted zeolite, (f) carbon paste-imprinted zeolite.

Table 3. The weight Concentration of Samples According to EDX Analysis

Element	Weight Concentration (%)					
	Zeolite	NIZ	IZ	Carbon paste-zeolite	Carbon paste-NIZ	Carbon paste-IZ
O	51.17	49.63	47.33	22.91	30.81	25.04
Si	15.94	15.80	17.50	3.21	2.67	3.43
Al	15.74	14.62	16.90	2.23	3.06	1.43
Na	11.33	10.94	10.24	0.59	0.87	0.21
C	4.04	6.82	5.88	51.85	59.43	55.87
N	1.77	2.20	1.75	3.78	4.26	3.67
Ca				6.10	5.92	6.11
Mg				0.32	0.42	0.43
K				0.27	0.34	0.39

the IZ is expected to increase the selectivity of the electrode because it has a selective recognition site for the analyte. Paraffin serves as an adhesive between the mixture of activated carbon and IZ to ensure the adhesion of the mixture to the electrode during measurements. Moreover, the activated carbon and IZ content are assumed to affect electrode performance, whereas the content of paraffin is kept constant. The Nernst factor, measurement range, and linearity of the measurement results of the AMT solution without the addition of buffer using varying compositions of the carbon-IZ paste electrode are listed in Table 4.

Table 4 shows that the working electrode E2 had a Nernst factor of 28.38 mV decade⁻¹, which was closest to that of the theoretical value. The experimental data of linear range detection of electrodes are shown in Fig. 5. According to this data, The E2 electrode had a wide measurement range of 10⁻⁵-10⁻² M and linearity close to 1, indicating a strong relationship between the x (log[AMT]) and y (electrode potential) variables. Furthermore, a zeolite-modified carbon paste electrode (EZ) and a NIZ-modified carbon paste electrode (ENIZ) composed of activated carbon, paraffin, and IZ or NIZ were fabricated, respectively, with the same composition as the optimum working IZ electrode (E2). The Nernst factor values obtained for EZ and ENIZ were 4.1 and 1.85 mV decade⁻¹, respectively, with a narrower measurement range of 10⁻⁴-10⁻² M. The detection mechanism of the modified electrode is beginning with the diffusion of AMT molecules into the IZ channel of the

electrode. Diffusion occurs whenever the concentration of an ion or molecule at the surface of the electrode is different from that in bulk solution. When the electrode has a specific site for the analyte, the equilibrium condition is faster to reach.

The analysis of AMT using EZ and ENIZ at the time of measurement did not experience any disturbance from the equilibrium at the surface of the electrode, and thereby no variation in the potential difference was observed owing to the electrode. Therefore, the measured potential values did not obey the Nernst equation. Moreover, the lack of imprinting material in EZ and ENIZ, that is, the absence of an active site, limits the ability of the electrode to recognize AMT analytes and thereby reduces the electrode performance.

Optimization of Solution pH

The pH of the solution greatly affects the stability of the AMT compound. The influence of acids or bases on the measurement of the AMT solution was investigated using pH optimization to ensure equilibrium takes place optimally at the electrode surface interface with the analyte solution. The optimum pH for electrode E2 was 8. When measuring the AMT solution at pH 5, 6, and 7, the obtained Nernst factors were far from the theoretical value. If the analyte solution is mostly neutral, AMT is likely to be present in the molecular form instead of its ionized form [28]. The acid dissociation constant (K_a) for AMT is as low as 10^{-9.76}, which indicates

Table 4. Nernst Factor, Measurement Range, and Linearity of the Amitriptyline Solution Measurements by Electrodes with Varying Compositions

Electrode	Composition (wt%)			Nernst factor (mV decade ⁻¹)	Measurement range (M)	Linearity (R ²)
	Activated carbon	Paraffin	IZ			
E1	65	35	0	16.42	10 ⁻⁵ -10 ⁻²	0.9325
E2	60	35	5	28.38	10 ⁻⁵ -10 ⁻²	0.9994
E3	55	35	10	19.32	10 ⁻⁵ -10 ⁻²	0.9780
E4	50	35	15	19.87	10 ⁻⁵ -10 ⁻²	0.9498
E5	45	35	20	16.76	10 ⁻⁵ -10 ⁻²	0.8861
E6	40	35	25	17.16	10 ⁻⁵ -10 ⁻²	0.8054
EZ	60	35	5	4.10	10 ⁻⁴ -10 ⁻²	0.9982
ENIZ	60	35	5	1.85	10 ⁻⁴ -10 ⁻²	0.9939

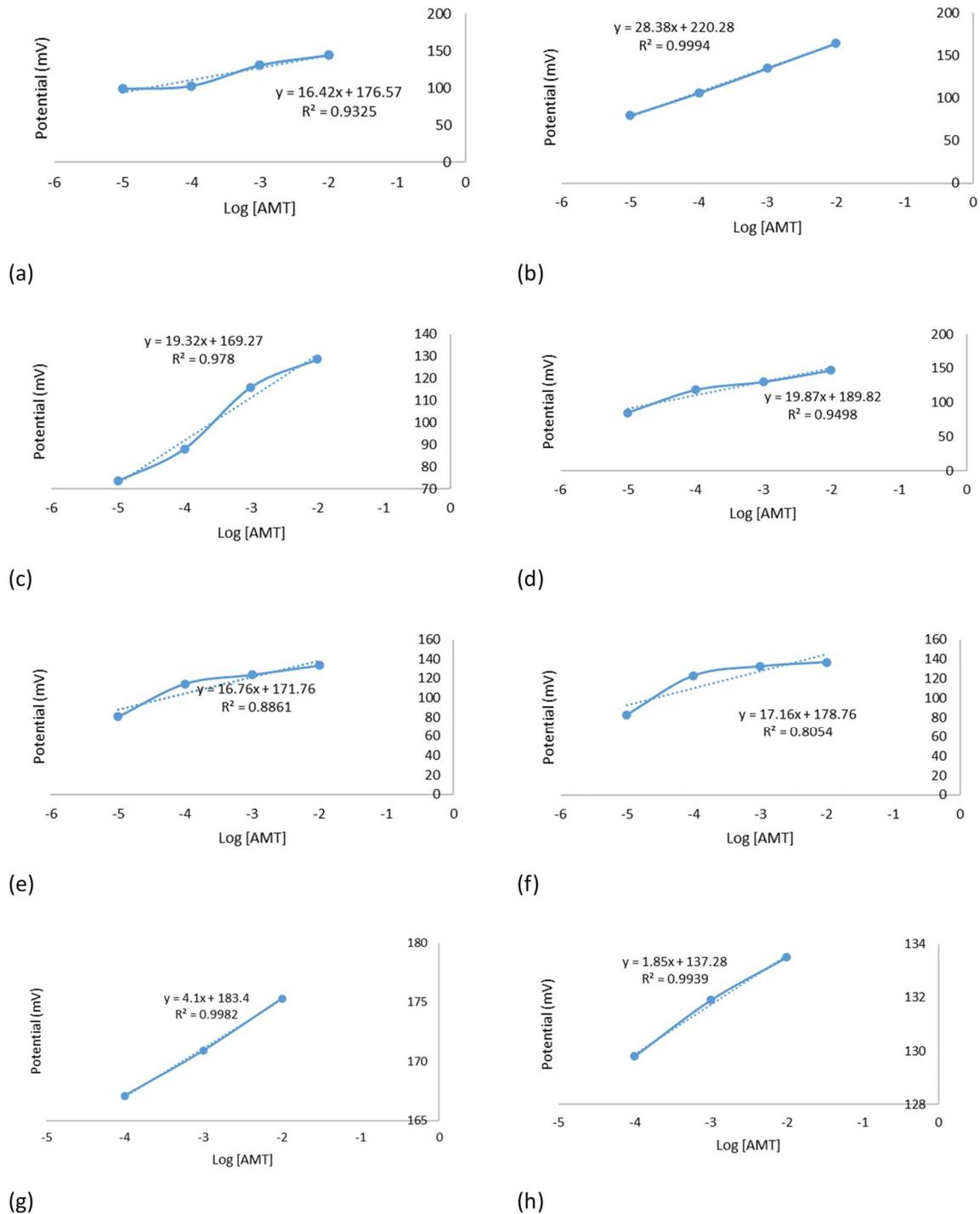


Fig. 5. The graphic of linear range detection of (a) E1 (b) E2 (c) E3 (d) E4 (e) E5 (f) E6 (g) EZ (h) ENIZ.

that the AMT molecules in the solution are barely ionized; therefore, larger amounts of AMT will be present in its molecular form. Additionally, AMT is present in its

molecular form at an alkaline pH (pK_a of AMT = 9.76); that is, the responses during measurement at an alkaline pH are generated from the molecular form of AMT.

Performance of the Modified Electrode and Method Validation

Electrodes in potentiometric measurements are known to exhibit good performance if they have a wide measuring range and Nernst factor of approximately $59/n \text{ mV decade}^{-1}$, where n is the number of electrons involved. The AMT molecule has one electron involved in the oxidation reaction process, with a Nernst factor that is close to the theoretical value of a monovalent ion ($59.2 \text{ mV decade}^{-1} \text{ mV decade}^{-1}$). The relationship of $\log[\text{AMT}]$ with the electrode potential and the standard curve of AMT analysis are shown in Fig. 6.

Based on Fig. 6, the linear regression equation $y = 28.38x + 220.28$ was obtained, and the Nernst factor of $28.38 \text{ mV decade}^{-1}$ was derived based on the slope of the graph. Previous studies have reported the detection of AMT using zeolite TS-1- and A-modified carbon paste electrodes, which exhibited Nernst factors of 58.3 [16] and $60.8 \text{ mV decade}^{-1}$ [17], respectively. Another study has reported that the detection of AMT using a dibutyl phthalate-coated graphite electrode exhibited a Nernst factor of 58.8 [17].

The measurement range of the potentiometric analysis exhibited a linear curve for the plot of $\log[\text{AMT}]$ versus the electrode potential, which was Nernstian. The measurement range is one of the factors used to determine the optimal electrode composition. The wider the measurement range, the better the performance of the electrode [29]. Based on the curve in Fig. 6, the measurement range was 10^{-5} - 10^{-2} M with a linearity value of 0.9994 .

The potentiometric detection of AMT using modified molybdovanadate and molybdotungstate electrodes resulted in a measurement range of 10^{-4} - 10^{-2} M [19]. In contrast, the electrode developed in this study exhibited a linear

measurement range that is ten times wider. The detection limit was evaluated to determine the lowest and highest AMT concentrations in the solution to which the electrodes respond well. The lower the detection limit, the higher the sensitivity of the method. In this study, the equation for the linear line was $y = 28.38x + 220.28$, whereas that for the lower non-linear line was $y = 13.45x^2 + 147.55x + 480.71$. The lower detection limit of the carbon-IZ paste electrode was $1.15 \times 10^{-5} \text{ M}$, which is higher than the detection limit of AMT analysis using ionic liquid carbon electrodes modified with nanoclay composites ($2.40 \times 10^{-8} \text{ M}$) [30]. Based on the obtained detection limit, the developed electrode may be applied to measure AMT levels with the lowest dose in pharmaceutical preparation, which is 5 mg ($1.5 \times 10^{-5} \text{ M}$). The detection limits and linear ranges of various analytical methods for AMT analysis are listed in Table 5.

The precision value is thought to be good if the AMT concentration range of 10^{-5} - 10^{-2} M has a corresponding CV value below 11% [34]. Therefore, the smaller the CV value, the better the precision because of smaller deviations in results between measurements. As listed in Table 5, the CV value obtained from repeated measurements of 10^{-5} - 10^{-2} M AMT solutions was in the range of 0.28 - 1.73% (98.27 - 99.72% precision). The precision value obtained in this study is better than that of previous studies using the PVC-molybdovanadate, which had a precision value of 97.5 - 98.3% [19]. The reproducibility was also determined using CV value. The smaller of CV value, the higher of reproducibility.

Accuracy was determined by comparing the analytical response (the potential measured by the electrode) with the actual value (based on the standard curve). Accuracy was determined in the measurement range of AMT concentrations (10^{-5} - 10^{-2} M) using the E2 electrode. The accuracy value for

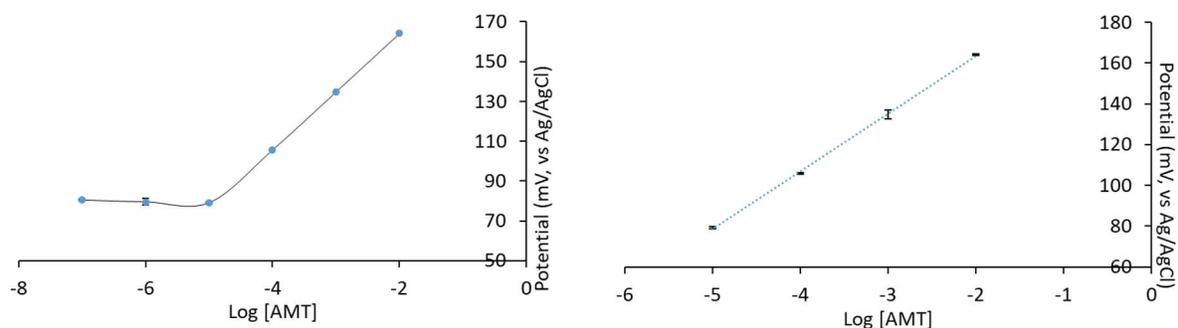


Fig. 6. (a) Plot of $\log[\text{amitriptyline}]$ vs. electrode potential and (b) amitriptyline standard curve.

Table 5. Limits of Detection (LODs) and Linear Measurement Range of Various Methods for Amitriptyline Analysis

Methods	LOD	Measurement range	Ref.
	(M)	(M)	
HPLC	3.6×10^{-5}	8.0×10^{-7} - 1.6×10^{-4}	[30]
RP-HPLC	1.3×10^{-5}	5.0×10^{-4} - 1.5×10^{-3}	[10]
Gas chromatography	1.6×10^{-7}	1.6×10^{-7} - 9.6×10^{-6}	[11]
Gas chromatography	2.2×10^{-6}	6.4×10^{-5} - 6.4×10^{-4}	[31]
DPV-carbon nanotube paste electrode	1.6×10^{-6}	0 - 3.0×10^{-7}	[6]
Voltammetry-Nafion Aunps@Branched Polyethyleneimine-Derived Carbon Hollow Spheres	3.4×10^{-8}	1×10^{-7} - 7×10^{-4}	[14]
Voltammetry-Graphite Screen Printed-CuO Nanoparticles	4×10^{-7}	1×10^{-6} - 2×10^{-4}	[13]
Voltammetry-Fe ³⁺ -zeolit-grafit	2.2×10^{-7}	5×10^{-7} - 5×10^{-5}	[32]
Voltammetry-carbon nanocomposite ionic electrode	1.9×10^{-8}	5×10^{-8} - 9×10^{-5}	[33]
Spectrophotometry-molybdenum-thiocyanate	6.4×10^{-7}	3.2×10^{-6} - 7.9×10^{-5}	[7]
Potentiometry-Ion exchange-based PVC membrane ISEs	5×10^{-9}	10^{-8} - 10^{-3}	[18]
Potentiometry-molybdovanadate and molybdotungstate	6.9×10^{-5}	10^{-4} - 10^{-2}	[19]
Potentiometry-liquid carbon ionic-nano clay	2.4×10^{-8}	1×10^{-7} - 8×10^{-6}	[35]
Potentiometry-DBP-coated graphite electrode	4.8×10^{-7}	10^{-6} - 10^{-1}	[17]
Potentiometry-carbon paste IZ-X	1.15×10^{-5}	10^{-5} - 10^{-2}	This study

LOD: limit of detection; HPLC: high-performance liquid chromatography; RP-HPLC: reverse phase-high performance liquid chromatography; DPB: dibutylphtalate; PVC: polyvinyl chloride; ISEs: ion selective electrodes.

Table 6. The Precision of the E2 Electrode in Amitriptyline Analysis

log[AMT]	E (mV, vs. Ag/AgCl)			SD	CV (%)		Precision (%)
	1	2	3		This study	AOAC	
-4	91.0	91.5	91.7	0.36	0.39	8.0	99.61
-3	119.4	122.9	123.2	2.11	1.73	5.7	98.27
-2	144.8	145.2	145.6	0.40	0.28	4.0	99.72

AMT: amitriptyline; CV: coefficient of variation; SD: standard deviation; AOAC: association of official analytical chemists.

Table 7. Accuracy of the Amitriptyline Analysis Method Using Carbon Paste-imprinted Zeolite (E2)

The concentration of AMT (M)		Accuracy (%)	
True value	Experimental value	This study	AOAC*
10^{-5}	1.07×10^{-5}	107	80-110
10^{-4}	9.12×10^{-5}	91.2	80-110
10^{-3}	9.70×10^{-4}	97	90-107
10^{-2}	1.00×10^{-2}	100	97-103

*Source: Taverniers *et al.* [34]. AMT: amitriptyline; AOAC: Association of Official Analytical Chemists.

an analytical method is thought to be good if it is in the range of 80-110% for AMT concentrations of 10^{-5} - 10^{-2} M [34]. The accuracy results for the AMT analysis method are listed in Table 7.

The accuracy of the developed method falls in the acceptable range for chemical analyses, which is 91.2-107% [34]. In a previous study, the accuracy was 85.1-107.1% [17], which was determined using standard addition techniques for pharmaceutical preparations.

The response time of the electrode was determined based on electrode contact with the analyte until a stable state (constant potential) was obtained. The faster the response time of the electrode, the more sensitive it is to the analyte. The response time results for the electrodes in the AMT solution at concentrations of 10^{-5} - 10^{-2} M are listed in Table 8. The response times show that the greater the AMT concentration, the smaller the time taken to attain equilibrium between the bulking solution and electrode surface. This is because, in a concentrated solution, intermolecular collisions often occur, and the reaction takes place more rapidly. Moreover, the response time can also be affected by stirring. Therefore, the response time of the electrode can also be used as an indicator of electrode sensitivity.

In this study, the electrode lifetime was expressed by the number of electrodes used, which still showed a good performance. In this case, it is apparent from the value of the Nernst factor obtained at a concentration of 10^{-5} - 10^{-2} M. Observations were carried out for four weeks. The electrode lifetime data are presented in Table 9.

The electrode performance reduced after 70 uses (within four weeks), as indicated by the decrease in the Nernst factor. When an electrode is used continuously, its surface becomes saturated owing to the dissolution of the electrode

Table 8. Response Times of the Carbon Paste-imprinted Zeolite Electrode During Amitriptyline (AMT) Analysis

[AMT]	Potential (mV, vs. Ag/AgCl)	Response time (s)
10^{-5}	80.5	211
10^{-4}	102.8	144
10^{-3}	129.7	31
10^{-2}	148.2	24

Table 9. Nernst Factor Values Derived from Amitriptyline Analysis by Potentiometry Using the E2 Electrode

Number of measurements	Measurement range (M)	Nernst factor (mV decade ⁻¹)
21	10^{-5} - 10^{-2}	28.38
46	10^{-5} - 10^{-2}	27.61
70	10^{-5} - 10^{-2}	19.7
106	10^{-5} - 10^{-2}	19.6

components, especially the mold. This leads to a deviation in the values.

In this study, the recovery test was performed using zeolite X-modified carbon paste electrodes for AMT analysis on AMT-HCl pharmaceutical tablet samples at a dose of 25 mg (3.18×10^{-3} M). The recovery value obtained was 96.2-102.4% (Table 10), which indicates the developed electrode exhibits high recovery within the range acceptable for chemical analysis methods [35].

Table 10. Recovery Data for the Amitriptyline (AMT) Analysis Method

Solution	Concentration (M)	Recovery (%)
Standard AMT 10^{-3} M	1.09×10^{-3}	
Tablet AMT 1	8.04×10^{-4}	102.4
Tablet 1 + AMT 10^{-3} M	1.92×10^{-3}	
Tablet AMT 2	7.82×10^{-4}	96.2
Tablet 2 + AMT 10^{-3} M	1.83×10^{-3}	
Tablet AMT 3	8.23×10^{-4}	97.3
Tablet 3 + AMT 10^{-3} M	2.15×10^{-3}	

The selectivity of the electrode is expressed by the selectivity coefficient K_{ij} , which was evaluated to determine the level of selectivity of the electrode to the AMT analyte in different matrices, as listed in Table 11. The selectivity test was performed using separate matrix solutions of glucose, lactose, and mannitol. These solutions were used because they are thought to be carriers of AMT pharmaceutical tablets. Moreover, the three solutions have functional groups that can form hydrogen bonds with the IZ, similar to AMT. In addition to the three matrix solutions, a selectivity test for the electrodes was performed using a $ZnSO_4$ solution, because this solution is also thought to be a carrier for AMT. Notably, the gastrointestinal side effects of AMT include diarrhea, which is closely related to the addition of one metal, especially zinc, because when a large amount of Zn is consumed, the secretory cycle is inhibited.

The selectivity coefficients for glucose, lactose, mannitol and $ZnSO_4$ solutions are listed in Table 11. The presence of glucose, lactose, and mannitol interfered with the analysis of AMT by both the carbon paste and carbon paste-IZ electrodes, which was indicated by the obtained K_{ij} value that was greater than one. Based on the obtained K_{ij} value for the $ZnSO_4$ matrix solution, it was concluded that the presence of $ZnSO_4$ in high concentrations (10^{-2} M) does not interfere with the AMT analysis using either a carbon paste electrode or carbon paste-IZ electrode. However, the K_{ij} value of the carbon paste-IZ electrode was much lower than that of the carbon paste electrode, which indicated that the addition of IZ could increase the electrode selectivity for AMT analysis

Table 11. The K_{ij} Value of E1 and E2 on Glucose, Lactose, Mannitol, and $ZnSO_4$

Solution	Concentration (M)	K_{ij}	
		E2	E1
Glucose	10^{-5}	3.0×10^3	3.0×10^{-2}
	10^{-4}	6.0×10^2	4.0×10^{-2}
	10^{-3}	7.9×10^2	3.9×10^{-2}
	10^{-2}	8.0×10^2	7.9×10^{-2}
	10^{-5}	1.0×10^3	4.0×10^{-2}
Lactose	10^{-4}	2.0×10^2	5.1×10^{-2}
	10^{-3}	3.0×10^1	1.2×10^{-2}
	10^{-2}	2.0×10^1	10.1×10^{-2}
	10^{-5}	1.5×10^3	5.6×10^{-2}
	10^{-4}	1.7×10^2	9.0×10^{-2}
Mannitol	10^{-3}	2.0×10^1	1.04×10^{-2}
	10^{-2}	3.0×10^0	1.86×10^{-2}
	10^{-5}	7.0×10^{-3}	2.0×10^{-1}
	10^{-4}	9.0×10^{-2}	4.0×10^{-1}
	10^{-3}	1.0×10^0	1.0×10^{-2}
$ZnSO_4$	10^{-2}	1.4×10^1	2.0×10^{-2}

in solutions containing $ZnSO_4$.

In this study, the presence of glucose and lactose in concentrations of 10^{-7} - 10^{-3} M did not interfere with the AMT analysis. Similarly, a previous study reported that the presence of glucose and fructose did not interfere with the potentiometric analysis of AMT using dibutyl phthalate-coated wire and graphite electrodes [17].

CONCLUSIONS

The modified electrode composed of activated carbon, paraffin, and IZ X with a mass ratio of 12:7:1 exhibited the optimum performance. The analysis of AMT using the developed electrodes resulted in a Nernst factor of 28.38 mV decade⁻¹, measurement range of 10^{-5} - 10^{-2} M, and linearity of 0.9994. The modified electrode showed a lower detection limit of 1.15×10^{-5} M, with a precision of 98.3-99.7% and accuracy of 91.2-107% for AMT solution concentrations of 10^{-5} - 10^{-2} M. The electrode showed high selectivity for AMT in the matrices of glucose, lactose,

mannitol, and ZnSO₄. The electrodes showed a response time of 24-211 s and a lifetime of more than four weeks (up to 70 uses). The application of the developed electrode for the analysis of AMT in pharmaceuticals exhibited a recovery of 96-102%. Therefore, it is recommended for application in the field of pharmaceuticals or medicines.

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REFERENCES

- [1] R. Ramasubbu, A. Burgess, I.G. Valdez, F. Cortese, D. Clark, A. Kemp, B. Goodyear, G. Macqueen, N.T. Bech-Hansen, J. Foster, V.A. Diwadkar, *Hum. Psychopharmacol.* 31 (2016) 144.
- [2] H. Li, M.W. Sumarah, E. Topp, *Environ. Toxicol. Chem.* 32 (2013) 509.
- [3] K. Kamari, A. Taheri, *J. Taiwan Inst. Chem. Eng.* 86 (2018) 230.
- [4] G.W. Kerr, A.C. McGuffie, S. Wilkie, *Tricyclic antidepressant overdose: A review*, *Emerg. Med. J.* 18 (2001) 236.
- [5] J. Gudeman, M. Jozwiakowski, J. Chollet, M. Randell, *Drugs R&D* 13 (2013) 1.
- [6] E.H. Duarte, W.P. dos Santos, F.F. Hudari, J.L. Bott Neto, E.R. Sartori, L.H. Dall'Antonia, A.C. Pereira, C.R. Tarley, *Talanta* 127 (2014) 26.
- [7] G.G. Mohamed, F.A. Nour El-Dien, N.A. Mohamed, *Spectrochim. Acta A: Mol. Biomol. Spectrosc.* 65 (2006) 1221.
- [8] J. Karpinska, B. Starczewska, *J. Pharm. Biomed. Anal.* 29 (2002) 519.
- [9] A. Petruczynik, M. Brończyk, T. Tuzimski, M. Waksmundzka-Hajnos, *J. Liq. Chromatogr. Relat. Technol.* 31 (2008) 1913.
- [10] B. Ramadevi, V.P. Kumar, S. Karishma, P. Divya, B. Sivagami, M.N. Babu, *Int. J. Res. Pharm. Sci. Technol.* 1 (2018) 12.
- [11] A.B. Melent'ev, S.S. Kataev, E.P. Ivanova, *Sud. Med. Ekspert.* 50 (2007) 31.
- [12] G. Vaghar-Lahijani, M. Saber-Tehrani, P. Aberoomand-Azar, M. Soleimani, *J. Anal. Chem.* 73 (2018) 145.
- [13] H. Beitollahi, F.G. Nejad, S. Tajik, S. Jahani, P. Biparva, *Int. J. Nano Dimens.* 8 (2017) 197.
- [14] Z.R. Zad, S.S.H. Davarani, A.R. Taheri, Y. Bide, *Biosens. Bioelectron.* 86 (2016) 616.
- [15] M. Guzinski, E. Lindner, B. Pendley, E. Chaum, *Talanta* 239 (2022) 123072.
- [16] J.P. Marco, K.B. Borges, C.R.T. Tarley, E.S. Ribeiro, A.C. Pereira, *J. Electroanal. Chem.* 704 (2013) 159.
- [17] M.S. Ali, A.I. Khaleel, *J. Phys. Conf. Ser.* 1879 (2021) 022071.
- [18] C.Y. Wang, X.Y. Hu, Z.Z. Leng, G.D. Jin, *Electroanalysis* 15 (2003) 709.
- [19] N. Rahman, S. Khan, *J. Electroanal. Chem.* 777 (2016) 92.
- [20] M. Khasanah, A.A. Widati, S.A. Fitri, *AIP Conf. Proc.* 1718 (2016) 070003.
- [21] M. Khasanah, M. Harsini, A. A. Widati, P.M. Ibrani, *J. Chem. Technol. Metall.* 52 (2017) 1039.
- [22] M. Khasanah, U.S. Handajani, A.A. Widati, Abdulloh, R.R. Rindarti, *Anal. Bioanal. Chem.* 10 (2018) 429.
- [23] A. Athiroh, T. Fadillah, D.F. Damayanti, A.A. Widati, A. Abdulloh, M. Khasanah, *IOP Conf. Ser.: Earth Environ. Sci.* 217 (2019) 012003.
- [24] M. Khasanah, A.A. Widati, U.S. Handajani, M.R. Shofiyah, S.A. Rakhma, *AIP Conf. Proc.* 2237 (2020) 020011.
- [25] S.K. Masoudian, S. Sadighi, A. Abbasi, *Bull. Chem. React. Eng. Catal.* 8 (2013) 54.
- [26] K. Tohda, D. Drago, M. Shibata, Y. Umezawa, *Anal. Sci.* 17 (2001) 733.
- [27] M.M.J. Treacy, J.B. Higgins, *Collection of Simulated XRD Powder Patterns for Zeolites*, fifth Rev. ed., Structure Commission of the International Zeolite Association, Amsterdam, 2001.
- [28] A.S. Yazdi, N. Razavi, S.R. Yazdinejad, *Talanta* 75 (2008) 1293.
- [29] J. Wang, *Analytical Electrochemistry*, third ed., John Wiley & Sons, Inc., New York, 2006.
- [30] R.S. Farag, M.Z. Darwish, W.M. Fathy, H.A. Hammad,

- Int. J. Chem. Anal. Sci. 4 (2013) 120.
- [31] S. Khanahmadzadeh, A. Tarigh, J. Chromatogr. B
Analyt. Technol. Biomed. Life Sci. 972 (2014) 6.
- [32] M. Madej, K. Fendrych, R. Porada, M. Flacha, J.
Kochana, B. Baś, Microchem. J. 160 (2021) 1.
- [33] F. Farjami, F. Fasihi, M. Valibeigi, S.E. Moradi, J.
Anal. Chem. 75 (2020) 941.
- [34] I. Taverniers, M. De Loose, E. Van Bockstaele, TrAC
Trends Anal. Chem. 23 (2004) 535.
- [35] E. Eslami, F. Farjami, P. Aberoomand Azar, M. Saber
Tehrani, Electroanalysis 26 (2014) 424