Research Article

Free Chlorine Determination in Disinfectant Product using Visible Spectrophotometry based on Prussian Blue Degradation

Hendri Wasito[1]*, Defi Srium Siagian[1], Muhamad Salman Fareza[1]

[1] Department of Pharmacy, Faculty of Health Sciences, Jenderal Soedirman University, Jl Dr. Soeparno Karangwangkal, Purwokerto, Central Java, 53123, Indonesia.

*E-mail: hendri.wasito@unsoed.ac.id

Abstract

Disinfectant products with excessive chlorine could be dangerous for health and need quality control. It is important to develop an analytical method for monitoring product quality. The main objective of this work is to develop an alternative method and evaluate the analytical performance of visible spectrophotometry for determining free chlorine in disinfectant products based on the Prussian blue decomposition process. The capability of chlorine to oxidize ferrous to ferric ions makes the Prussian blue generated by ferrosulfate and potassium ferricyanide is decomposed and measured by spectrophotometer. The formation of Prussian blue was improved by optimizing some reaction conditions and assessing incubation time. Linearity, analytical concentration range, precision, accuracy, detection limit, and quantitation limit parameters were among the examined analytical parameters. The results showed that the optimum concentration of ferrosulfate, potassium ferysianide, and hydrochloric acid for Prussian blue formation was 2.0 mmol L^{-1}, 3.0 mmol L^{-1}, and 0.5 mol L^{-1}, respectively, with 15 minutes incubation time after chlorine addition. Analytical performance parameters seemed appropriate for routine analysis purposes. The developed method can also be applied as an alternative analytical method to determine the free chlorine concentration of disinfection products in the market.

Keywords: Spectrophotometry, Validation, Chlorine, Prussian Blue

INTRODUCTION

Chlorine can be found in disinfection products in two forms, namely sodium hypochlorite and calcium hypochlorite. This substance can be decomposed into hypochlorite acid, chlorine monoxide, and chlorine when dissolved in water. The free chlorine content refers to the amount of these molecules (March and Simonet, 2007). Chlorine has strong antibacterial properties and can effectively destroy certain bacteria, spores, fungus, and viruses in moderate quantities. It is also widely available and inexpensive, making it a suitable alternative as an active ingredient in disinfectants (Tri, 2010). Hypochlorite is an unstable compound that can decompose into chloride and oxygen (March and Simonet, 2007). Hypochlorite is commonly used as an active ingredient in disinfectant products (Parnomo, 2003). Products containing an excessive amount of chlorine can irritate eyes and skin by direct physical contact. Furthermore, it can harm the respiratory system when inhaled (Nickmilder et al., 2007). According to the Food and Agricultural Product Research and Technology centre, the chlorine content in the disinfectant products used to sanitize equipment should not exceed 2000 ppm (McGlynn, 2004). Therefore, it is necessary to control the quality of the chlorine content in the disinfectant product.

Several analytical techniques have been developed for the determination of chlorine. For instance, using interferometry methods (Xu et al., 2011), spectrophotometry assisted with N,N-diethyl-p-phenylenediamine (Moberg and Karlberg, 2000), potentiometry (Soldatkin et al., 1997), amperometry (Kodera et al., 2005; Ordeig et al., 2005), and green analytical methods (March and Simonet, 2007). One promising method that can be used as an alternative method for determining chlorine levels in the sample is the spectrophotometric method. This method has advantages in terms of accuracy, simplicity, and fast processing (Watson, 2020). A flow injection analysis method was developed with the decolonization reaction of methyl orange and through the formation of complex yellow compounds with *o*-toidine compound, then measured the intensity of the colour formed by a spectrophotometer (Leggett et al., 1982). However, this method has several disadvantages. For instance, a lot of organic solvents and complex sample preparation are required.

Chemically, Prussian blue is formed by reacting ferrous (Fe^{II}) with potassium ferricyanide ($K_3[Fe^{III}(CN)_6]$) (Teepoo et al., 2012). Chlorine has strong oxidizing characteristics that enable it to convert ferrous (Fe^{II}) to ferric (Fe^{III}) (Ropp, 2012). Therefore, degradation of Prussian blue can occur. The degradation of Prussian blue makes the intensity of the blue colour in the solution decrease due to this oxidation, which releases bonds between ferrous and potassium ferricyanide. To the best of our knowledge, the spectrophotometric method based on Prussian blue decomposition reaction, which is quite simple for determining chlorine content in the disinfectant product, has never been carried out. Therefore, this study aimed to develop a simple spectrophotometric method based on the decomposition reaction of Prussian blue for determining chlorine levels and evaluating their analytical performance. Additionally, the proposed method was applied for determining chlorine content in disinfectant products. The developed method is found to be potential as an alternative analytical method for quality control of disinfection products.

MATERIALS AND METHODS

Materials and instrumentations

The analytical grade of anhydrous ferrosulfate, potassium ferricyanide (purity ≥99.0%), hydrochloric acid, potassium dichromate, and sodium thiosulfate were obtained from Merck (Darmstadt, Germany). Distilled water and sodium hypochlorite working solution was purchased from Bratachem (Purwokerto, Indonesia), and disinfectant product samples were obtained from a market in Purwokerto. All other chemicals were analytical grade.

The colour intensity was measured using a UV/Vis-3000 single-beam spectrophotometer (Cecil CE 3021, Cambridge, England). In addition, analytical balance, shaker incubator Memmert EN 60529 (Schwabach, Germany), micropipette (Socorex, Switzerland), and laboratory glassware were used for the preparation of solutions and titration.

Standardization of chlorine working solution

The iodometric titration method was used to determine the concentration of working solutions of chlorine (Dietz Jr et al., 1996). Firstly, 0.1 mol L^{-1} sodium thiosulfate solution was standardized by 2.0 g potassium iodide diluted with 80 mL of water in a 250 mL Erlenmeyer flask. After that, 25 mL of 0.1 mol L^{-1} hydrochloric acid and 10 mL 0.01 mol L^{-1} potassium dichromate was added and stirred homogeneously in five minutes. Afterwards, the mixture solution was titrated with 0.1 mol L^{-1} sodium thiosulphate until the colour of the solution changed from dark brown into yellowish-green, then 1.0 mL of starch was added as the endpoint indicator. Titration was carefully continued, and the endpoint of titration was reached that indicated by observing a clear solution. Titration was performed in three replicates, and the accurate value of sodium thiosulphate concentration in the solution was calculated. Finally, after the sodium thiosulphate solution was standardized, chlorine working solution was determined. Fifty mL of water were used to dilute the 2.0 g potassium iodide, and after that, 1.0 mL chlorine solution and 2.0 mL concentrated glacial acetic acid was added, followed by homogenously stirred in five minutes. The dark brown mixture solution was titrated with standardized 0.1 mol L^{-1} sodium thiosulphate until a yellowish-green solution was formed, then added a 1.0 mL starch indicator. The titration was continued up to the clear endpoint colour solution reached. Determination was conducted in three replicates, and the concentration of chlorin working solution was calculated in mmol L^{-1} unit.

Spectrophotometric method development

The intensity of blue colour from Prussian blue and after their degradation was measured by visible spectrophotometry. Firstly, the measured wavelength of Prussian blue was determined by measuring the absorbance of solutions in the range of between 400 nm and 800 nm, then the obtained maximum wavelength (e.g. 750 nm) was observed and selected for further measurements. The conditions of Prussian blue formation were optimized by varying each concentration in terms of ferrosulfate (0.5 - 3.0 mmol L^{-1})), potassium ferricyanide (1.5 - 4.0 mmol L^{-1}), and hydrochloric acid (0.0 - 2.0 mol L^{-1}). Technically, 1.0 mL of ferrous sulfate and 1.0 mL of potassium ferricyanide with various concentrations of hydrochloric acid in a volumetric flask were mixed. Then distilled water was added up to the mark (e.g. 10 mL) followed by stirring until homogenous solutions were

obtained. A spectrophotometer measured the absorbance of the mixture solutions at 750 nm. The optimum conditions were selected by monitoring the blue colour intensity's absorbance reached the maximum value without the precipitation formed. In addition, the incubation time (1.0 – 30 min) or operating time for colour degradation was evaluated by measuring the solutions constituted of formed Prussian blue and 1.0 mL of 2.8 mmol L^{-1} chlorine working solution. The colour intensity was monitored until the absorbance value remained constant.

Spectrophotometric determination of free chlorine

Determination of chlorine in the samples was performed based on Prussian blue degradation. Briefly, 1.0 mL of ferrosulfate, 1.0 mL of hydrochloric acid, and 1.0 mL of potassium ferricyanide were added into a 10.0 ml volumetric flask, with each concentration was set at optimal conditions stirred for 1 minute. Afterwards, 1.0 mL of the sample that contained chlorine was added and stirred until homogeneous, and then the mixture solution was incubated with the optimal incubation time for the Prussian blue decomposition process. The absorbance was measured using a spectrophotometer at 750 nm. The obtained absorbance was recorded and fitted into a calibration curve to determine the chlorine concentration in the samples. The percentage of degradation of Prussian blue was calculated as follow, with Abs_{sample} was the absorbance value of the solution from Prussian blue with the sample; Abs_{blank} was the absorbance value of the sample without Prussian blue (e.g. sample and their solvent), and $Abs_{control}$ was the absorbance value of the Prussian blue without sample:

$$\%\ Degradation = 100 - \left[\frac{(Abs_{sample} - Abs_{blank)\ x\ 100}}{Abs_{control}}\right]$$

Analytical performance evaluation

Evaluation of some analytical performances in terms of linearity and range, precision, accuracy, and limit of detection as well as limit quantification was carried out according to the guideline from the International Conference on Harmonisation for validation of analytical procedures (ICH-Q2(R1)) (Borman and Elder, 2017). Six dilution concentration series of chlorine solutions (1.5 – 11.0 mmol L^{-1}) were analyzed for linearity and analytical range assessment with three replicates. The linearity was represented by a correlation coefficient (r^2) between the chlorine concentration and their percentage of Prussian blue degradation in the concentration range of measurement. Accuracy assay was performed based on the standard addition method with spiked and unspiked samples. Three spiked sample solution was prepared by adding chlorine working solution with different concentrations, namely 50 % concentration level (2.8 mmol L^{-1}), 100% concentration level (5.6 mmol L^{-1}), and 150% concentration level (8.4 mmol L^{-1}). Afterwards, the percentage of recovery was calculated based on comparing the actual and theoretical concentrations of chlorine in the samples. Precision was evaluated using the same concentration composition of chlorine working solution used in accuracy assessments. The repeatable precision (intra-day) and intermediate precision (inter-day) were evaluated for precision assay. Intra-day precision was measured three times on the same day, while inter-day precision was measured three times on three different days. The relative standard deviation (*% RSD*) value of the obtained measurement data was used to evaluate analytical precision. In addition, the residual standard deviation and the slope value from the linear equation acquired from the calibration

curve calculation were used to estimate the detection and quantitation limits. The detection limit and quantification limit were calculated based on the analytical result's three times and ten times of standard deviation, respectively.

Application of the developed method on disinfectant products

Two disinfection products were employed for applying the developed spectrophotometric method for the real samples. The samples were selected based on the products commonly available in the market (e.g. Purwokerto area) and stated the chlorine as an active ingredient on the label. The samples were labelled and kept at room temperature in sealed containers. The chlorine concentration of each sample of disinfectant product was measured and descriptively presented in the form of the average concentration. Afterwards, the measured concentrations were compared to the concentration listed on each product's packaging.

RESULTS AND DISCUSSION

Spectrophotometric method development

Standardization of chlorine working solution was carried out by iodometric titration method in order to accurately determine the concentration of chlorine in the solution because this solution will be used as a reference solution for further quantification. After the assessment and quantification processes, the result showed that the obtained concentration of chlorine in the working solution was 560 mmol L^{-1}. The titration method was selected because the process was quite simple, robust, and precise. Because of its ability to oxidize iodide (I^-) to iodine (I_2), chlorine (Cl_2) is one compound that can be analyzed using this method. Afterwards, thiosulfate ($S_2O_3^{2}$) will diminish the iodine that has been generated (Watson, 2020). The following are the reactions that occur:

$$Cl_2 + 2I^- \rightarrow 2Cl^- + I_2$$

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$$

Scanning the wavelength of Prussian blue solutions formed by the reaction of ferrous sulfate with potassium ferricyanide in acidic solution was carried out in the range of 500 nm to 800 nm to determine the measuring wavelength of a spectrophotometer. The observed measurement wavelength was selected at 750 nm, giving the higher absorbance in accordance with increasing the investigated concentrations (Figure 1). Prussian blue ($KFe^{III}[Fe^{II}(CN)_6]$)) is a dark blue pigment that can be synthesized chemically by reacting ferrous with ferricyanide (Samain et al., 2013) ions as follows:

$$FeSO_4 \rightarrow Fe^{2+} + (SO_4)^{2-}$$

$$Fe^{II} + K_3[Fe^{III}(CN)_6] \rightarrow KFe^{III}[Fe^{II}(CN)_6] + 2K^+$$

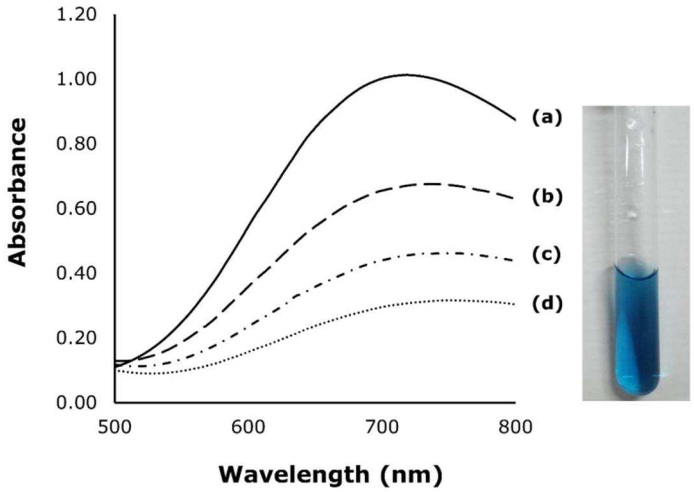

Figure 1. Visible spectrophotometric spectrum profile of formed Prussian blue and their solution colour. (a) Prussian blue without the addition of chlorine. Prussian blue with the addition of (b) 1.12 mmol L^{-1}, (b) 2.8 mmol L^{-1}, and (d) 11.2 mmol L^{-1} of chlorine. Conditions were 2.0 mmol L^{-1} ferrosulfate, 2.5 mmol L^{-1} potassium ferricyanide, and 2.0 mol L^{-1} hydrochloric acid with an incubation time of 1 min.

The measurement wavelength for spectroscopic analysis is carried out at the wavelength that gives maximum absorbance value because the change in absorbance for each unit of concentration is most prominent, and the absorbance curve seems to be flat around. Furthermore, the Lambert-Beer law will be fulfilled by minimizing errors caused by repeated measurement (Watson, 2020). Figure 1 shows the measurement wavelength of formed Prussian Blue at 750 nm, consistent with the visible light spectrum and complementary blue-green colour at 610-750 nm (Day and Underwood, 2002). This finding is also in accordance with the findings of other studies, which indicate that the optimum measurement wavelength for Prussian blue determination is around 700 nm (Matei et al., 2012; Wasito et al., 2021; Zhai et al., 2012).

After the measurement wavelength was determined, the formation of Prussian blue was optimized to obtain the proper conditions for generating Prussian blue before it was degraded by chlorine. Several factors, including the concentration of ferrosulfate, potassium ferricyanide, hydrochloric acidic, and the time for incubation after degradation by chlorine, were optimized and illustrated in Figure 2. It can be summarized that the optimized condition to generate Prussian blue can be obtained from ferrous sulfate, potassium ferricyanide, and hydrochloric acid with the concentration of 2.0 mmol L^{-1}, 3.0 mmol L^{-1}, and 0.5 mol L^{-1}, respectively. Afterwards, 15 minutes' incubation is adequate for degradation of Prussian blue with chlorine in spectrophotometric measurement.

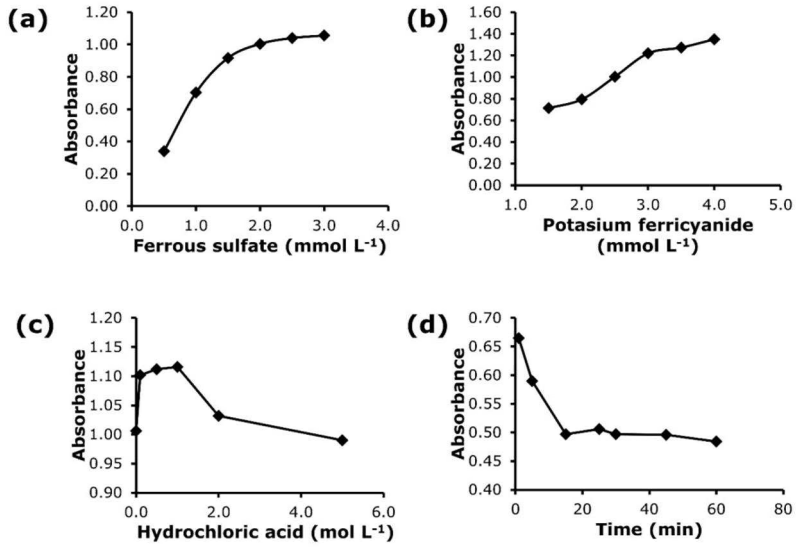

Figure 2. Optimization of sample preparation for Prussian blue formation by visible spectrophotometer at 750 nm. (a) Effect of ferrous sulfate concentration (0.5 – 3.0 mmol/L) on Prussian blue formation under the condition of 2.5 mmol L^{-1} potassium ferricyanide and 2.0 mol L^{-1} hydrochloric acid. (b) Effect of different potassium ferricyanide concentrations (1.5 – 4.0 mmol L^{-1}) on Prussian blue colour development under 2.0 mmol L^{-1} ferrous sulfate and 2.0 mol L^{-1} hydrochloric acid. (c) Effect of the concentration of hydrochloric acid (0.1 – 2.0 mol L^{-1}) on Prussian blue formation under 3.0 mmol L^{-1} potassium ferricyanide and 3.0 mmol L^{-1} ferrous sulfate. (d) Profile of the incubation time (1-60 min) after 3.0 mmol L^{-1} chlorine was added and the absorbance observed under the conditions of 2.0 mmol L^{-1} ferrous sulfate and 0.5 mol L^{-1} hydrochloric acid.

Chemicals such as potassium ferricyanide and ferrous sulfate are needed to generate Prussian blue. More Prussian blue is generated as the concentration of the two reagents increases, resulting in increased absorbance of the solutions (Teepoo et al., 2012). Figures 2(a) and 2(b) show that potassium ferricyanide and ferrous sulfate with concentrations of 3.0 mmol L^{-1} and 2.0 mmol L^{-1}, respectively, provide absorbance with a greater and more stable value. Furthermore, Figure 4(c) shows that hydrochloric acid at a concentration of 0.5 mol L^{-1} produces an appropriate absorbance value. However, at concentrations over 1 mol L^{-1}, the absorbance value decreases. Prussian blue is stable in acidic environments. Therefore the acid concentration used in its production should be optimized (Bai et al., 2009).

Meanwhile, a higher alkaline condition is incompatible with the synthesis of Prussian blue because ferrous hydroxide can develop in this pH range, making Prussian blue more unstable (Zargar and Hatamie, 2014). However, excessively hydrochloric acid can cause a reduction in pH value, which can reduce the solubility of inorganic compounds such as potassium ferricyanide that can reduce the formation of Prussian blue (Meeussen et al., 1992). In addition, to determine the correlation between the absorbance measurements and incubation time, the operating time of Prussian blue degradation after adding chlorine is optimized. The amount of Prussian blue that decomposes is proportional to the amount of chlorine added to the mixture solutions. The absorbance of Prussian blue degradation remains stabilized after 15 minutes, as shown in Figure 2(d).

Analytical performance and application

Prussian blue was mixed with a six-series concentration of chlorine working solution to evaluate their analytical performance in terms of linearity, which was repeated three times of measurements. The linearity (r^2) value obtained using linear regression calculation between the percentage of degradation of Prussian blue and the concentration of chlorine was 0.9577 in the range of 1.5-11.0 mmol L^{-1} (Figure 3(a)). The relationship between chlorine concentration and compound absorption from the reaction of chlorine with Prussian blue revealed that the higher the chlorine concentration, the more Prussian blue decomposes, as demonstrated by the disappearance of the blue colour in the solution (Figure 3(b)).

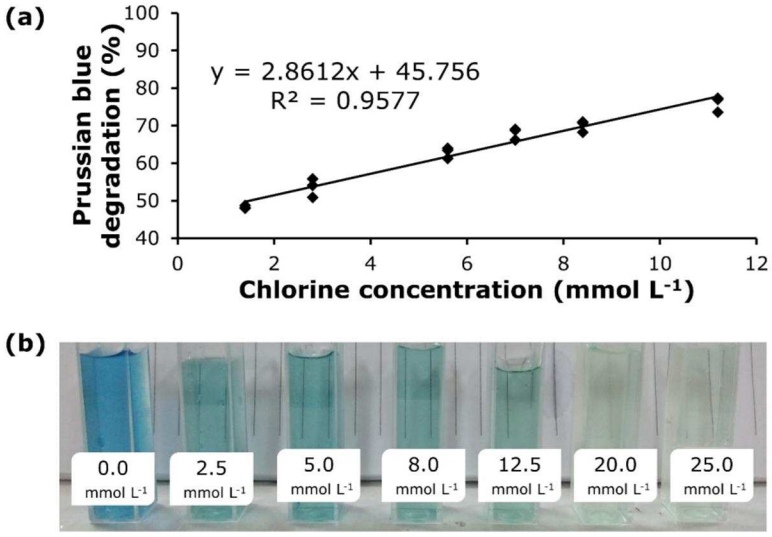

Figure 3. Prussian blue degradation profile after incubated with chlorine. (a) Linear regression from the calibration curve and (b) solutions colour of Prussian blue degradation by various chlorine concentrations. Conditions were 2.0 mmol L^{-1} ferrosulfate, 3.0 mmol L^{-1} potassium ferricyanide, and 0.5 mol L^{-1} hydrochloric acid with an incubation time of 15 min.

In this study, the assessment of analytical precision and accuracy was carried out using the standard addition technique with three different concentration levels in three replicates (Table 1). The obtained percentage recovery values for measuring the chlorine concentration in the solution were between 91.67 % and 100.41 %. The percentage of recovery of an analytical method close to 100 % meets the analytical requirement (Borman and Elder, 2017). Afterwards, the precision evaluation showed an RSD value between 1.87% and 8.72 % for inter-day precision and between 2.46 % and 11.69 % for intra-day precision. In addition, the limit of detection and quantification of the chlorine determination was performed based on the Prussian blue decomposition reaction utilizing visible spectrophotometry. The findings revealed that the detection and quantification limits were 0.7 mmol L^{-1} and 2.3 mmol L^{-1}, respectively.

Table 1 Accuracy and precision assessment of chlorine determination by visible spectrophotometric (n = 3)

Level (%)	Sample	Chlorine added	Intra-day		Inter-day	
			Chlorine found	Recovery	Chlorine found	Recovery
		(mg/L)	(mg/L)	(%)	(mg/L)	(%)
50	1	2.80	2.80	100.00	4.80	100.60
	2	2.80	2.57	91.78	4.17	106.99
	3	2.80	3.60	109.28	6.80	106.58
	Average			**100.35**		**104.72**
	% RSD			**8.72**		**3.40**
100	1	5.60	5.29	94.46	7.59	99.72
	2	5.60	5.48	99.64	6.81	100.60
	3	5.60	5.41	96.60	9.20	96.02
	Average			**96.90**		**98.78**
	%RSD			**2.60**		**2.46**
150	1	8.40	7.55	89.88	9.85	93.39
	2	8.40	7.80	91.67	9.44	73.85
	3	8.40	7.84	93.33	11.06	86.26
	Average			**100.35**		**84.50**
	% RSD			**8.72**		**11.69**

The developed method was then used to determine the chlorine concentration in commercially available products. Table 2 shows the findings of the chlorine content determination, which reveal that the chlorine concentration of more than 85 % is in line with the amounts mentioned on the product label. Chlorine is a volatile, unstable molecule that easily decomposes into other compounds, allowing for the measurement of the products found to be lower than the initial condition due to storage environments (March and Simonet, 2007).

Tabel 1 Chorine concentration in the investigated products that determined by spectrophotometry based on Prussian blue degradation

Product	Claimed concentration in the product (%)	Measured concentration (%)	Percentage of label claimed found in the product (average ± SD in %)
1	5,25	4,49	85,19 ± 0,79
2	5,25	4,57	86,58 ±1,08

CONCLUSION

Visible spectrophotometry can be used to monitor the degradation of Prussian blue by the presence of chlorine. The visible spectrophotometry method was successfully developed for free chlorine determination based on Prussian blue degradation with the analytical performances, namely linearity, accuracy, and precision, were still acceptable for analysis purposes. The developed method can also be used as an alternative routine analysis of chlorine concentration in disinfection products.

REFERENCES

Bai, J., Qi, B., Ndamanisha, J.C., Guo, L., 2009. Ordered mesoporous carbon-supported Prussian blue: Characterization and electrocatalytic properties. *Microporous and Mesoporous Materials* 119, 193–199. https://doi.org/10.1016/j.micromeso.2008.10.030

Borman, P., Elder, D., 2017. Q2 (R1) validation of analytical procedures. *ICH Quality guidelines* 5, 127–166.

Day, R., Underwood, A., 2002. *Analisis Kimia Kuantitatif Edisi Keenam*. Jakarta: Erlangga.

Dietz Jr, E.A., Cortellucci, R., Williams, M., 1996. A study of analysis errors caused by nitrite and free available chlorine during iodometric titration of total residual chlorine in wastewater. *Water Environment Research* 68, 974–980.

Kodera, F., Umeda, M., Yamada, A., 2005. Determination of free chlorine based on anodic voltammetry using platinum, gold, and glassy carbon electrodes. *Analytica Chimica Acta* 537, 293–298. https://doi.org/10.1016/j.aca.2005.01.053

Leggett, D.J., Chen, N.H., Mahadevappa, D.S., 1982. Rapid determination of residual chlorine by flow injection analysis. *Analyst* 107, 433–441. https://doi.org/10.1039/AN9820700433

March, J.G., Simonet, B.M., 2007. A green method for the determination of hypochlorite in bleaching products based on its native absorbance. *Talanta* 73, 232–236. https://doi.org/10.1016/j.talanta.2007.03.027

Matei, N., Dobrinas, S., Radu, G.L., 2012. Spectrophotometric determination of ascorbic acid in grapes with the Prussian blue reaction. *Analele Universitatii Ovidius Constanta. Seria Chimie* 23, 174–179.

McGlynn, W., 2004. *Guidelines for the use of chlorine bleach as a sanitizer in food processing operations.*

Meeussen, J.C.L., Keizer, M.G., Van Riemsdijk, W.H., De Haan, F.A.M., 1992. Dissolution behavior of iron cyanide (Prussian blue) in contaminated soils. *Environ. Sci. Technol.* 26, 1832–1838. https://doi.org/10.1021/es00033a019

Moberg, L., Karlberg, B., 2000. An improved N,N′-diethyl-p-phenylenediamine (DPD) method for the determination of free chlorine based on multiple wavelength detection. *Analytica Chimica Acta* 407, 127–133. https://doi.org/10.1016/S0003-2670(99)00780-1

Nickmilder, M., Carbonnelle, S., Bernard, A., 2007. House cleaning with chlorine bleach and the risks of allergic and respiratory diseases in children. *Pediatric allergy and immunology* 18, 27–35.

Ordeig, O., Mas, R., Gonzalo, J., Del Campo, Fco.J., Muñoz, F.J., de Haro, C., 2005. Continuous detection of hypochlorous acid/hypochlorite for water quality monitoring and control. *Electroanalysis* 17, 1641–1648. https://doi.org/10.1002/elan.200403194

Parnomo, A., 2003. *Pembuatan Cairan Pemutih*. Jakarta: Puspa Swara.

Ropp, R.C., 2012. *Encyclopedia of the alkaline earth compounds*. Newnes.

Samain, L., Grandjean, F., Long, G.J., Martinetto, P., Bordet, P., Strivay, D., 2013. Relationship between the synthesis of prussian blue pigments, their color, physical properties, and their behavior in paint layers. *J. Phys. Chem. C* 117, 9693–9712. https://doi.org/10.1021/jp3111327

Soldatkin, A.P., Gorchkov, D.V., Martelet, C., Jaffrezic-Renault, N., 1997. New enzyme potentiometric sensor for hypochlorite species detection. *Sensors and Actuators B: Chemical* 43, 99–104. https://doi.org/10.1016/S0925-4005(97)00144-5

Teepoo, S., Chumsaeng, P., Jongjinako, S., Chantu, K., Nolykad, W., 2012. A New Simple and Rapid Colorimetric Screening Test for Semi-qualitative Analysis of Vitamin C in Fruit Juices Based on Prussian Blue. *Journal of Applied Sciences* 12, 568–574. https://doi.org/10.3923/jas.2012.568.574

Tri, J., 2010. Unit Produksi Dalam Sistem Penyediaan Air Minum. Yogyakarta: Graha Ilmu.

Wasito, H., Purnamasari, D., Fareza, M.S., 2021. Mefenamic acid determination in tablet formulations using a selective and accurate spectrophotometric method based on prussian blue formation. *Malaysian Journal of Analytical Sciences* 25, 71–80.

Watson, D.G., 2020. P*harmaceutical analysis E-book: a textbook for pharmacy students and pharmaceutical chemists*. Elsevier Health Sciences.

Xu, J., Feng, K., Weck, M., 2011. Free chlorine sensing using an interferometric sensor. *Sensors & Actuators: B. Chemica*l.

Zargar, B., Hatamie, A., 2014. Prussian blue nanoparticles: a simple and fast optical sensor for colorimetric detection of hydralazine in pharmaceutical samples. *Anal. Methods* 6, 5951–5956. https://doi.org/10.1039/C4AY00618F

Zhai, J., Yong, D., Li, J., Dong, S., 2012. A novel colorimetric biosensor for monitoring and detecting acute toxicity in water. *Analyst* 138, 702–707. https://doi.org/10.1039/C2AN36160D

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENT

The authors would like to thank the Institute for Research and Community Services of Jenderal Soedirman University, Purwokerto for financial support of this research project and thanks to Pharmaceutical Chemistry Laboratory, Jenderal Soedirman University for the facilities support.

AUTHORS CONTRIBUTIONS

Hendri Wasito: Conceptualization, methodology, validation, investigation, resources, visualization, supervision. Defi Srium Siagian: Methodology, formal analysis, validation, investigation, visualization, data curation. Muhamad Salman Fareza: Methodology, supervision. Methodology, formal analysis, validation, investigation. All authors contribute to the data interpretation, writing, and approval of the final version of the manuscript.