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Chlorine Identification in Rice Samples through a Method Validation Approach using Cyclic Voltammetry with Platinum Electrodes

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Abstract

Determination of chlorine in rice samples with a method validation approach using cyclic voltammetry based on platinum electrodes has been conducted. This study aimed to determine whether the cyclic voltammetry method with platinum electrodes is acceptable and can be recommended for routine chlorine testing in the laboratory. The basis of the analysis is the reduction reaction of hypochlorite ions to chloride ions. The initial step taken by optimizing the electrolyte with the optimal electrolyte for testing is a pH 7 phosphate buffer solution. Method validation parameters consist of determining linearity, detection limit, quantitation limit, repeatability, recovery, and measurement uncertainty. The range of standard solutions used for testing was from 10 mg / L to 150 mg / L and analyzed at a rate of 0.1 V / s. Linearity is determined based on the calculation of the coefficient of determination (R2) of 0.998, while the limits for detection and quantization are 7.49 mg / L and 24.96 mg / L, respectively. Repeatability is determined by calculating the relative standard deviation with a result of 1.77% while for recovery it is obtained at 88.19%. The result of the chlorine content test for rice samples was 0.011% with a measurement uncertainty value of 0.0011%. The major contributor to uncertainty came from the concentration of the calibration curve plot of 87.91%.

Keywords: Reduction reaction, hypochlorite ions, chloride ions, electrolyte, phosphate buffer solution, uncertainty

Introduction

Indonesia is one of the countries in Southeast Asia where most of the population consumes rice as a staple food. Many people view the good quality of rice can be seen from its white physical form. Dull-colored rice will have a cheap price, but on the other hand white rice has a high selling value. However, many parties use hazardous chemicals to whiten rice so the selling value is high. In 2015 at a market in Indonesia's West Java province, it was found that rice was mixed with bleach to make it look like high-quality rice. One type of chemical that is sometimes used as bleach is chlorine. The Regulation of the Indonesian Minister of Health issued in 2018 states that chlorine is prohibited from being used as a food additive.

Chlorine is generally used as a bleaching agent in the process of making paper or tissue in the form of NaOCl and $Ca(OCl)_2$. Effects that can occur due to the consumption of NaOCl can cause corrosive gastrointestinal injury and systemic effects, including metabolic acidosis, hypernatremia, and hyperchloremia (Dutta & Saunders, 2012; Slaughter et al., 2019). Based on the Regulation of the Minister of Health of the Republic of Indonesia No.033/Menkes/Per/IX/2012, it is stated that chlorine is not included in the permitted food additives. Several regions in Indonesia are suspected of having rice circulating in the community that contains chlorine for bleaching purposes (Astuti et al., 2020; Sammulia et al., 2020; Bunyanis et al., 2021).

The identification of chlorine in rice samples can be tested using the Volhard titrimetric method as with chlorine testing in other samples. This method is based on the reaction of giving excess silver ions with the formation of silver chloride precipitate so that the remaining silver ions react with potassium thiocyanate to form a permanent red color of silver thiocyanate (KCNS). This method is less efficient because it is only able to test at high chlorine concentrations.

Another method to determine chlorine in rice samples is to use a UV Vis spectrophotometer based on the reaction between chlorine and N, N′-diethyl-p-phenylenediamine (DPD) which is detected at wavelengths between 525 – 550 nm. This method is less sensitive because it is only able to detect chlorine concentrations between 0.7 – 56 µmol L-1 or about 50 – 450 ppm (Moberg & Karlberg, 2000).

The chlorine content of solid samples has also been tested using a high-resolution continuum source graphite furnace molecular absorption spectrometry method (Guarda et al., 2017), but this method is used for testing chlorine in the form of CaCl. Radiochemical neutron activation (RNAA) has also been used for the assay of chlorine and other halogen elements such as bromine and iodine (Sekimoto & Ebihara, 2017; Palme & Zipfel, 2022). The Radiochemical Neutron Activation Analysis (RNAA) method is not an easy method and not all laboratories and analysts can immediately practice it. Instruments associated with neutron activation must be located in special locations and their potential radiation exposure must be monitored, so this is also a problem if the laboratory uses such instruments. Therefore, laboratories that will carry out routine testing require test methods that are simple, inexpensive, and can be easily practiced by analysts.

Chlorine in solid samples can also be detected using a combination of X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) (Mazurek et al., 2021), but these methods are also expensive because both instruments are very expensive and impractical to use in laboratories performing routine examinations.

Based on these reasons, it is necessary to develop a test method to determine the chlorine content in rice samples that have high sensitivity. One such method is cyclic voltammetry using platinum as an electrode. Chlorine analysis by cyclic voltammetry can be used routinely for testing if the method has been previously validated. The validation parameters used to validate by determining the value of linearity, the limit of detection (LOD), the limit of quantification (LOQ), repeatability or precision (%RSD), and accuracy by calculating the recovery of chlorine analysis by cyclic voltammetry method using the optimal electrolyte. Uncertainty is also taken into account because it is one part of method validation (Mohamed et al., 2020)

The purpose of this validation is to ensure that the chloride test method on rice samples using cyclic voltammetry with platinum electrodes can be used routinely in the laboratory because it meets the specified requirements.

Methods

Chemicals and instrumentations

The materials used for this research are chemicals produced by Merck which consist of potassium chloride (99.5%), potassium nitrate (99.0%), sodium thiosulfate (99.5%), sodium carbonate (99.9% Merck), disodium hydrogen sulfate (99.0%), sodium hypochlorite (12%), sodium dihydrogen sulfate (99.0%), sulfuric acid (95-97%), potassium iodate (99.7 - 100.4%), potassium iodide (99.5%), and starch. Rice samples that have been treated with chlorine. The instruments used in this research are Electroanalyzer Cyclic Voltammetry from Metrohm, a volumetric apparatus made by Iwaki, and an analytical balance produced by Ohaus.

Sample preparation

The first step is to prepare a rice sample which is a simulated sample that has been treated with chlorine as bleach. The weighed rice was then put into a bottle and added to distilled water which was then shaken vigorously for 2 minutes. The solution was allowed to stand for 30 minutes and then filtered. The filtrate was allowed to stand for 2 hours to separate the starch and impurities that precipitated.

Electrolyte optimization

Twenty-five mL of a standard 100 mg / L chlorine solution was put into an electrolysis cell then added 5 mL of 0.1 M KCl was which served as an electrolyte and analyzed at a speed of 0.1 V/s using a Pt electrode and repeated with variations of KNO_3 phosphate buffer solution. 0.1 M, and pH 7.

Analysis of chlorine by electron analyzer cyclic voltammetry

A total of 25 mL of precipitated rice washing water was put into an electrolysis cell, then 5 mL of phosphate buffer pH 7 was added and analyzed by electron analyzer cyclic voltammetry at a speed of 0.1 V/s. The chlorine content was calculated by plotting the current obtained from the sample measurements into a regression equation made with variations of standard chlorine solution with a concentration of 10 - 150 mg / L. Precision was carried out by 7 tests and accuracy was determined by the spike method (recovery test). In this study, the detection limit, quantization limit, and measurement uncertainty evaluation are also calculated.

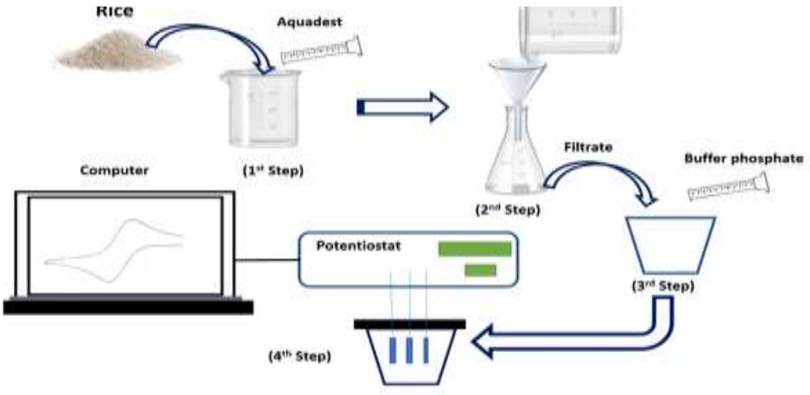


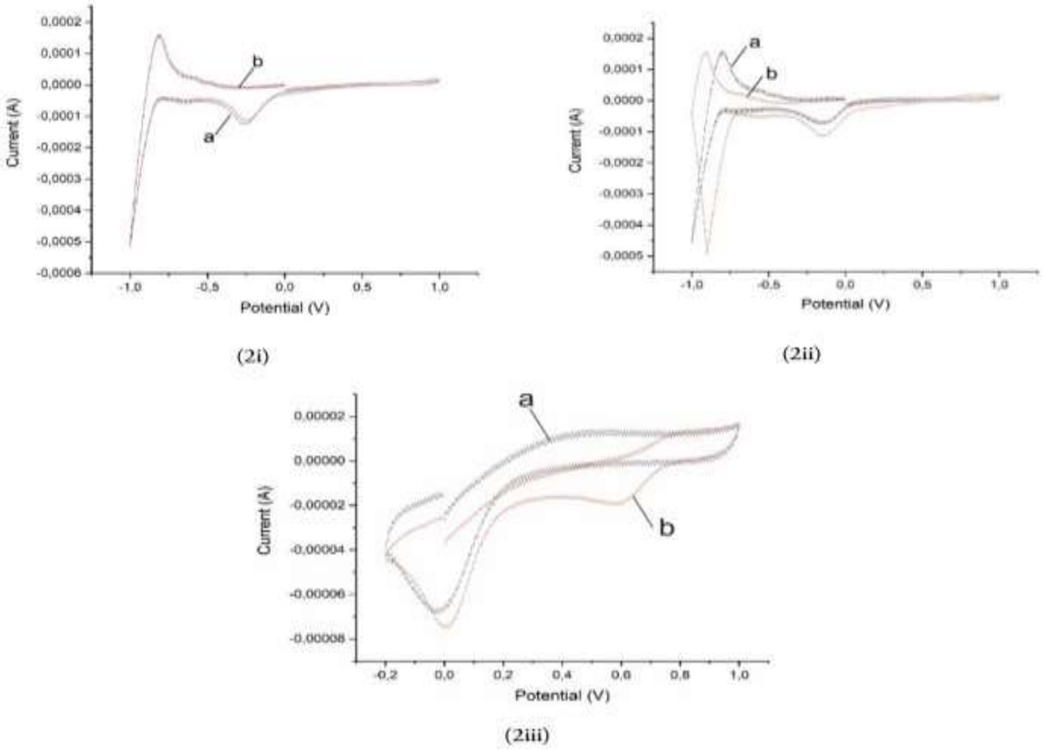
Figure 1. Steps to identify chlorine in rice samples using the cyclic voltammetry method

Results and Discussion

The principle of chlorine analysis in rice samples is based on the reduction reaction of hypochlorite ions (OCl^-) to chloride ions (Cl^-) as shown in reaction equations (1) and (2) (Kabir et al., 2019).

$ClO^{-}_{(aq)} + H_2O_{(l)} + 2e \rightleftharpoons Cl^{-}_{(aq)} + 2OH^{-}_{(aq)}$ (1)

$HClO_{(aq)} + 2e \rightleftharpoons Cl^{-}_{(aq)} + OH^{-}_{(aq)}$ ....(2)



(2i)

(2ii)

(2iii)

Figure 2. Electrolyte optimization for chlorine analysis with platinum electrodes (2i) Voltammogram pattern with 0.1 M KCl electrolyte, (2ii) Voltammogram pattern with 0.1 M KNO_3 electrolyte, and (2iii) Voltamogram pattern with phosphate buffer electrolyte

In this study, electrolyte optimization was carried out for the determination of chlorine in rice samples consisting of 0.1 M KCl, 0.1 M KNO_3, and a phosphate buffer solution of pH 7. The results of the optimization determination for the three electrolytes can be seen in **Figure 2. Figure (2i)** shows that the voltammogram produced by the blank and the sample has the same sweep so that it cannot be distinguished between the peaks produced by the blank and the sample. The voltammogram produced by the blank and the sample has a slight difference as shown in **Figure (2ii)**, but the difference is not so significant that it is difficult to determine the peak of chlorine with the electrolyte. **Figure (2iii)** shows the voltammogram produced from different blanks and samples with sweeping starting from potential 0 and producing a reduction peak at a potential of ± 0.55 volts. The resulting voltammogram is a type of voltammogram with an irreversible system because only one reduction peak is formed at a positive potential. Therefore, for quantitative analysis using the cyclic voltammetry method, then using phosphate buffer as a support for chlorine testing on rice samples with platinum electrodes.

Quantitative analysis was carried out through a method validation approach that began with measuring the current of a standard chlorine solution whose concentration was varied from 10 to 150 mg L^{-1} using a phosphate buffer electrolyte. The results of measuring chlorine standard solutions with concentrations between 10 and 150 mg L^{-1} using cyclic voltammetry can be seen in **Figure 3.**

Figure 3 (b) is a graph of the relationship between concentration and electric current which is a linear graph with a negative correlation. This is indicated by the greater the concentration, the smaller the current. This study obtained a correlation coefficient (R) of -0.9991. The results of standard chlorine measurements with cyclic voltammetry showed good linearity because it was more than 0.990 validation (Mohamed et al., 2020).

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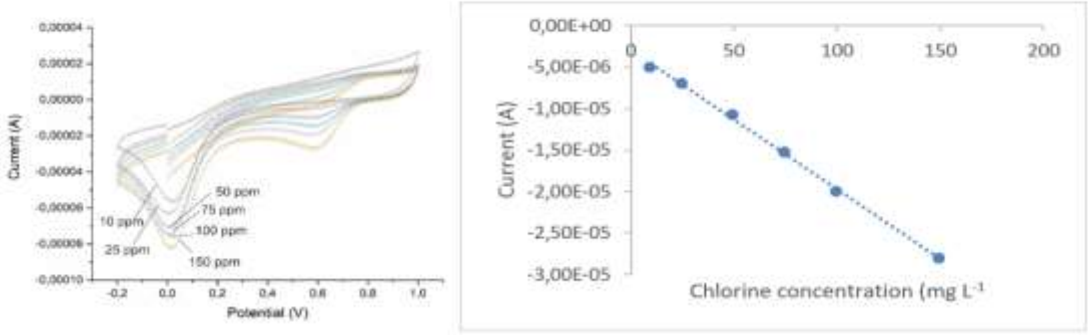

(a) (b)

Figure 3. Observation of chlorine standard solution (a) Voltamogram of chlorine standard solution (b) calibration curve between chlorine concentration and current

Determination of precision is done by testing as many as six repetitions. Measurements were made by testing the selected standard chlorine solution with a concentration of 100 mg L^{-1}. The results of the voltammogram observations are shown in **Figure 4** with the current observations listed in **Table 1**. **Figure 4** shows the average current generated with six repetitions of the chlorine test -1.98E - 05 A with a relative standard deviation of 1.79% as shown in **Table 1** which indicates that the repeatability or precision is acceptable because the result is less than 2% (Chan et al., 2004; Taverniers et al., 2004; Thompson et al., 2002).

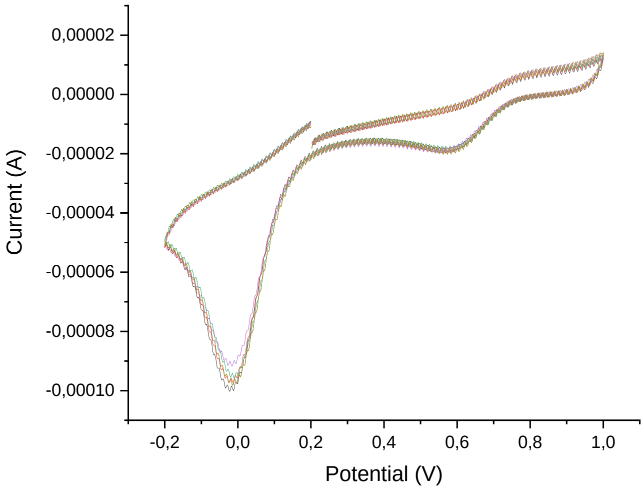

Figure 4. The results of the analysis of the standard precision voltammogram for chlorine measurements of 100 mg L-1 with a phosphate buffer solution of pH 7

Acceptance of precision can also be made by comparing the RSDr value in percent with the RSDr predicted from the Horwitz equation (PRSDr) known as the Horwitz ratio or Horrat value. Acceptance of repetitions seen from the value of horrat then the number should not be more than 1. In this study, the value of Horrat obtained results of 0.22 or in other words, the repeatability or precision of the chlorine test on rice is acceptable (Horwitz & Albert, 2006).

The accuracy of the chlorine test on rice samples was carried out using the spike method or known as percent recovery, which is the addition of a standard to the sample whose content is known. The calculation of percent recovery is done by dividing the difference between the spike concentration test results and the sample concentration with the target concentration, all three of which are determined based on the results of plotting the resulting current into the Ilkovic equation obtained previously with units of mgL^{-1}. Observations of the voltammetric cyclic pattern for the determination of chlorine in rice samples and percent recovery are presented in **Figure 4.**

Table 1. Observational data for six repetitions of chlorine testing

Repetition	Current (A)	Concentration (mg L^{-1})
1	-1.98×10^{-5}	100.05
2	-1.99×10^{-5}	100.90
3	-2.00×10^{-5}	101.39
4	-1.93×10^{-5}	97.28
5	-1.98×10^{-5}	99.78
6	-2.02×10^{-5}	102.46
Average		100.31
Standard Deviation		1.77
Relative Standard Deviation (RSD_r)		1.78%
PRSDr		8.00%

Based on **Figure 4**, the observation data and calculation results for the concentration and percent recovery values are obtained as listed in **Table 2**.

The standard chlorine solution added to the sample as a target had a concentration of 74.07 mg L^{-1}, so the percent recovery obtained in the chlorine test on rice samples was 88.19%. These results are still included in the acceptance criteria set by the AOAC, where the analyte concentration of 43.52 mg L-1 is in the range of 80 - 115% (Horwitz & Albert, 2006).

Table 2. Data to determine the recovery of chlorine testing

Description	Total Volume (mL)	Current (A)	The concentration of Chlorine (mg L^{-1})
Analysis of chlorine in rice samples	25	-1.03E-05	43.52
Determination of % recovery	27	-2.13E-05	108.84

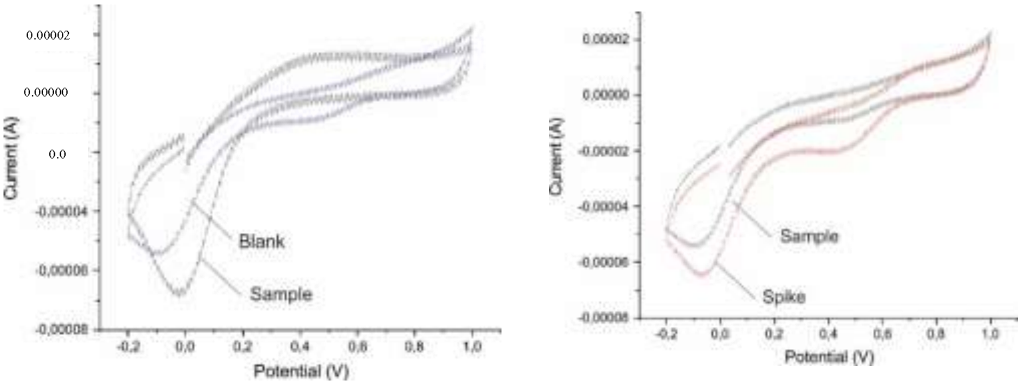

Figure 5. (5a) voltammogram for determination of chlorine in rice samples, (5b) voltammogram for determining recovery

Each chemical test including the analysis of chlorine in rice samples must determine the value of the measurement uncertainty and evaluate its value following the process requirements in ISO 17025:2017, especially in clause 7.6. Determination of the estimated uncertainty of the chlorine test on this rice sample refers to the steps that have been set by Eurachem (Ellison & Williams, 2012). The equation for determining the complete chlorine content in rice is the most important step in identifying the source of uncertainty. Furthermore, the formula is developed into a fishbone diagram which is part of the causal approach. In this study, there are three sources of measurement uncertainty, namely the chlorine concentration resulting from plotting the sample current value into a straight line equation or the Ilkovic equation, sample volume, sample mass, and test repetition. Overall the results of the calculation of uncertainty in this study can be presented in **Table 3**.

Table 3. Data processing to determine the value of the uncertainty of the chlorine test on rice samples

Uncertainty Sources	Value (x)	Units	Standard Uncertainty (µx)	Relative Standard Uncertainty (µx/x)	$(\mu x/x)^2$
Concentration	4.,52	mg/L	-2.11, E+00	-4.84, E-02	2.35, E-03
Volume of sample	25.00	mL	3.14, E-02	1.25, E-03	1.57, E-06
Mass of sample	20.00	G	5.77, E-05	2.89, E-06	8.33, E-12
Repeatability	1.00		1.77, E-02	1.77, E-02	3.12, E-04
				Combine uncertainty	**0.00056%**
		Expanded Uncertainty with 95% interval confidence			**0.0011%**

The results of the test of chlorine levels in rice samples that have been simulated with chlorine and referring to **Table 3** ways of reporting test results are 0.011 ± 0.0011%. These results indicate that the ratio of the uncertainty value compared to the chlorine content is 10% so the chlorine analysis procedure using the cyclic voltammetry method using platinum electrodes and phosphate buffer solution pH 7 as a support can be stated well because the uncertainty is less than 25% (Bennett & Zion, 2005).

The biggest contribution in calculating the uncertainty of chlorine in the rice sample is the concentration of chlorine which is plotted into the calibration curve equation, this is because the concentration is not only influenced by the standard deviation value of the calibration curve but is also influenced by the standard chlorine mother liquor first with sodium thiosulfate. Several studies have shown that mass concentration greatly affects the uncertainty that exceeds 50% of the total source of uncertainty (Lara-Almazán et al., 2021). The percentage of uncertainty contributors for each source can be seen in **Figure 5**.

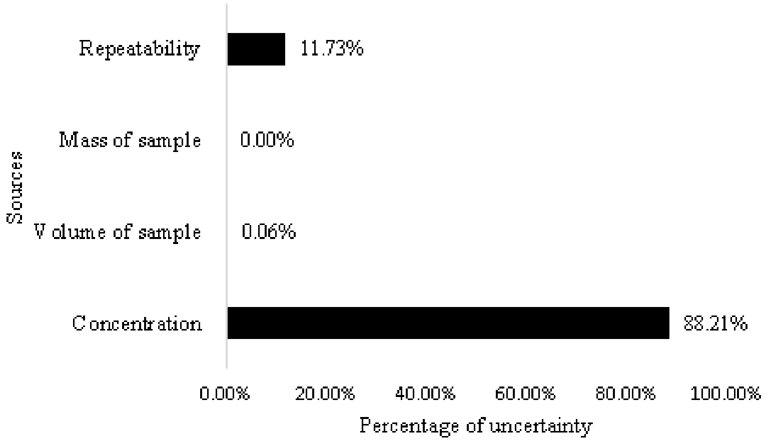

Figure 6. Percentage contributing to uncertainty in chlorine testing in rice samples

Conclusions

The results of the chlorine test on rice tested using the cyclic voltammetry method obtained results of 0.011 ± 0.0011%. The resulting linearity is determined by the correlation coefficient value of 0.9991. The limit of detection and limit of quantization in the test results obtained are 7.49 mg L^{-1} and 24.96 mg L^{-1} respectively. The RSD value and the percent recovery were 1.77% and 88.19% which met the AOAC requirements. The highest test uncertainty contribution of 87.91% came from the value of the chlorine concentration plotted on the standard curve.

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