# Carbon Paste Electrode-Modified Imprinted Zeolite X and Its Performance as a Potentiometric and Voltammetric Sensor for Cholesterol Analysis

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***Abstract:*** *Carbon paste electrode-modified imprinted zeolite X has been developed as a potentiometry and voltammetry sensor to monitor cholesterol levels in the body. This is crucial to detect early health risks caused by high cholesterol levels. The modified electrode was fabricated with a mass ratio of activated carbon, paraffin, and imprinted zeolite X of 12:7:1. Potentiometric measurement produced a linear dynamic range of $10^{-6}$ – $10^{-3}$ M, Nernst factor of 27.12 mV/decade, a detection limit of $1.12 \times 10^{-6}$ M, precision of 99.7% (n = 3), and accuracy of 99.8% (n = 5). Using the electrode for up to 56 measurements over 6 weeks did not significantly decrease its performance. The presence of glucose did not interfere with cholesterol analysis by potentiometry. The modified electrode was applied to analyze cholesterol voltammetrically at the optimum deposition potential of 0.4 V, deposition time of 60 s, and a scan rate of 100 mV/s. Voltammetric analysis of cholesterol resulted in a detection limit of 7.2 mg/L (1.86 μM), precision of 96–99%, accuracy of 74–114%, sensitivity of 7.1 nA.L/mg/cm$^2$, and recovery of 87.2% (n = 3). The glucose and urea in various concentrations cause less than 5% current deviations.*

***Keywords:*** *cholesterol detection; electrometry; imprinted zeolite X; carbon paste electrode; health risk*

## ■ INTRODUCTION

Cholesterol is a structural component of biological membranes, nerves and brain cells. Cholesterol is important in producing sex hormones and vitamin D, carrying out nerve and brain functions, and functioning as a cell membrane. Increased cholesterol levels in the blood are due to excess production by the liver and the consumption of animal fat diets. Accumulation of cholesterol in the body can increase the risk of atherosclerosis, diabetes mellitus, thyroid disorders, pancreatitis, coronary heart disease, liver disease, and kidney disease [1]. One way to avoid these diseases is to check cholesterol levels regularly.

Some methods for analyzing cholesterol levels have been developed in many previous studies, namely enzymatic methods using ESI-MS [2], HPLC [3], and electrometry. Analysis using the HPLC method is sensitive but requires expensive instruments and complicated sample preparation; this is inefficient when applied in real conditions. The ESI-MS enzymatic method is less selective because cholesterol oxidase can react with other sterols [2]. Ascorbic acid and bilirubin in blood samples can react with resulting hydrogen peroxide, which will cause several errors in quantification. Currently, the measurement of cholesterol levels using the enzymatic method converts cholesterol into colored compounds that are measured by spectrophotometry. Another method of measuring high-density lipoprotein (HDL) is the direct HDL assay, which is determined by precipitation methods using various reagents [4]. Both methods require specific reagents and are highly dependent on the quality and stability of these reagents. High levels of triglyceride, bilirubin, and hemoglobin can disrupt the analysis. These methods are also less sensitive to low cholesterol levels. A reliable, economical, sensitive method is needed to measure cholesterol without affecting

the interfering matrix in the blood.

The electroanalytical methods developed to analyze organic compounds are potentiometry and voltammetry. Analysis using the potentiometric method involves a simple analysis process, a robust and effective technique to determine the concentration of ions [5]. The potentiometric method also has high accuracy, low detection limit (LOD), good selectivity, and affordable costs, so many researchers have developed this method to analyze components in solution. Analysis using the voltammetric method is simple, does not require complicated sample preparation, and is able to measure the low levels of components with high sensitivity. The working electrode is an important component in electrochemical analysis, especially in the composition of the material that makes it up, because it will affect the performance of the electrode [6]. Carbon paste electrodes can be modified to increase their performance. The advantage of the carbon paste is the possibility of surface and bulk modification by depositing a layer on the electrode surface and adding a material directly into the carbon paste, respectively [7].

Materials that are widely used to modify electrochemical sensors are molecularly imprinted polymer (MIP) [8-10] and imprinted zeolite (IZ) from various types of synthetic zeolite. The IZ-type TS-1 modified carbon paste electrode has previously been developed for the potentiometric analysis of uric acid [11]. The imprinted zeolite LTA-modified carbon paste electrodes have also been applied as sensors for creatinine [12] and glucose [13] in the human serum sample. The sensors showed a low LOD and high selectivity. In this research, an imprinted zeolite X-based sensor was developed for voltammetric and potentiometric analysis of cholesterol.

Zeolite X belongs to the faujasite zeolite group, known for its highly ordered crystal structure. Its structure has large silica and alumina spaces, allowing ion adsorption and exchange. It shows higher cation exchange selectivity; therefore, zeolites are widely used as selective adsorbents [14]. Zeolite X is resistant to thermal stability and can be used in acidic or basic conditions. Zeolite X has a large surface area, cavities in wide channels, and cages with large openings. Zeolite X has good selective adsorption, ion exchange, and hydrophilic properties and has been widely studied and applied in the chemical industry [15]. The printing technique is a specific recognition method using template molecules so that its pores are more selective. IZ X has been applied as a carbon paste electrode modifier in amitriptyline analysis, resulting in a low LOD and high selectivity under the presence of glucose, lactose, mannitol, and $ZnSO_4$ as interferences [16]. In this study, conductive carbon paste electrodes are modified with IZ X, and cholesterol as a template was printed into zeolite X. The imprinted cholesterol molecule on the electrode material causes an increase in the detection performance of cholesterol on the electrode surface. The sensor will only interact with cholesterol, even though many other ions exist in the complex solution. The IZ X-modified sensor is expected to show good performance on cholesterol in blood samples, as shown by a previous study.

In this research, modification of carbon paste electrodes was carried out by mixing IZ X, activated carbon powder, and paraffin pastilles and heating the mixture to form a paste. Several measurement parameters were optimized to obtain optimum results. The developed electrode's performance and the analytical method's validity have been studied to ensure its applicability for measuring cholesterol in human serum samples.

## ■ EXPERIMENTAL SECTION

### Materials

The chemicals used in this study were $C_{27}H_{46}O$ (99%, Merck, Rahay, NJ, USA), $C_6H_{12}O_6$ (99.5%, Sigma Aldrich, St. Louis, MO, USA), $CH_4N_2O$ (99%, Merck, Rahay, NJ, USA), $CH_3COOH$ (100%, Merck, Rahay, NJ, USA), $CH_3COONa{\cdot}3H_2O$ (99.5%, Merck, Rahay, NJ, USA), $NaH_2PO_4{\cdot}2H_2O$ (98%, Merck, Rahay, NJ, USA), $Na_2HPO_4{\cdot}2H_2O$ (99%, Merck, Rahay, NJ, USA), TEOS (98%, Merck, Rahay, NJ, USA), $NaAlO_2$ (50%, Sigma Aldrich, St. Louis, MO, USA), NaOH (99%, Merck, Rahay, NJ, USA), silver wire (Ag, 99.9%, Sigma Aldrich), $C_6H_{14}$ (Merck, Merck, Rahay, NJ, USA), paraffin granule (Merck, Merck, Rahay, NJ, USA), and activated carbon (Sigma Aldrich). The solvents used are ethanol and distilled water.

## Instrumentation

The instruments used in this study were a set of Cyberscan 510 potentiometers, a digital potentiostat (eDAQ ER 461 Echem), Ag/AgCl as reference electrode, and platinum wire as auxiliary electrode, X-ray diffraction (XRD, Shimadzu, Kyoto, Japan), Fourier transform infrared spectrophotometer (FTIR, Shimadzu Kyoto Japan in the range of 4000–400 $cm^{-1}$), pH-meter (Cyberscan Eutech Instrument pH 510, Frankfurt, Germany), vacuum Oven (Napco 5851, Fischer Scientific, NY, USA), centrifuge (Hettich EBA 20, Westphalia, Germany), furnace (Nabertherm), hotplate (Thermolyne S46410-26), magnetic stirrer, agate mortar, 1000 µL micropipette tip, polypropylene bottle, spatula, and a set of glassware commonly used in chemistry laboratories.

## Procedure

### *Synthesis of the zeolite X, non-IZ X, and IZ X*

The zeolite X was synthesized by mixing $NaAlO_2$, NaOH, TEOS, and distilled water at a molar ratio of $Na_2O$, $Al_2O_3$, $SiO_2$, and $H_2O$ of 4.5:1.0:3.0:315.0 [17]. The TEOS was added drop by drop with stirring for 1 h. The homogeneous mixture was left at room temperature for 1 h, transferred to a stainless autoclave, and heated hydrothermally at 100 °C in the oven for 24 h. The one-third portion of the mixture was washed with distilled water using centrifugation and dried in an oven at 80 °C, resulting in zeolite X powder. The two-thirds other portions of the mixture were added cholesterol, previously dissolved in ethanol, with a cholesterol/Si ratio of 0.0306. Stirring was carried out for 30 min while adding the cholesterol solution. The mixture was kept for 3 h at room temperature. Half a portion of the mixture was centrifuged, and then the precipitate non-imprinted zeolite (NIZ) was dried using an oven.

IZ was obtained by extracting cholesterol from the zeolite framework using acetone with the help of centrifugation. The completion of the extraction is determined by a qualitative test using $FeCl_3$ solution to the filtrate. The undetectable reddish-brown color in the tested filtrate indicates the absence of cholesterol. The precipitates were dried in an oven at a temperature of 80 °C. Characterization was done using XRD and FTIR.

### *Electrochemical sensor fabrication*

The preparation of carbon paste electrode modified with IZ X (CPE-IZ X) is carried out by mixing activated carbon, paraffin, and IZ-X in a ratio of 12:7:1 with a total mass of 0.3 g. The mixture is heated to 60 °C to form a paste. The CPE-IZ X for potentiometric and voltammetric measurements was fabricated by inserting a silver wire into the tip micropipette, then filling ¼ with carbon paste IZ-X and ¾ with paraffin. The tip of the micropipette is cut to obtain a specific surface area. The construction of CPE-IZ X for potentiometric and voltammetric measurements is shown in Fig. 1.

The difference between CPE-IZ X for potentiometric and voltammetric measurements is in the electrode construction. For potentiometric measurements, CPE-IZ X is positioned at the tip end with a larger surface area, as shown in Fig. 1(a). Meanwhile, the CPE-IZ X is positioned at the tip end with a smaller surface area for voltammetric measurements, as shown in Fig. 1(b).

### *Potentiometric measurements*

The pH optimization for potentiometric measurements was carried out in the linear concentration

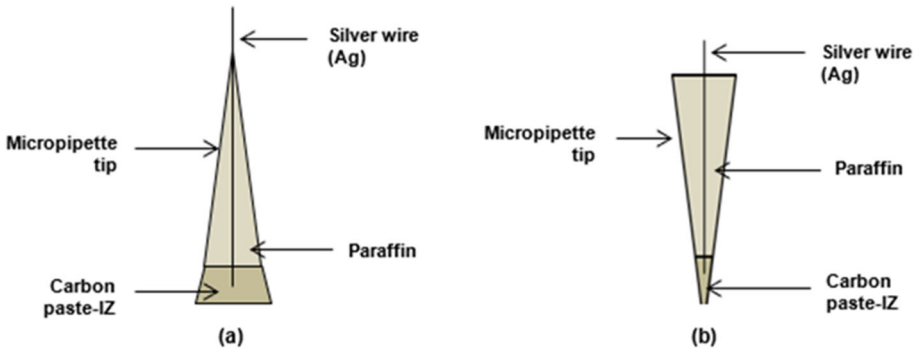


**Fig 1.** Construction of the electrode for (a) potentiometric and (b) voltammetric sensor

range $10^{-6}$ – $10^{-3}$ M with pH 4, 5, 6, 7, and 8. The electrode potential data is used to create a curve relating the electrode potential (mV) to the log[cholesterol] by obtaining a linear regression equation. The optimum pH can be seen from the results of measurements, which show that the Nernst factor value is close to the theoretical value, $\left[\frac{59.2}{n} \pm 2\right]$ mV/decade.

### *Voltammetric measurements*

In the voltammetric analysis, deposition potential, deposition time, scan rate, and pH solution are carried out using a 50 mg/L cholesterol solution. The results of the optimum measurement conditions are used to measure standard cholesterol solutions at concentrations of 10–50 mg/L, and a curve was created between cholesterol concentration and peak current.

### *Electrode performance and method validation*

In this research, the parameters used to determine electrode performance and method validity include linearity of the standard curve, LOD, precision, accuracy, selectivity, and sensitivity. The potentiometric analysis also studies linear dynamic range, Nernst factor, response time, and electrode lifetime. The response time and lifetime of the electrode are studied using a series of solutions that provide a linear relationship between the log concentration of cholesterol and the electrode potential.

The linearity is expressed by the standard curve's correlation coefficient (R). In contrast, the precision of the method is expressed by relative standard deviation (RSD), calculated in Eq. (1) and (2).

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

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

That SD is the standard deviation, xi is the signal response of every i-th measurement, $\bar{x}$ is the average value of the measurement signal and n is the number of repetitions of measurements. The accuracy value is calculated with Eq. (3);

$$Accuracy = \frac{Csp}{Ks} \times 100\% \quad (3)$$

where Csp is the found concentration and Ks is the actual concentration of the analyte.

The LOD in the potentiometric analysis was determined by intersecting the linear and non-linear curve relationship of the log[cholesterol] with the electrode potential and substituting the potential intersection of the two curves to the linear regression equation to obtain the log[cholesterol] and [cholesterol]. While in voltammetric analysis, the LOD was calculated using the data of linear regression of the calibration curve with Eq. (4) and (5) [18];

$$S_{y/x} = \sqrt{\frac{\sum\left(yi - \dot{y}i\right)^2}{n-2}} \quad (4)$$

$$Y_{LOD} = Yb + 3S_{y/x} \quad (5)$$

where $S_{y/x}$ is the standard deviation of the regression line and Yb is the blank response (intercept of the standard curve). The $Y_{LOD}$ value is substituted into the linear regression equation to obtain LOD.

The selectivity of the electrode in potentiometric analysis was studied by measuring the potential of a standard solution and an interfering solution. Electrode selectivity is determined through the selectivity coefficient value ($K_{ij}$) using the matched potential method (MPM) based on Eq. (6) [19];

$$K_{ij\ pot} = \frac{\Delta ai}{aj} \quad (6)$$

with the Δai as the main ion activity (cholesterol) and the aj as the concentration of the interfering compound. The sensor selectivity test was also carried out using voltammetry, which was studied by observing the measured current deviation between the peak current of the cholesterol solution and the cholesterol solution containing the interfering compound.

### *Pre-treatment of blood serum samples*

The sample pre-treatment was conducted by pipetting 500 μL of blood serum into a microcentrifuge tube and then adding 10–20% TCA solution. The mixture was homogenized with a vortex mixer and then incubated at 4 °C for 10–30 min to ensure the protein was completely precipitated. The mixture was centrifuged for 15 min; then, the supernatant was separated into another tube and diluted with double distilled water. This procedure was carried out by a Health Laboratory.

## ■ RESULTS AND DISCUSSION

### Synthesis and Characterization of Zeolite X

Zeolite X was synthesized from basic materials $NaAlO_2$, NaOH, TEOS, and $H_2O$. Synthesis was carried out in the polypropylene bottle to maintain the mole ratio of Si/Al in zeolite X. The XRD pattern in Fig. 2(a) explains that the 2θ position of synthetic zeolite X was observed at 6.52°; 10.41°; 12.72°; 16.35°; 18.22°, 20.07°; 27.35°; and 37.35°. There are similar peaks with standard zeolite X at 6.12°; 10.00°; 12.25°; 15.43°; 21.00°; 24.64°, 27.37°; 30.30°; and 34.17°, as shown in Fig. 2 [20]. The formation of zeolite X is characterized by the peak with the highest intensity (main peak) at 18.22°, the most dominant phase. FTIR characterized the zeolite X, NIZ-X, IZ-X, and cholesterol, which were characterized by FTIR to provide information on the functional group characteristics of each material. The FTIR spectra of zeolite X, NIZ X, IZ X, and cholesterol are shown in Fig. 3, while the wave number data is displayed in Table 1.

The absorption bands at 455, 584, 692, and 1003 $cm^{-1}$ indicate the typical zeolite wavenumbers. The spectra of NIZ and IZ show absorption in the wave numbers of ~453, ~583, ~687, and ~1000 $cm^{-1}$, which are characteristic of the absorption of the zeolite X. NIZ-X and IZ-X also show absorption in the wave number ~1450 $cm^{-1}$ which is characteristic of cholesterol compounds with C–H cyclohexane bending vibrations and wavenumbers ~2940 $cm^{-1}$ which are C–H stretching vibrations of $CH_3$. This proves the cholesterol molecule has been printed as a template on IZ-X. The appearance of the low peak at wavenumbers 1456 and 2941 $cm^{-1}$ in the IZ spectra illustrates that the cholesterol molecule has not been completely extracted from the zeolite framework.

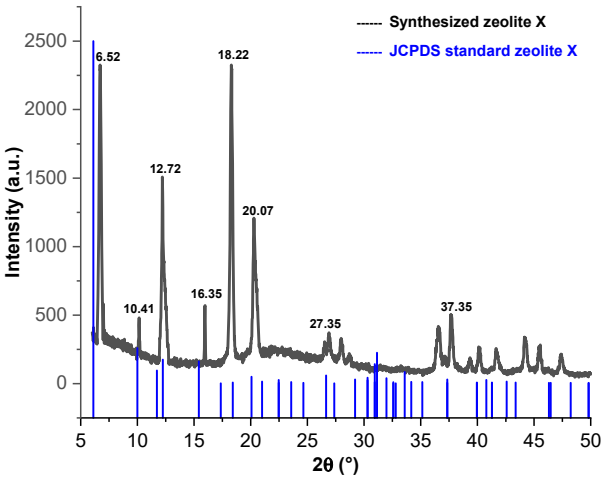


**Fig 2.** XRD pattern of synthesized zeolite X and standard zeolite X [16]

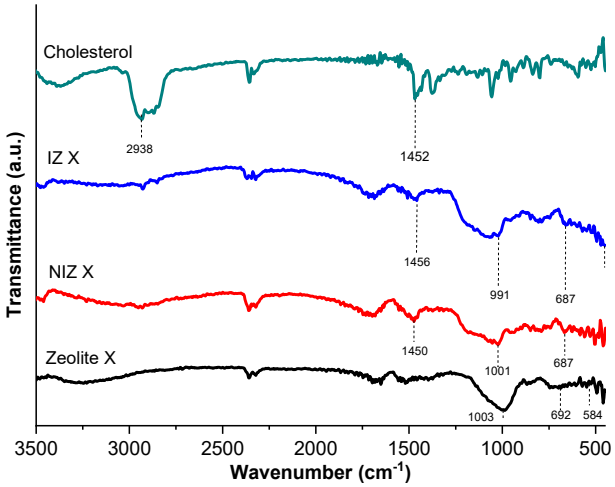


**Fig 3.** FTIR spectra of zeolite X, NIZ, IZ, and cholesterol

**Table 1.** Wavenumber data of zeolite X, NIZ, IZ, and cholesterol

| Wavenumber ($cm^{-1}$) | | | | Functional groups |
|---|---|---|---|---|
| Zeolite X | NIZ X | IZ X | Cholesterol | |
| 455 | 453 | 457 | - | Si–O/Al–O bending vibration |
| 584 | 583 | 602 | - | double ring vibration of zeolite framework |
| 692 | 687 | 687 | - | Si–O–Si or Al–O–Al symmetric stretching vibration |
| 1003 | 1001 | 991 | - | Si–O-Si or Al–O–Al asymmetric stretching vibration |
| - | 1450 | 1456 | 1452 | C–H bending vibration of cyclohexane |
| - | 2936 | 2941 | 2938 | C–H stretching vibration of $CH_3$ |

### The Electrode Performance

The mass ratio of activated carbon, paraffin, and IZ-X of 12:7:1 is used as a ratio to make CPE-Z and CPE-NIZ. The purpose of making CPE-Z and CPE-NIZ is to determine the effect of cholesterol molding on CPE-IZ electrode performance. The three electrodes are used to measure cholesterol solutions $10^{-8} - 10^{-3}$ M by potentiometry. The electrode potential data obtained from the measurements are used to create a relationship curve between log[cholesterol] and electrode potential (mV) so that a comparative data of CPE-IZ, CPE-NIZ, CPE-Z performance which includes linear concentration range and Nernst factor (slope) value are obtained which are shown in Table 2 and Fig. 4. The CPE-IZ provides performance the most optimal in measuring cholesterol with a linearity value that is closest to the theoretical, namely 0.9993, and the Nernst factor value is close to the theoretical value for divalent ions, namely 30.9 mV/decade [21].

The CPE-Z and CPE-NIZ show the non-Nernstian curve because the two electrodes do not contain imprinting material (do not contain active sites), so the electrodes do not recognize the cholesterol well. Apart from that, in the CPE-NIZ material, the pores and bond sites in the zeolite framework are still occupied by cholesterol molecules, which means no bond sites are available for interaction with cholesterol molecules.

### Optimization of Solution pH in Potentiometric Measurement

The pH optimization was carried out by measuring the potential of the cholesterol solution in the linear concentration range $10^{-6} - 10^{-3}$ M with pH variations of 4, 5, 6, 7, and 8 using CPE-IZ X. Data from the pH optimization results of the cholesterol solution are shown in Table 3. Table 3 shows that the cholesterol solution measurement at pH 8.17 (without pH adjustment) using CPE-IZ showed better performance with a Nernst factor approaching the theoretical value of 27.12 mV/decade. Cholesterol molecules have a pKa value of 18.2 [22], so cholesterol measurements without pH adjustment are in their molecular form. Thus, the binding sites available on IZ are suitable for cholesterol molecules. On the other hand, measurements at pH 8 with pH adjustment using phosphate buffer show a Nernst factor value that is much lower than the theoretical value. This is likely because the addition of phosphate buffer can cause changes in the structure of cholesterol in solution so that the cholesterol exchange process at the membrane and solution interface is not perfect, resulting in a non-Nernstian response.

### Potentiometric Measurements

The cholesterol standard curve was obtained by measuring the potential of cholesterol solution in the concentration range $10^{-8} - 10^{-3}$ M without pH adjustment using CPE-IZ X, potential data is shown in Table 4, and the cholesterol standard curve is shown in Fig. 5. Based on the standard curve of the cholesterol solution, the regression equation obtained is y = 27.12x + 428.09 with

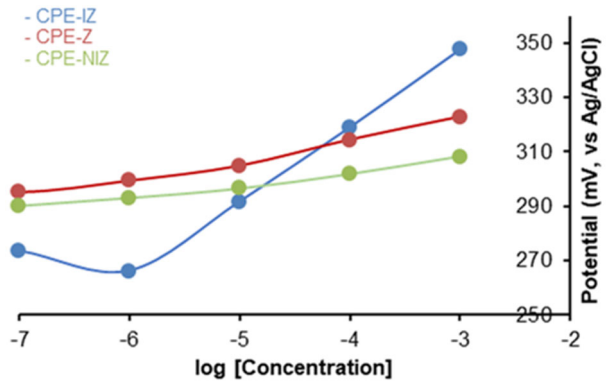


**Fig 4.** The curve of the relationship between log[concentration] and electrode potential

**Table 2.** Comparative data of CPE-IZ, CPE-NIZ, CPE-Z performance

| Electrode | Nernst factor (mV/decade) | Concentration range (M) | Linearity (R) |
|---|---|---|---|
| CPE-IZ | 27.12 | $10^{-6}$–$10^{-3}$ | 0.9993 |
| CPE-NIZ | 8.01 | $10^{-6}$–$10^{-3}$ | 0.9892 |
| CPE-Z | 5.06 | $10^{-6}$–$10^{-3}$ | 0.9825 |

**Table 3.** The Nernst factor resulting from the varies pH

| pH | Nernst factor (mV/decade) |
|---|---|
| 4 | 19.01 |
| 5 | 25.34 |
| 6 | 22.68 |
| 7 | 17.45 |
| 8 | 14.11 |
| Without pH | 27.12 |

**Table 4.** Data resulting of the electrode potential from potentiometry without pH adjustment

| Concentration of cholesterol (M) | Electrode potential (mV, vs Ag/AgCl) |
|---|---|
| $10^{-8}$ | 278.9 |
| $10^{-7}$ | 273.5 |
| $10^{-6}$ | 266.2 |
| $10^{-5}$ | 291.6 |
| $10^{-4}$ | 318.9 |
| $10^{-3}$ | 347.5 |

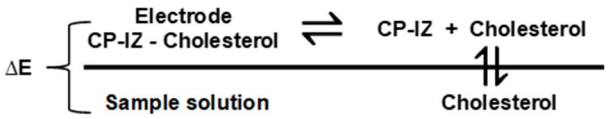


**Fig 5.** Mechanism of interaction between the CPE-IZ X with cholesterol

a correlation coefficient ($R^2$) of 0.9993, and the dynamic linear range is $10^{-6}$ – $10^{-3}$ M.

The electrode responds to changes in the concentration of cholesterol ions that undergo electrochemical reactions on the electrode surface. The mechanism of interaction between the electrode and the analyte in the detection process is shown in Fig. 5. When CPE-IZ X is dipped in a cholesterol solution, the presence of cholesterol ions in the solution will disrupt the balance on the electrode surface, resulting in a potential difference at the interface between the electrode and the sample solution.

### *Nernst factor and linearity*

Cholesterol is a divalent compound because cholesterol can undergo oxidation by releasing two electrons [21]. Based on the standard curve in Fig. 6, a Nernst factor of 27.12 mV/decade is obtained with a R value of 0.9996. The electrode developed in this research has a Nernst factor value closer to the theoretical value.

### *Detection limit*

Carbon paste electrode-IZ can detect cholesterol to a concentration of $1.12 \times 10^{-6}$ M. Therefore, the IZ-X carbon paste electrode can be applied for cholesterol analysis at normal concentrations in blood ($< 5.18 \times 10^{-3}$ M) and cholesterol concentrations in hypercholesterolemic patients ($> 6.18 \times 10^{-3}$ M) through a sample dilution process.

### *Precision*

Each cholesterol standard solution concentration range $10^{-6}$ – $10^{-3}$ M was measured 3 times repetition. The Association of Official Analytical Chemists (AOAC) provides a maximum RSD limit for the concentration of 3.7–11% [23]. The RSD and precision data of the potentiometric method to determine cholesterol using the CPE-IZ X are shown in Table 5.

### *Accuracy*

Accuracy was determined in the cholesterol concentration range of $10^{-6}$ – $10^{-3}$ M. According to the AOAC, the accuracy value of an analytical method is said to be good if it is in the range of 80–110% for a concentration of $10^{-6}$ – $10^{-3}$ M [23]. The accuracy values of the potentiometric method in this study were 99.7–99.8%.

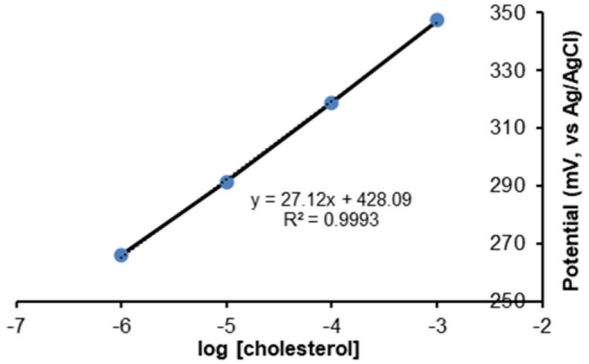


**Fig 6.** Standard curve of cholesterol solution

**Table 5.** Precision values of potentiometric measurements of cholesterol using CPE-IZ X

| log[cholesterol] | Electrode potential (mV) | | | % RSD | | Precision (%) |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | This study | AOAC | |
| −6 | 265.9 | 266.1 | 266.5 | 0.11 | 11 | 99.89 |
| −5 | 291.0 | 291.5 | 292.2 | 0.21 | 7.3 | 99.79 |
| −4 | 317.6 | 318.7 | 320.2 | 0.41 | 5.3 | 99.59 |
| −3 | 346.4 | 347.6 | 348.7 | 0.33 | 3.7 | 99.67 |

It can be concluded that the potentiometric method using ECP-IZ X has good accuracy and is acceptable as a chemical analysis method. The accuracy was better than the previous study using various electrodes [24-26].

### *Response time*

The greater the concentration, the faster the ability of cholesterol compounds to reach equilibrium on the electrode surface. In the CPE-IZ X, equilibrium will occur between the CPE-IZ-cholesterol and the cholesterol molecules in the solution. This equilibrium occurs at the solution interface with the membrane surface on the electrode. The cholesterol in the analyte solution will disturb the cholesterol-IZ equilibrium, so exchange will occur until equilibrium is reached. This equilibrium will cause a potential change that can be responded to by the potentiometer. Response time data of the CPE-IZ X to various cholesterol concentrations is shown in Table 6.

### *Electrode lifetime*

The electrode lifetime refers to the duration over which it maintains its functionality and performance within acceptable parameters for its intended application. The Nernstian calibration curve's slope expressed the modified electrode's lifetime (Nernst factor value). After being used more than 56 times over 6 weeks, the modified electrode decreased in performance, characterized by a drastic decrease in the Nernst factor Fig. 7. Electrode lifetime is affected by the mechanical properties of electrode material, such as the solubility of the material, the flexibility of the material, and the pH of the solution. Continuous electrode usage can cause material loss from the electrode surface, change in surface morphology, and decrease electrode performance. An electrode often used will form holes on the electrode surface because several electrode materials are dissolved, thereby reducing electrode performance. The material's flexibility causes the analyte to quickly enter and exit the site active of the electrode, making the measurement results unstable. In addition, continuously used electrodes will be saturated on the electrode surface so that they can not bind analyte molecules in solution and affect the electrode's potential. This phenomenon causes a decrease in the Nernst factor.

### *Selectivity*

The selectivity test was studied using a glucose solution, which is thought to interfere with cholesterol analysis because it has the same functional group as cholesterol, namely hydroxyl. Therefore, glucose and cholesterol compete for hydrogen bonds with the imprinted zeolite X. Based on Table 7, the CPE-IZ X produces a $K_{ij}$ value that is smaller than the $K_{ij}$ value on value on the CPE, indicating that the CPE-IZ has better selectivity for cholesterol. Adding IZ to the carbon paste electrode causes the specific active site of the electrode only to recognize cholesterol molecules, so the IZ X - modified electrode is more selective to cholesterol molecules.

**Table 6.** Response time of CPE-IZ X to cholesterol solution

| Concentration (M) | Response time (s) |
|---|---|
| $10^{-6}$ | 102 |
| $10^{-5}$ | 54 |
| $10^{-4}$ | 29 |
| $10^{-3}$ | 11 |

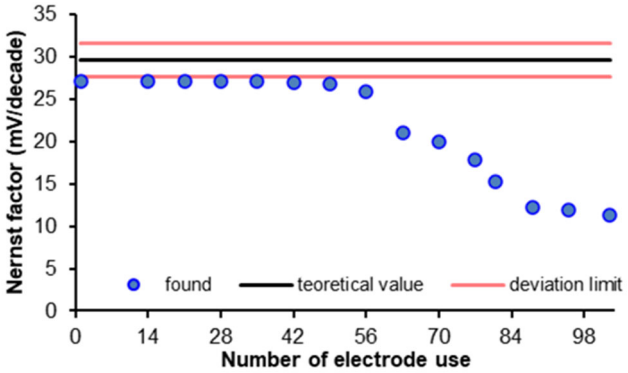


**Fig 7.** The influence of electrode use on the value of the Nernst factor

**Table 7.** Selectivity coefficient ($K_{ij}$) value of the electrode in the glucose solution

| Concentration (M) | $K_{ij}$ | |
|---|---|---|
| | CPE | CPE-IZ |
| $10^{-6}$ | $3.16 \times 10^{-2}$ | $1.99 \times 10^{-6}$ |
| $10^{-5}$ | $2.19 \times 10^{-3}$ | $1.35 \times 10^{-7}$ |
| $10^{-4}$ | $3.39 \times 10^{-4}$ | $1.07 \times 10^{-8}$ |
| $10^{-3}$ | $5.01 \times 10^{-5}$ | $1.32 \times 10^{-9}$ |

## Optimization of Voltammetric Measurements

### *Deposition potential*

Measurements of the cholesterol concentration are performed using differential pulse stripping voltammetry (DPSV) and cyclic voltammetry techniques. The amount of analyte that undergoes a redox reaction on the electrode surface is influenced by the deposition potential, deposition time, and scan rate. The voltammograms of 50 mg/L cholesterol at pH 5 with various deposition potentials were presented in Fig. 8. The largest current was obtained at a deposition potential of 0.40 V with no significant difference in current when varying the deposition potential. Besides the high peak current, the narrow peak base is another reason for selecting the optimum deposition potential. The voltammogram with a broad peak base may be caused by prolonged current reading.

The peak potential resulting from stripping shifts more to the left (0.21 V) than the deposition potential (0.40 V). Therefore, oxidation occurs during deposition, and reduction occurs during stripping. Thus, the cholesterol analysis process in this study belongs to cathodic stripping DPV. Cholesterol undergoes oxidation to form cholest-4-en-3-one and hydrogen peroxide [27]. This oxidation reaction occurs on the electrode surface when voltammetric analysis is carried out. The oxidation reaction of cholesterol is illustrated in Fig. 9.

### *Deposition time*

The optimization of deposition time is performed with the optimum deposition potential (0.4 V) and a scan rate of 100 mV/s. The analysis results with various deposition times are presented in Fig. 10. Deposition time represents the duration given during electrolysis. Theoretically, the longer the deposition time, the more analyte is deposited on the electrode surface, leading to an increase in peak current until the electrode surface becomes saturated at a specific deposition time [28].

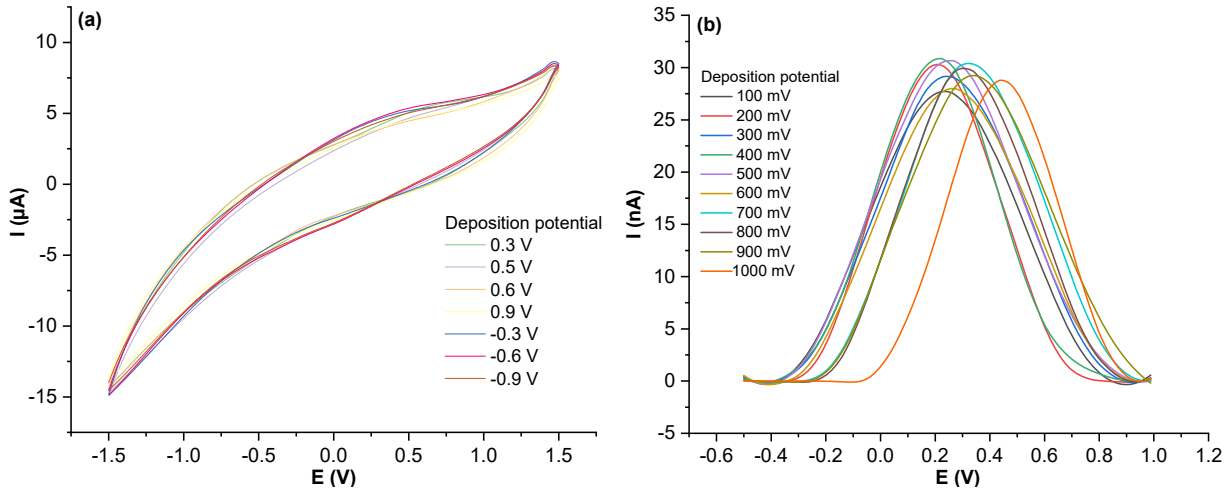


**Fig 8.** Cyclic (a) and differential pulse (b) voltammogram of cholesterol 50 mg/L at various deposition potentials

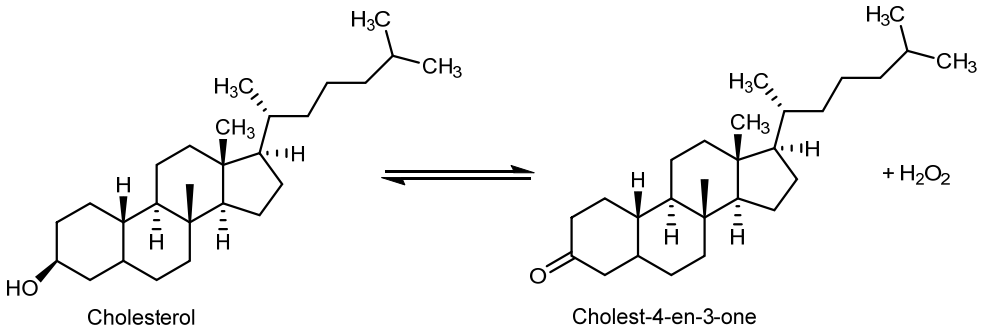


**Fig 9.** Cholesterol oxidation reaction [27]

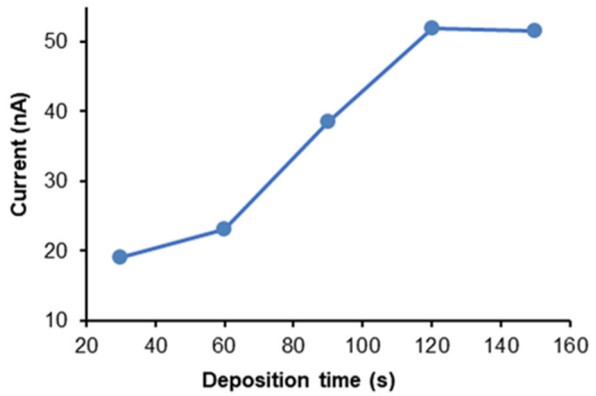


**Fig 10.** The curve of current response of cholesterol 50 mg/L at various deposition time

Based on Fig. 10, it can be observed that the peak current at 120 and 150 s does not show significant changes. This is because the electrode surface becomes saturated with the analyte. The optimum deposition time for further measurements is 60 s, considering the high current and time efficiency.

### *Scan rate*

The higher the scan rate, the faster the electrolysis reaction runs. This is caused by the thinner diffusion layer on the electrode surface, which causes electron transfer to occur more quickly [28]. Based on Table 8, the scan rate of 100 mV/s was selected for subsequent measurements. A linear correlation between root scan rate and peak current was not found in the plot. The analyte mass transfer process does not influence the peak current from the bulk solution to the electrode surface. The reaction that occurs on the electrode surface is an electron transfer reaction (electrochemical reaction). The results of peak current measurements at voltammetric scan rate variations are shown in the cyclic voltammogram on Fig. 11.

### *pH solution*

The optimization of pH for voltammetric measurements is performed with pH 4 to 8, using the optimum deposition, time deposition, and scan rate. The plots between pH and peak potential and current response are shown in Fig. 12. The Nernst equation, which expresses the relationship between the concentration of $H^+$ ions and the peak potential, is given in Eq. (7),

$$E = E^0 - \frac{0.059}{n} \times \log\left[H^+\right] \quad (7)$$

where n is the number of electrons. If the optimization of pH yields a slope value of 0.059 V/pH, then the electrode acts as a sensor for $H^+$ ions.

Based on Fig. 12, a slope of 0.031 V/pH is obtained, indicating that the measured analyte is cholesterol, with a valency of 2 for cholesterol molecules [27]. In addition, it shows that the higher the pH level of the solution, the shift in peak potential toward negative values. This is due to the decreasing accumulation of $H^+$ ions on the electrode surface, making it difficult to reduce them and resulting in a shift of the peak potential toward the negative direction [28]. In Fig. 12, it can be observed that the pH with the highest peak current is pH 5. A cholesterol solution without pH adjustment yields a peak current of 16.69 nA, whereas a cholesterol solution with pH adjustment using a buffer yields a peak current of 40.83 nA.

**Table 8.** Data of potential and peak current at various scan rate

| Scan rate (mV/s) | Peak potential (V) | Peak current (nA) |
|---|---|---|
| 25 | 0.20 | 27.91 |
| 50 | 0.18 | 30.51 |
| 75 | 0.33 | 30.35 |
| 100 | 0.24 | 32.28 |

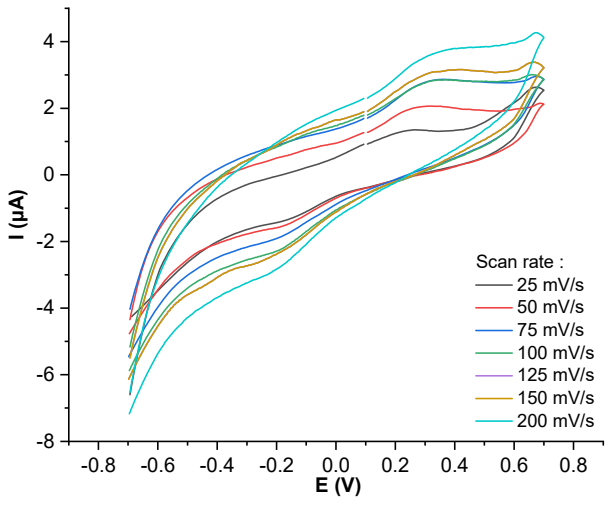


**Fig 11.** Voltammogram measurement of cholesterol solution scan rate

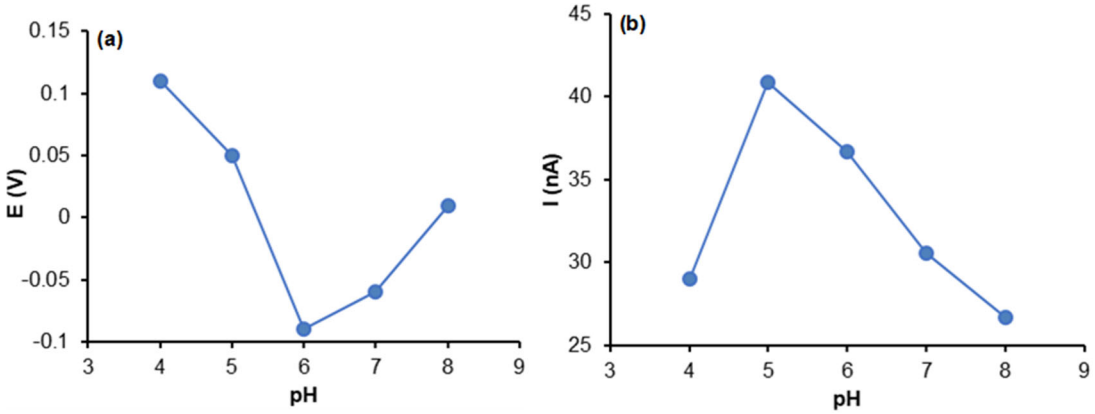


**Fig 12.** The plot of peak (a) potential and (b) current at various pH

## Voltammetric Measurements and Method Validity

A standard cholesterol solution of 10–50 mg/L was measured using the differential pulse voltammetry technique. The current data from these measurements is shown in Table 9, and the cholesterol standard curve is presented on Fig. 13.

Based on the standard graph of the cholesterol solution, the regression equation obtained is $y = 0.1776x + 20.185$. This study obtained an R-value close to 1, namely 0.9915. There is a linear relationship between the current response and the analyte concentration. Sensitivity in this study is expressed by the slope value of the standard curve of 0.1776 nA.L/mg.

### *Detection limit*

The LOD is defined as the smallest concentration of cholesterol that can be detected by the voltammetry method using CPE-IZ X. The LOD of the voltammetric method using developed CPE-IZ X for cholesterol analysis is 7.2 mg/L. This value is much lower than the normal concentration of cholesterol in the blood (1000 mg/L), and the previous study used AgNPs modified glassy carbon (AgNPs/GCE) [29] and zinc oxide nanorods based electrode [30].

### *Precision and accuracy*

Precision is carried out by measuring the cholesterol standard solution 3 times in repetition. In this study, the %RSD value obtained at a concentration of 10–50 mg/L was around 1.5–4.5%, while the AOAC sets an RSD limit of 5.3–7.3% [23]. In the cholesterol concentration of 30–50 mg/L, the accuracy of the voltammetric method using CPE-IZ X is 102.8–97.5%. At the low concentration (10–20 mg/L), were 74.5 and 114.4%, respectively. The values deviated from the limit AOAC set, namely 80–110%.

### *Selectivity*

The selectivity of the electrode was studied by analyzing the current of a 40 mg/L cholesterol standard solution and a 40 mg/L cholesterol standard solution containing glucose and urea carried out using CPE-IZ and CPE. The current deviation data in the selectivity test is shown in Table 10. The CPE-IZ is about 26 times

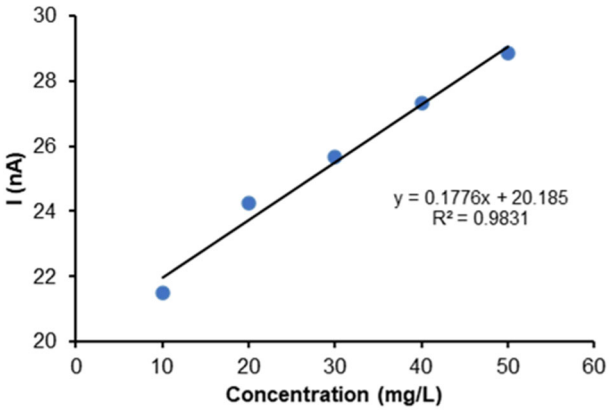


**Fig 13.** Cholesterol standard curve

**Table 9.** The current cholesterol standard solution

| Concentration (mg/L) | I (nA) | | | Average of current (nA) |
|---|---|---|---|---|
| | I | II | III | |
| 10 | 21.86 | 21.33 | 21.31 | 21.50 |
| 20 | 25.05 | 23.70 | 23.99 | 24.25 |
| 30 | 26.21 | 25.66 | 25.10 | 25.66 |
| 40 | 27.10 | 27.88 | 26.99 | 27.32 |
| 50 | 29.97 | 29.13 | 27.43 | 28.84 |

**Table 10.** Current deviations of cholesterol due to the addition of glucose and urea

| Concentration comparison (mg/L) | | | CPE IZ-X | | CPE | |
|---|---|---|---|---|---|---|
| CHOL | GLU | UREA | Current (nA) | % Current deviation | Current (nA) | % Current deviation |
| 1 | - | - | 173.48 | - | 190.16 | - |
| 1 | 0.50 | - | 177.26 | 2.18 | 282.80 | 48.72 |
| 1 | 1.00 | - | 178.55 | 2.92 | 352.01 | 85.11 |
| 1 | 2.00 | - | 221.13 | 27.47 | 372.38 | 95.82 |
| 1 | - | - | 173.48 | - | 299.06 | - |
| 1 | - | 0.25 | 179.13 | 3.26 | 373.07 | 87.86 |
| 1 | - | 1.00 | 166.09 | 4.26 | 342.53 | 112.49 |
| 1 | - | 1.00 | 241.35 | 39.12 | 299.06 | 114.52 |

more selective to cholesterol than CPE. Glucose and urea give significant current deviations in cholesterol analysis using CPE-IZ if their concentration is twice as high as the cholesterol concentration, namely at a ratio of 1:2. This occurs predictably because the interfering matrix has hydrogen atoms in the hydroxyl functional a, which can form a hydrogen bond with oxygen atoms in the zeolite. However, CPE-IZ can still be applied for cholesterol analysis in actual blood samples because in general, the concentration of cholesterol in blood serum is higher than urea [31].

## Application on Real Sample

A modified electrode was used to analyze cholesterol in blood serum samples to determine its usability and study the recovery method. Measurements were carried out using the standard addition technique. The recovery values resulting from the measurement of the three serum samples are shown in Table 11. The voltammetric method using CPE-IZ X to analyze cholesterol in serum samples shows a recovery of 87.2% (n = 3). This value is within the acceptance range established by the AOAC for a chemical analysis method for the concentration used, namely 80–110%. Thus, a voltammetric method using the developed sensor is recommended as an alternative to measure and monitor cholesterol levels in real samples.

## Comparison of Potentiometric and Voltammetric Methods

Data on the validity of potentiometric and voltammetric methods to analyze cholesterol using CPE-IZ X are entirely presented in Table 12. The developed electrode shows superior performance as a sensor for detecting cholesterol in the sample. The potentiometric analysis gives better results than voltammetry, as shown by accuracy, precision, and selectivity in Table 12. The

**Table 11.** Recovery in application of electrode to analyze cholesterol in blood serum sample

| Sample | Concentration (mg/L) | | Recovery (%) |
|---|---|---|---|
| | Spiked | Found | |
| Sample 1 | 1.79 | 1.56 | 86.90 |
| Sample 2 | 1.79 | 1.58 | 88.40 |
| Sample 3 | 1.79 | 1.54 | 86.20 |

**Table 12.** Validity of potentiometric and voltammetric methods to analyze cholesterol

| Parameter | Potentiometric | Voltammetric |
|---|---|---|
| Measurement range (M) | $10^{-6} – 10^{-3}$ | $2.58 \times 10^{-5} – 1.29 \times 10^{-4}$ |
| Linearity (R) | 0.9996 | 0.9915 |
| LOD (M) | $1.12 \times 10^{-6}$ | $1.86 \times 10^{-6}$ |
| Precision (%RSD) | 0.11 – 0.33 | 1.45 – 4.49 |
| Accuracy (%) | 99.7 – 99.8 | 74.0 – 114.4 |
| Selectivity | The presence of glucose does not interfere with the analysis | Good selectivity to the concentration of the interfering matrix ≤ cholesterol concentration |

**Table 13.** LOD and accuracy of potentiometric and voltammetric method using various electrode to measure cholesterol level

| Methods | Electrode | LOD (M) | Accuracy (%) | Ref. |
|---|---|---|---|---|
| Potentiometric | Wire membrane coated with phosphomolybdic acid | $5.00 \times 10^{-7}$ | 98.93 | [24] |
| | Wire membrane coated with phosphotungstic acid | $2.50 \times 10^{-7}$ | 99.14 | [24] |
| | Screen-printed nanoporous gold | $8.36 \times 10^{-6}$ | 92.67–106.67 | [25] |
| | Cobalt oxide ($Co_3O_4$) nanocrystal hydrolyzed poly polyethyleneimines | $1.00 \times 10^{-6}$ | 98.5–102.00 | [26] |
| | Carbon paste-IZ X | $1.12 \times 10^{-6}$ | 99.68–99.78 | This study |
| Voltammetric | Silver nanoparticle modified glassy carbon | $2.50 \times 10^{-5}$ | 99.6–100.80 | [29] |
| | Zink oxide nanorods | | 96.00 | [30] |
| | Nanocomposite-NiO-$MoS_2$ | $6.20 \times 10^{-6}$ | 94.30–95.70 | [32] |
| | Carbon paste-IZ X | $1.86 \times 10^{-6}$ | 74.04–114.44 | This study |

potentiometry method showed a wide linear dynamic range for cholesterol analysis in samples up to a 43.23 mg/dL concentration. The voltammetry method needs easier sample preparation and has a low LOD. The electrometric method using the developed electrode is recommended as an alternative method for determining cholesterol in the medical field. The LOD and accuracy of various analytical methods in previous studies for the analysis of cholesterol are shown in Table 13.

## ■ CONCLUSION

The CPE-IZ X acts as a superior sensor in cholesterol analysis using potentiometric and voltammetric methods. The CPE-IZ X showed a wide linear dynamic range and lower detection limit. The modified electrode was stable for 6 weeks and had more than 56 uses. The presence of glucose with various concentrations did not interfere with the analysis of cholesterol. This electrometric method using the developed sensor can later be used as an alternative method for detecting cholesterol in the blood.

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## ■ CONFLICT OF INTEREST

Authors declare that there is no conflict of interest related to the writing of this manuscript.

## ■ AUTHOR CONTRIBUTIONS

Miratul Khasanah is responsible for methodology, data validations, writing, and revisions. Alfa Akustia Widati is responsible for characterizing the material, writing, and revising it. Nadya Maya Severia and Citra Marantika Nur Oktaviana served as researchers and writers. Naftalia Wirdatul Ummah served for writing draft preparation and revisions. Evrillia Puspitasari and Ziana Alviani served with writing and revisions.

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