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# Validation Method of the Cellulose Triacetate-Based Optode Membrane for Fe(III) Detection in Water Samples

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## Abstract

Iron contamination in water is a threat to human health and the environment. This contamination requires a fast and efficient detection method. Method validation is integral to method development, ensuring alignment with the intended objectives. This research aims to validate a method for selective detection of Fe(III) based on cellulose triacetate (CTA) optode membrane. The optode membrane was prepared by mixing CTA, plasticizers, Aliquat-336, and thiocyanate as selective reagents. The validation of the optode membrane was evaluated based on validation performance parameters. The Fe(III) optode membrane detection demonstrated a linear response with a determination coefficient of 0.9972 within a concentration range of 0.1–4.0 mg/L, the detection limit of 0.0553 mg/L, quantitation limit of 0.1676 mg/L, precision 3.01%, intermediate precision of 3.03% and 3.01%, the accuracy of 101.62%. The optode membrane exhibited good selectivity with a value of -0.4580 and -0.2748 against Pb(II) and Cr(VI), respectively, sensitivity of 1.05 × $10^7$ $M^{-1}$ $cm^{-1}$ and color formation stability %RSD of 3.14%. The application of real samples shows no significant difference between the UV-Vis spectrophotometry and optode membrane methods at a 95% confidence level ($\alpha = 0.05$). The validation results offer a valuable perspective into whether this method can be adopted as a new approach or as an alternative to existing methods for cation analysis in water samples.

## 1. Introduction

Iron, a heavy metal, is used in many fields, both in the environment and human life. Iron is useful for the muscles and transport system of the human body. However, excessive intake will impact human health with hemochromatosis symptoms in the liver, cardiac problems, and pancreatic dysfunction [1]. Plants use iron for growing and developing critical physiological processes, such as photosynthesis, respiration, and nitrogen metabolism. Plants maintain a concentration of $10^{-4}$ to $10^{-9}$ M for optimal metabolism. Excessive iron uptake results in toxicity, causing damage to cell membranes, inhibiting growth, reducing crop yields, and compromising overall plant health [2]. Iron contamination in environments comes from various industrial, non-industrial, and anthropogenic activities. Excess iron contamination in environments endangers organisms, plants, and the quality of the environment [3]. Iron typically occurs in the Fe(0) and Fe(II) or Fe(III) cation forms. The Fe(II) cation is easily oxidized to Fe(III) in relatively neutral aquatic environments, making Fe(III) the dominant form present in such conditions. At an appropriate pH, Fe(III) reacts with thiocyanate to form a deep red complex, whereas Fe(II) produces a pale green to colorless solution when reacted with thiocyanate [4, 5]. According to the United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO), the maximum allowable concentration of iron in water intended for human use is 0.3 mg/L [6, 7].

Iron detection in samples can be carried out using various techniques, including UV-Vis spectrophotometry [8, 9], atomic absorption spectrometry [10, 11], electrochemical methods [12, 13], fluorescence spectroscopy [14, 15], ICP spectrometry [16, 17], and

X- ray fluorescence (XRF) [18, 19]. These methods offer acceptable levels of sensitivity, selectivity, and accuracy. However, their application is often limited by factors such as high instrumentation costs, specialized operational skill requirements, and the inability to conduct in situ measurements. To address these limitations, an optode membrane is proposed as a simpler and more practical approach for Fe(III) detection in environmental samples.

Optode membranes function by immobilizing color-forming compounds or ligands within a membrane, enabling the detection of specific metal ions through a color change [20]. These membranes offer several advantages, including the absence of the need for an electrical power supply, simplicity, the ability to perform in situ measurements, and low cost. Optode membranes are widely used for detecting various gases and metal ions, such as Hg, Pb, Cd, and Cu [21, 22, 23]. Cellulose triacetate (CTA) membranes are particularly suitable as polymer matrices for optode fabrication. CTA-based optode membranes are stable, relatively easy to produce, and exhibit durable properties. Furthermore, the CTA membrane binds analytes without absorbing water, ensuring that it does not interfere with either color formation or the stability of the produced color [24, 25].

This study used method validation of optode membranes for detecting Fe(III). The initial development of the optode membrane was carried out by Arif *et al.* [26]. Method validation is essential to ensure that the methods are appropriate for their intended purposes and provide consistent measurement performance [27]. Validation was conducted based on the procedures outlined in [28] and [29], assessing the method's accuracy, precision, sensitivity, and reproducibility. The objective of this analysis is to confirm the reliability of the optode membrane for Fe(III) detection across various environmental and industrial applications. The novelty of this study lies in the optimization and validation of the optode membrane method for Fe(III) detection, presenting a more robust, sensitive, and cost-effective alternative to existing techniques, while also demonstrating its applicability in real-world sample analysis.

## 2. Experimental

The research began with fabricating an optode, which was subsequently used in the method validation process. Performance parameters were derived from prior experiments. The validation was conducted in accordance with the guidelines set by the International Council for Harmonisation (ICH) [28], encompassing parameters such as linearity, detection limit, quantitation limit, precision, intermediate precision, accuracy, selectivity, color stability, sensitivity, and robustness. The acceptance criteria were then compared with the standard values provided by the Association of Official Analytical Chemists (AOAC) [29].

### 2.1. Materials and Equipment

The materials used in this study included cellulose triacetate (CTA), N-Methyl-N,N,N-trioctylammonium chloride (Aliquat-336), oleic acid, acetophenone, chloroform, methanol, $FeCl_3.6H_2O$, $Pb(NO_3)_2$, $H_3PO_4$, $H_2SO_4$, acetone, $K_2Cr_2O_7$, $NH_4SCN$, HCl, $HNO_3$ (4 M), KSCN (2 M), distilled water, and water samples. All chemicals were purchased from Merck (Darmstadt, Germany) and were of analytical grade quality.

The equipment used included glassware, pipettes, an analytical balance (OHAUS AX224/E), a magnetic stirrer, a sonicator (AS ONE), a micrometer screw gauge (Fowler 52-224), a pH meter (Hanna HI 2211), an oven (Memmert UM 400), a solid UV-Vis spectrophotometer (Thermo Scientific GENESYS 10S), an FTIR spectrophotometer (Thermo Fisher Scientific Nicolet iS50), and a UV-Vis spectrophotometer (Thermo Scientific Genesys 140/150).

### 2.2. Fabrication and Characterization of the Optode Membrane

An optode membrane of CTA was prepared by mixing CTA solutions, a plasticizer, Aliquat-336, and a 1 M $NH_4SCN$ solution, followed by thorough mixing and drying at room temperature. The membrane was then cut into a 1 × 3 cm rectangular shape. The optode membrane was characterized using an FTIR spectrophotometer to obtain the infrared spectrum, facilitating functional group analysis. Measurements were taken within the wavenumber range of 4000–400 $cm^{-1}$. Scanning electron microscopy (SEM) was used to observe the formation of pores and the surface structure of the membrane at various magnifications. The methods used for membrane preparation and characterization are detailed in reference [26].

### 2.3. Linearity, Detection limit, and Quantitation limit

The parameters for linearity, limit of detection (LoD), and limit of quantification (LoQ) were determined by immersing the optode membrane in 25 mL of a standard Fe(III) solution with concentrations ranging from 0.10 to 4.00 mg/L for 15 minutes at pH 2. The color formed on the optode membrane was subsequently measured for absorbance using a UV-Vis spectrophotometer designed for solid material, at the maximum wavelength. The experiment was repeated at least six times. The LoD and LoQ were calculated based on the standard deviation of the intercept and the mean slope of the calibration curve. A minimum of six series of measurements were conducted. The equation used to calculate the LoD and LoQ values is provided in Equation 1.

$$LoD = \frac{3.3 \times SD}{S}; LoQ = \frac{10 \times SD}{S} \quad (1)$$

Where, $LoD$ is the limit of detection, $LoQ$ is the limit of quantification, $SD$ is the standard deviation, and $S$ is the slope.

### 2.4. Precision (Repeatibility and Intermediate Precision)

Precision is expressed in terms of repeatability and intermediate precision. The optode membranes were immersed in standard Fe(III) solutions. Repeatability, as a measure of precision, is represented by the percentage of relative standard deviation (%RSD) from a series of repeated measurements, with a minimum of six repetitions of standard measurements. Intermediate precision data were obtained from tests conducted on different days. The %RSD was then compared between experiments, as outlined in Equation 2.

$$RSD = \frac{SD}{\bar{X}} \times 100\% \qquad (2)$$

Where, $SD$ is the standard deviation, $\bar{X}$ is the average absorbance, and $RSD$ is the relative standard deviation (%).

### 2.5. Accuracy

Accuracy was calculated using the percentage of recovery of the standard measurements. The optode membrane was immersed in standard Fe(III) solutions at three different concentration levels, with each level used to prepare a new optode membrane. The color formed on the optode membrane was then measured using a solid UV-Vis spectrophotometer. The percentage of recovery (PR) was calculated by comparing the concentration detected in the measurement with the known value of the standard concentration. The experiment was repeated at least three times for each of the three concentration levels, as outlined in Equation 3.

$$\mathrm{PR} = \left(\frac{[\mathrm{Fe(III)}]_{\mathrm{Calc}}}{[\mathrm{Fe(III)}]_{\mathrm{Theoretical}}}\right) \times 100\% \qquad (3)$$

Where, $[\mathrm{Fe(III)}]_{\mathrm{Calc}}$ is the concentration of Fe(III) obtained from the measurements, and $[\mathrm{Fe(III)}]_{\mathrm{Theoretical}}$ is the known concentration of the standard.

### 2.6. Sensitivity and Selectivity

The sensitivity parameter of the optode was determined by calculating the molar absorptivity value using the Lambert-Beer equation. The optode was immersed in a standard solution and measured using a UV-Vis spectrophotometer designed for solid materials. These measurements were repeated six times. A selectivity test was conducted to evaluate the potential interference from other cations that could affect the optode membrane's response. This test assessed the selectivity of the optode membrane toward specific metal cations, specifically Cr(VI) and Pb(III) solutions. The experiment was repeated six times, and the responses were used to calculate the selectivity coefficient. Sensitivity was calculated using Equation 4, and selectivity was determined using Equation 5.

$$\varepsilon = \frac{A}{b \times c} \qquad (4)$$

Where, $A$ is the absorbance, $b$ is the optode thickness (cm), $c$ is the solution concentration (M), and $\varepsilon$ is the molar absorptivity ($M^{-1}cm^{-1}$).

$$S_{sample} = S_A + S_I;\ S_{sample} = K_A C_A + K_I C_I;\ K_{A,I} = \frac{K_I}{K_A} \qquad (5)$$

Where, $S_{\mathrm{sample}}$ is the signal in the sample solution, $S_A$ is the analytical signal, $S_I$ is the interference signal, $K_A$ and $K_I$ are the analyte and interference coefficients, respectively, and $C_A$ and $C_I$ are the concentrations of the analyte and interfering ions.

### 2.7. Color Stability

The stability of the color complex was tested to observe its behavior after the reaction. The optode membrane was immersed in standard Fe(III) solutions for 15 minutes at the optimal pH of 2. The absorbance of the resulting color complex was measured daily using a solid-state UV-Vis spectrophotometer.

### 2.8. Robustness Test

Robustness refers to the ability of a method to remain stable despite small variations in its parameters. In this study, robustness was evaluated by varying pH as the parameter. The optode membrane was immersed in a standard Fe(III) solution at pH levels 1, 2, and 3 for 15 minutes. The absorbance of the color formed on the membrane was then measured using a solid-state UV-Vis spectrophotometer. Each experiment was repeated six times, and the results at each pH level were subjected to a significance test with a 95% confidence level.

### 2.9. Detection of Fe(III) in Water Samples

The water sample was collected from the area around Bogor, West Java, and was intended for household use. The sample solution was prepared by mixing 15 mL of the water sample with 10 mL of distilled water. The optode membrane was immersed in the sample solution for 15 minutes at pH 2 to detect Fe(III). The color formed on the optode membrane was measured for its absorbance at the maximum wavelength using a solid-state UV-Vis spectrophotometer, allowing for the calculation of Fe(III) concentration. For comparison, a standard spectrophotometric method was also used, where 15 mL of the water sample was mixed with 1.25 mL of 2 M KSCN and 0.75 mL of 4 M $HNO_3$ in a 25 mL volumetric flask, diluted to the mark, homogenized, and measured for absorbance at 480 nm. Statistical analysis was conducted with a 95% confidence level.

## 3. Results and Discussion

### 3.1. CTA Optode Membrane

Optical sensors are a type of chemical sensor that connects chemical reactions with spectroscopic measurements. These sensors identify analytes at specific wavelengths within the UV-Vis spectrum [30]. One example of an optical sensor is the optode membrane, which analyzes the chemical reactions of analytes in samples through spectroscopic measurements such as reflectance, absorption, fluorescence, and/or luminescence. The working principle of the optode membrane involves an interface matrix between the analyte and the reagent. This reagent undergoes a binding reaction with ions, forming colored complexes within an organic or inorganic matrix [31, 32].

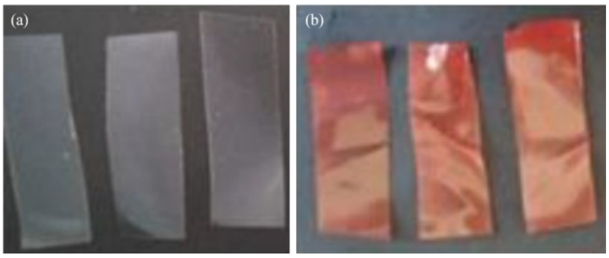

**Figure 1.** Color formation of optode membrane (a) before and (b) after contact with a standard solution of Fe(III) at 1 mg/L

The primary materials used in fabricating the optode membrane include CTA, plasticizers (acetophenone and oleic acid), Aliquat-336, and chromophores. CTA serves as the matrix carrier for the active substance, possessing hydrophobic properties due to the acetyl group replacing the hydroxyl group in the cellulose polymer. This modification enables the measurement of analytes in solution. Aliquat-336, a quaternary ammonium salt, functions as an extractor or carrier of metal ions into the membrane, where they react with the chromophore.

Fe(III) ions, transported into the membrane by Aliquat-336, react with thiocyanate ions ($SCN^-$) to form a blood-red complex, $[Fe(SCN)]^{2+}$, which is widely used as a qualitative indicator for the presence of $Fe^{3+}$ in solution. This complex exhibits a strong, deep red color due to its intense light absorption, making it highly sensitive for detecting trace amounts of ferric ions. The reaction's high sensitivity and distinct color change make it a preferred method in analytical chemistry. Furthermore, the color intensity is directly proportional to the concentration of the Fe(III)-thiocyanate complex, allowing quantitative measurement using a spectrophotometer at wavelengths between 450 and 480 nm.

The stoichiometry of the complex formed depends on the Fe(III) to $SCN^-$ ratio and solution conditions such as pH and ionic strength. Iron(III) reacts with thiocyanate ions to produce a red-orange color, as shown in reaction (1). The number of thiocyanate ions involved, represented by n, typically ranges from two to six times the concentration of Fe(III) ions, depending on the chemical environment. Therefore, when the reaction is complete, using six thiocyanate ions results in the formation of an octahedral complex structure [33, 34, 35]. The reaction and color formation are shown in Figure 1.

$$Fe^{3+} + nSCN^- \rightleftharpoons [Fe(SCN)_n]^{3-n} \quad (1)$$

Aliquat-336 acts as an extractor or carrier of Fe(III) ions into the CTA membrane, enabling the ions to react with the active components. The mechanism and interaction between Fe(III) and Aliquat-336 are illustrated in Reaction 2 and Figure 2. This process involves transferring Fe(III) ions into the membrane, facilitated by Aliquat-336 as an intermediary, performing an ion exchange. The exchanged ions are then more readily incorporated into the membrane and react with a specific reagent, as depicted in Figure 1 [36, 37].

$$Fe^{3+}_{(aq)} + 4Cl^-_{(aq)} + R_3NCH_3Cl_{(org)} \rightleftharpoons R_3NCH_3{\cdot}FeCl_{4(org)} + Cl^- \quad (2)$$

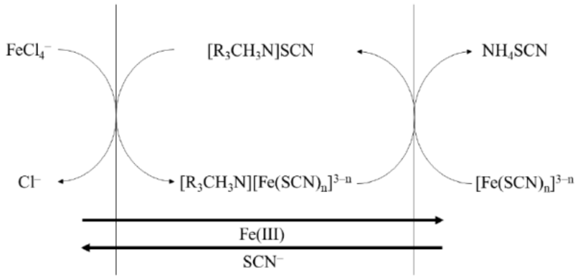

**Figure 2**. Transport mechanism of the ion through Aliquat-336 and reaction with thiocyanate

### 3.2. Characteristics and Properties

Fourier Transform Infrared (FTIR) spectroscopy is an instrument used to detect functional groups and identify compounds within a sample. The IR spectrum provides insights into the molecular structure through specific absorption bands characteristic of each molecule [38]. FTIR analysis of functional groups in the fabrication of CTA membranes was performed in the wavenumber range of 4000–400 $cm^{-1}$. CTA contains functional groups such as C–H, C=O, and C–O in its structure [24].

As shown in Figure 3, the peaks at wavenumbers 2876, 1734, and 1233–1051 $cm^{-1}$ correspond to the stretching vibrations of C–H $sp^3$, C=O, and C–O, respectively (Figure 3a). The IR spectra of the CTA+SCN and CTA+SCN+Fe(III) optode membranes show additional absorption at wavenumbers of 2058 $cm^{-1}$ and 2061 $cm^{-1}$, respectively, which correspond to the stretching vibration of S–C≡N (Figures 3b, 3c). These results indicate no significant differences in wavenumbers for the C–H, C=O, and C–O vibrations in the optode before or after immersion in the Fe(III) solution. The distinct peaks confirm the successful impregnation of thiocyanate into the membrane. Furthermore, the persistence of color over several days after immersion indicates that the CTA membrane can retain the iron thiocyanate complex color.

The thickness of the optode membrane for the detection of Cr(VI) was measured using a screw micrometre, with an average thickness of 0.0276 mm from three Petri dishes and a %SBR of 1.02%. In contrast, the optode membrane for detecting Fe(III) exhibited an average thickness of 0.0639 mm with a %SBR of 3.90%. This thickness is neither excessively thick (>100 μm) nor too thin (<5 μm) [24]. A two-tailed t-test for significance indicated no significant difference between the average thicknesses of the optode membranes. A uniform thickness is expected to yield a good chromophore distribution for detecting the metal analyte.

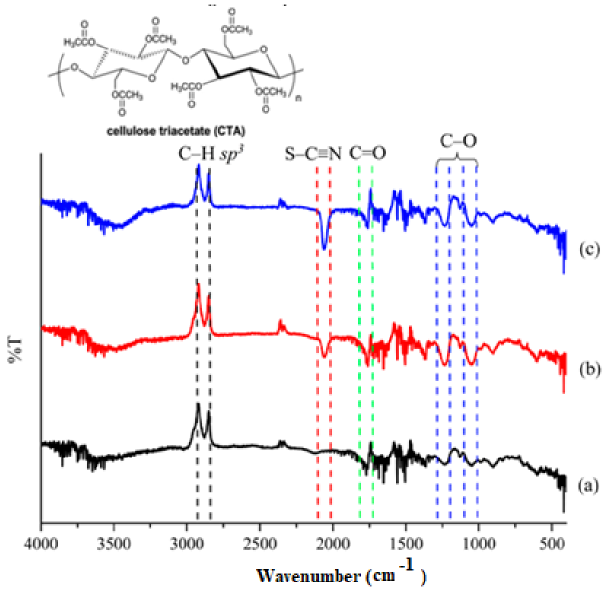

**Figure 3**. IR spectra of the optode membrane: (a) CTA, (b) CTA+SCN, (c) CTA+SCN+Fe(III)

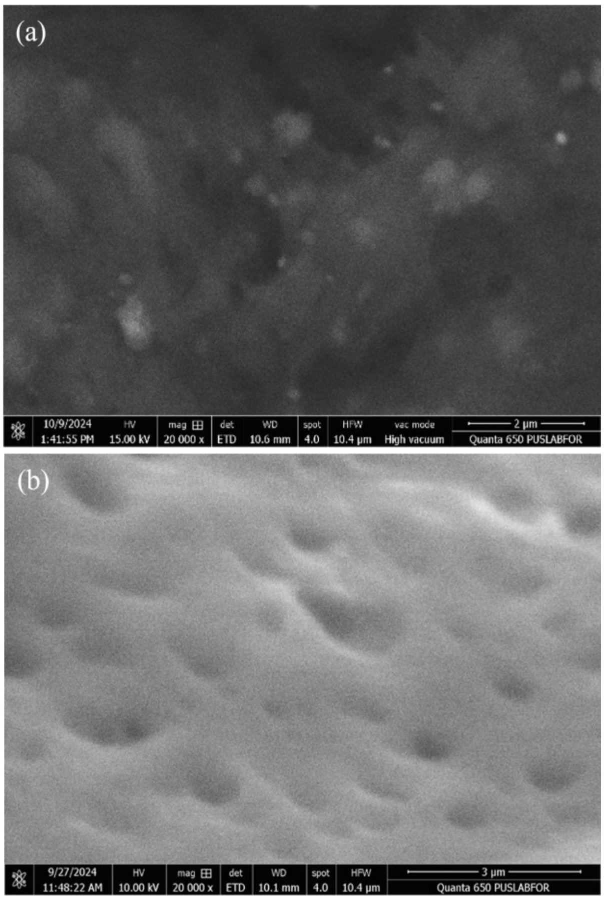

**Figure 4**. SEM imaging of the optode membrane at 20,000× magnification: (a) before and (b) after contact with the Fe(III) solution

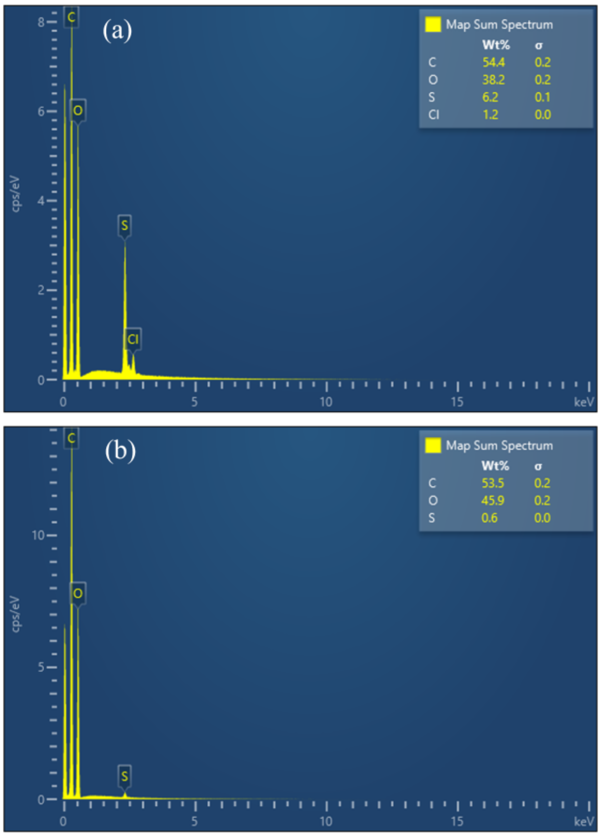

**Figure 5**. EDX profile of optode membrane (a) before and (b) after contact with Fe(III)

Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) analysis was employed to examine the surface of the optode membrane. SEM imaging was conducted at magnifications of 5000× and 20,000× to capture detailed surface features. The electron microscope was equipped with an EDX detector to identify the materials present on the membrane. The results of this analysis are presented in Figure 4.

A noticeable difference is observed between the initial optode and the optode membranes after immersion in the reaction solution. Before immersion, the surface of the optode membrane appears smooth. However, the surface becomes rougher after interacting with the $SCN^-$ solution (Figure 4b), and more pores are formed due to this interaction. The magnification at 20,000× provides a clearer view of these changes (Figures 4a, 4b). Before immersion, the surface is highly homogeneous. After immersion, slight alterations in the surface are evident, likely due to the components in the solution. This suggests that an interaction has occurred, potentially altering the surface characteristics.

Based on the SEM-EDX analysis of the optode membrane (Figure 5a), the elemental composition consists of carbon (C) at 54.4 wt%, oxygen (O) at 38.2 wt%, sulfur (S) at 6.2 wt%, and chlorine (Cl) at 1.2 wt%. The dominant carbon content reflects the organic nature of CTA and Aliquat-336, both of which contain alkyl and carbon groups in their molecular structures. Oxygen likely originates from the polar groups in the membrane and interactions with thiocyanate.

The detected sulfur confirms the presence of $SCN^-$, a key ligand in this membrane system, particularly in its interaction with metal ions like Fe(III). The small amount of chlorine is attributed to Aliquat-336, which includes chloride ions as counter ions in its structure. These results demonstrate that the components of the optode membrane—CTA, Aliquat-336, and $SCN^-$—have been successfully incorporated, with each component contributing to the elemental composition observed in the SEM-EDX spectrum.

Figure 5b shows the SEM-EDX analysis after the membrane's interaction with the Fe(III) solution. The detected elements include carbon (53.5 wt%), oxygen (45.9 wt%), and sulfur (0.6 wt%). The dominant carbon content indicates that the membrane retains its organic components, primarily from Aliquat-336, which is rich in carbon due to its long alkyl chains. The significant oxygen content suggests the presence of polar functional groups within the membrane, which may have interacted with Fe(III) ions or reflected hydration and possible oxidation processes on the membrane surface.

The low sulfur content confirms the presence of $SCN^-$, but at a reduced level compared to the initial membrane. This decrease in sulfur suggests that some thiocyanate ions may have reacted with Fe(III) to form Fe(SCN) complexes, which could have diffused into the solution or detached from the membrane. Overall, the results indicate that the optode membrane, containing Aliquat-336 and thiocyanate, underwent a chemical interaction with Fe(III). The reduction in sulfur content, coupled with the retention of high carbon and oxygen levels, suggests the membrane's stability and highlights the chemical interaction of thiocyanate with Fe(III).

### 3.3. Linearity, Detection, and Quantitation Limit Parameter

Linearity is the ability of an analytical method to provide a proportional response to the analyte concentration, measured through the linear regression equation of the standard curve between concentration and absorbance. In the 0.1–4.0 mg/L concentration range, the coefficient of determination ($R^2$) values obtained from six replicate measurements were 0.9972, 0.9946, 0.9910, 0.9907, 0.9880, and 0.9861. According to the Association of Official Analytical Chemists (AOAC) [29], an $R^2$ value greater than 0.99 indicates good analytical results, meaning that changes in the analyte concentration have a linear effect on the signal (Figure 6).

The detection and quantification limits for Fe(III) were 0.0553 mg/L and 0.1676 mg/L, respectively. The iron threshold for clean water is set at 0.3 mg/L by USEPA and WHO [6, 7], indicating that the optode membrane can detect iron in clean water. However, Fe(III) concentrations below the detection limit cannot be distinguished from noise, which may be attributed to the matrix, sample concentration, and reagent purity.

### 3.4. Precision (Repeatability and Intermediate precision)

Precision measures the closeness of analytical results obtained from repeated measurements of the same sample. Precision is expressed as the relative standard deviation (RSD). It can be represented in terms of repeatability, intermediate precision, or reproducibility. Precision (repeatability) refers to the repetition of measurements in a single session conducted by the same analyst using the same equipment, materials, and laboratory [39]. An analytical method demonstrates good precision when the relative standard deviation is less than 8% for concentrations below 10 mg/L [29]. The %RSD obtained for Fe(III) detection was 3.01%. The low RSD values indicate that the measurement method has a good response in detecting Fe(III), and random errors do not interfere with the analytical results.

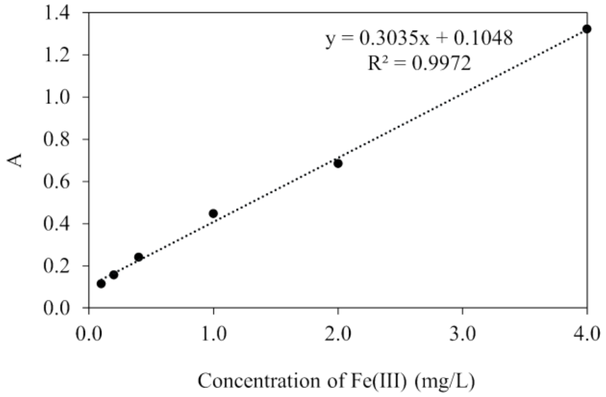

**Figure 6**. Correlation between absorbance and concentration of Fe(III) standard

Intermediate precision demonstrates the ability of the same method to maintain %RSD within the reference value. This intermediate precision testing is conducted across laboratory sessions, times, materials, and analysts [27]. However, the reference method must remain consistent and unchanged. Precision tests were performed on different days and with varying concentrations during the testing sessions [39]. The first test yielded a %RSD value of 3.03%, while another produced a %RSD value of 3.01%. These results indicate that the method demonstrates intermediate precision that meets the criteria outlined by AOAC [29].

### 3.5. Accuracy

Accuracy refers to the closeness of an analytical method's result to the actual value. In this study, accuracy was assessed using the recovery percentage method [39, 40]. Measurements at three concentrations showed a recovery percentage of 101.62%, within the validation criteria of 80-110% for concentrations between 1-10 mg/L [29]. These results demonstrate that the optode membrane accurately determines Fe(III) in real samples, with outcomes aligning closely with accepted values within the acceptable limits.

### 3.6. Sensitivity and Selectivity

Sensitivity is measured to evaluate the optode membrane's ability to detect small changes in analyte concentration. The sensitivity of the optode membrane was determined using the Lambert-Beer law. For Fe(III) detection, the optode membrane demonstrated an average molar absorptivity of $1.05 \times 10^7$ $M^{-1}$ $cm^{-1}$ (Table 1).

Selectivity measures a method's resistance to interference, as indicated by the selectivity coefficient ($K_{A,I}$) [39]. To determine selectivity, other metal ions are added to the solution. The $K_{A,I}$ value is calculated by comparing the absorbance of the optode membrane when exposed to the metal solution alone and when the metal solution is mixed with interfering metals (Table 2).

**Table 1**. Molar absorptivity of the optode membrane for Fe(III)

| Conc. of Fe(III) (mg/L) | Abs | Specific absorptivity | Conc. (M) | Molar absorptivity |
|---|---|---|---|---|
| 0.200 | 0.157 | 29.3 | $3.57 \times 10^{-6}$ | $1.64 \times 10^7$ |
| 0.400 | 0.241 | 22.6 | $7.14 \times 10^{-6}$ | $1.26 \times 10^7$ |
| 1.00 | 0.447 | 16.7 | $1.79 \times 10^{-5}$ | $9.37 \times 10^6$ |
| 2.00 | 0.685 | 12.8 | $3.57 \times 10^{-5}$ | $7.18 \times 10^6$ |
| 4.00 | 1.32 | 12.4 | $7.14 \times 10^{-5}$ | $6.93 \times 10^6$ |
| Average | | | | $1.05 \times 10^7$ |
| SD | | | | $4.01 \times 10^6$ |

**Table 2**. Selectivity coefficient values of the optode membrane

| Analyte | Interference | $K_{A,I}$ |
|---|---|---|
| Fe(III) | $Pb^{2+}$ | -0.4580 |
| | $Cr^{6+}$ | -0.2748 |

**Table 3**. The absorbance of the optode membrane after contact with metal at different pH levels

| Optode membrane | Abs | | |
|---|---|---|---|
| | pH 1 | pH 2 | pH 3 |
| Fe (III) | 0.317±0.005$^a$ | 0.385±0.002$^b$ | 0.371±0.005$^c$ |

Note: a, b, c different letter means significantly different at $\alpha$=0.05

Other metal ions do not interfere with the analyte if the $K_{A,I}$ value is < 1. However, if $K_{A,I}$ > 1 or $K_{A,I}$ < -1, the metal ions interfere with the analyte [39]. Table 2 shows that the addition of other metal ions did not interfere with the analyte, as the $K_{A,I}$ values for Fe(III) detection with the addition of Pb(II) and Cr(VI) ions were -0.4580 and - 0.2748, respectively. These values indicate that the optode membrane is selective towards Fe(III), with minimal interference from Pb(II) and Cr(VI). The investigation focused on testing cations potentially present in river water, concentrating on a selected range of probable candidates. The methodology relied on specific chemical reactions, applied under controlled pH conditions to ensure accurate identification and analysis. A smaller selectivity value indicates better discrimination of the analyte from interferences.

Selectivity is a crucial aspect of analytical chemistry as it influences method design. It ensures that signals remain unaffected by interference from other components, allowing for precise analyte identification and reflecting the representativeness of the samples. It also ensures alignment between the goals of the methods and the analytical results [41].

### 3.7. Color Stability

The stability test was conducted to determine whether the optode membrane could maintain the color of the complex under specific environmental conditions. The stability of the optode color complex was evaluated by calculating the percentage decrease in absorbance from day 0 to day 10. Over ten days, the absorbance of the Fe(III) detection membrane varied with an average value of 0.6677 and a %RSD of 3.14%. The low standard deviation values support the stability, indicating that the absorbance data is closely distributed around the average value. The plasticizer also contributes to the membrane's stability by binding the components, enhancing both physical and chemical stability [24]. Overall, the color complex produced by the optode membrane over ten days demonstrates good stability.

### 3.8. Robustness Test

Robustness refers to the ability of an analytical method to maintain consistent results despite minor variations in testing conditions [27]. This study conducted the significance test at three different pH levels to evaluate the optode membrane's ability to detect Cr(VI) and Fe(III). The results showed that the experimental values were lower than the critical value at $\alpha$ = 0.05, indicating a significant difference between the mean absorbance values across the measurements (Table 3). This suggests that the analytical method is sensitive to changes in pH.

**Table 4**. Metal concentration in water samples

| Detection | Optode (mg/L) | UV-Vis Spectrophotometer (mg/L) |
|---|---|---|
| Fe(III) | 0.0578±0.003 | 0.0564±0.005 |

The absorbance (Abs) of the optode membrane in the presence of Fe(III) ions was measured at three different pH levels: 1, 2, and 3. The results show that pH significantly influences the membrane's response. At pH 1, the absorbance value was 0.317±0.005, the lowest among the three conditions, suggesting limited interaction between Fe(III) and the membrane, potentially due to partial hydrolysis of Fe(III) or changes in the membrane's chemical affinity for the ion. At pH 3, the absorbance slightly increased to 0.371±0.005, possibly reflecting increased hydrolysis of Fe(III) or a reduced sensitivity of the membrane at this pH [4, 5].

The statistically significant differences, indicated by superscripts a, b, and c, emphasize the importance of pH in the Fe(III) coloring formation reaction and its interaction with the optode membrane. This variability in response highlights the critical role of pH control when designing and applying optode-based sensors for Fe(III) detection in analytical chemistry and environmental monitoring.

### 3.9. Detection of Fe(III) in Water Samples

The optode membrane was used to detect Fe(III) in water samples obtained from a household in Bogor, West Java, which was used for domestic purposes. The samples were odorless and colorless, with a pH range of 6.70–6.87. The Fe(III) concentration in the water was determined using the CTA-based optode membrane method and a UV-Vis spectrophotometer (Table 4). The testing with optodes was conducted at pH 2, with the pH adjusted using acid to align with the optimal conditions for color formation, as determined during the initial phase of the experiment.

The Fe(III) concentrations determined by the two methods were compared using a calibration curve derived from the experimental results. The linear equation for the calibration curve was $y = 0.1343x + 0.0083$ with an $R^2$ of 0.9986. A two-tailed Student's t-test was conducted to test the null hypothesis, positing no significant difference between the mean results obtained by the optode membrane method and the UV-Vis spectrophotometer method. The t-test results indicated no significant difference between the two methods at a 95% confidence level ($\alpha$ = 0.05). As a result, the null hypothesis was retained, confirming that both methods yielded comparable results. Furthermore, the determined Fe(III) concentrations were below the quality standard threshold of 0.2 mg/L, confirming the reliability and accuracy of the optode membrane method for Fe(III) detection in water samples [6, 7].

## 4. Conclusion

The cellulose triacetate (CTA)-based optode membrane, enhanced with thiocyanate, offers a reliable and effective alternative for Fe(III) detection. This method meets all required specifications, demonstrating excellent performance with an $R^2$ of 0.9972 across a concentration range of 0.1–4.0 mg/L. Validation parameters include a LoD of 0.0553 mg/L, a LoQ of 0.1676 mg/L, precision of 3.01%, intermediate precision of 3.03% and 3.01%, and an accuracy of 101.62% recovery. Additionally, the method demonstrates high sensitivity with a value of $1.05 \times 10^7$ $M^{-1}$ $cm^{-1}$, good selectivity with values of -0.4580 and -0.2748 against Pb(II) and Cr(VI), respectively, and stable color formation with a %RSD of 3.14%. The validation results confirm that the method aligns well with established reference standards, fulfilling the intended validation objectives. Furthermore, the optode membrane successfully detects Fe(III) in water samples, showing no significant differences from results obtained using a UV-Vis spectrophotometer. This highlights its practical applicability and reliability for real-world water analysis.

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