World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Determination of the optimum concentration of the coupling agent in chloramphenicol analysis

Navirra Ayu Khudzaifah and Maria Monica Sianita Basukiwardojo *

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Indonesia.

World Journal of Advanced Research and Reviews, 2022, 15(01), 525–533

Publication history: Received on 14 June 2022; revised on 20 July 2022; accepted on 22 July 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.15.1.0719

Abstract

The aim of this research is to determine the optimum concentration of the coupling agent used in chloramphenicol analysis, then calculate the limit of detection of that photometric reaction. Chloramphenicol is a colorless compound that is often abused in animals whose products are consumed by humans. Chloramphenicol has side effects on the human body. So, chloramphenicol must be analyzed. To facilitate the analysis process, chloramphenicol must be converted into colored compounds called azo compounds. To make azo compounds must go through the process of reduction, diazotization, and coupling. A coupling agent is an important compound that played a role in an azo compound formation and was analyzed using UV-Vis spectrophotometer. This research, used N-(-1-Naphthyl) ethylenediamine dihydrochloride (NEDA) with variations concentrations of 0.1%, 0.3%, 0.5% at 565 nm. The optimum coupling concentration that gives maximum absorbance was 0.1%. The detection limit (LOD) obtained by using the optimum coupling is 0.0498 µg/mL, the limit of quantitation (LOQ) is 0.1660 µg/mL, the sensitivity value is 0.1425, and a correlation coefficient (R2) of 0.999 with a concentration range of chloramphenicol is 0,14 - 1,4 ppm.

Keywords: Chloramphenicol; Photometry; NEDA; LOD; LOQ; Concentration

1. Introduction

Chloramphenicol (CAP) is a colorless compound used as an antibiotic with a broad spectrum originally isolated from the bacterium *Streptomyces venezuelae* but is now produced through a relatively straightforward synthesis [1]. Chloramphenicol is commonly used to treat Gram-positive and Gram-negative bacterial infections. Chloramphenicol can enter the human body through the food chain then accumulate, resulting in adverse effects. The chloramphenicol side effects include aplastic anemia, kidney damage, and diarrhea [2]. Therefore, it has been restricted and even banned in most country. The Maximum Required Performance Limit (MRPL) for chloramphenicol in foods of animal origin established by the European Union (EU) is 0.15 µg/kg [3]. However, there are still numerous illegal uses of chloramphenicol because it is cheap and widely available. According to a study titled "Natural antimicrobial resistance of the Chicken Food Chain" conducted by World Animal Protection and the Indonesian Consumers Foundation (YLKI), 120 samples contained *E.Coli* bacteria were resistant to five antibiotics, including chloramphenicol. It is anticipated that as many as 761.27 tons of antibiotics will be administered to animals in Indonesia by 2020 [4].

Therefore, research must be conducted on the analysis of chloramphenicol. So far, chloramphenicol analysis has been carried out using instruments including High Performance Liquid Chromatography (HPLC) [5] [6] [7], Liquid Chromatography Mass Spectrometer (LC-MS/MS) [8], ELISA [9] [10], immunoassays[11] and others. HPLC is typically the instrument of choice. However, there are several disadvantages to chloramphenicol analysis using HPLC, such as the high cost, infrequent HPLC instruments, complicated procedures, and long waiting time, so it is necessary to develop analytical methods that are simple to observe, quick, and inexpensive. One method that is fast and easy to observe is the

* Corresponding author: Maria Monica Sianita
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Indonesia.

photometric reaction-based method because it produces a colored solution that is easy to be observed [12]. The chemical structure of chloramphenicol is as follows:

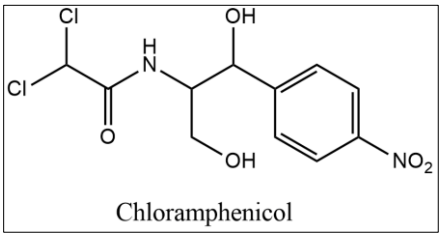

Figure 1 Structure of Chloramphenicol [13]

The photometric method for the analysis of chloramphenicol can be carried out with the formation of azo compounds [14]. A reduction, diazotization, followed by a coupling reaction, are necessary for the formation of azo compounds. *N-(-1-Naphthyl)ethylenediamine dihydrochloride* (NEDA) is used as coupling. The absorbance was then determined using UV-Vis spectrophotometer. (Hussein, K et al. 218) [15] conducted their experiments of analyzed chloramphenicol using a naphthalene-1,5-diamine coupling and found the *Limit of Detection* (LOD) of 0.241 g/mL and the Limit of Quantitation (LOQ) of 0.804 µg/mL. Then, based on the research form (Wafi A. et al., 2020) [16] analyzed chloramphenicol using a 2-naphthol coupling, the *Limit of Detection* (LOD) and the *Limit of Quantitation (LOQ) of 0.36 µg/mL and 1.19 µg/mL. This research utilized an* N-(-1-Naphthyl) *ethylenediamine dihydrochloride* (NEDA) coupling, and concentration was optimized to achieve a lower detection limit.

2. Material and methods

2.1. Materials and Tool

The following materials were used in this research: chloramphenicol (Sigma Aldrich), aquabidest, ethanol (Sigma Aldrich), formic acid (Merck), Zn powder (Sigma Aldrich), HCl 37% (Merck), $NaNO_2$ (Sigma Aldrich), ammonium sulfate (Sigma Aldrich), and *N-(-1-Naphthyl)ethylenediamine dihydrochloride* (Sigma Aldrich).

The tools used in this research is an analytical scale (OHAUS PA244), Vortex (Wizard IR Infrafed Vortex Mixer), Magnetic stirrer 8×40 mm (Velp Scientifica), Hot Plate Stirer (WiseStir MSH-20D), Glass Funnel, Beaker, Erlenmeyer, measuring pipette, volume pipette, and UV-Vis Spectrophotometer.

2.2. Research Method

2.2.1. Preparation of chloramphenicol 100 ppm

A total of 0.1 grams of chloramphenicol powder was weighed and dissolved in 100 mL of ethanol to make 1000 ppm chloramphenicol solution. Then, 1 mL of 1000 ppm chloramphenicol solution was taken and dissolved in 10 ml of ethanol. so that it becomes a 100 ppm chloramphenicol solution.

2.2.2. Preparation working solution of chloramphenicol

Diluted stock solution so that it becomes a chloramphenicol solution with a concentration of 0.14 - 1.4 ppm.

2.2.3. Reduction

A 5 mL of chloramphenicol solution was added to the Erlenmeyer, followed by 2.5 mL distilled water and 2.5 mL of 90% formic acid. Then, 0.3 grams of zinc (Zn) powder was added. Then, stirred at 350 rpm for 40 minutes. The filtrate and precipitate were then separated by filtration.

2.2.4. Diazotization

Diazo salts were prepared by mixing the reduced filtrate with concentrated HCl, $NaNO_2$, and ammonium sulfamate. After each addition, it was vortexed and cooled at a temperature of 0 – 5 ºC. It was then coupled with the coupling *N-(-1-Naphthyl) ethylenediamine dihydrochloride* (NEDA).

3. Results and discussion

3.1. Formation of Azo Compounds

This research analyzed chloramphenicol using a UV-Vis spectrophotometer instrument to convert it to a purple azo compound. Chloramphenicol contains -NO_2, -NH, and -OH groups. The first step is reduction of chloramphenicol. Figure 2 showed the mechanism of the reduction.

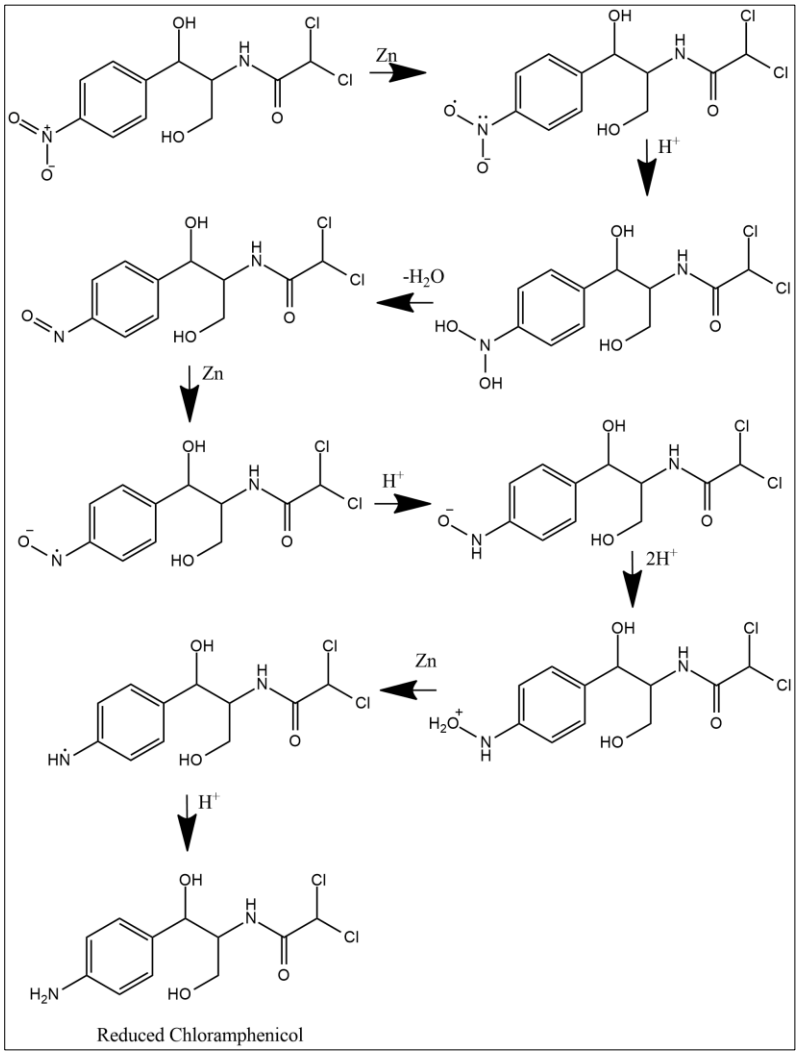

Figure 2 Reaction for the Formation of Reduced Chloramphenicol [17]

Reduced chloramphenicol is formed by adding formic acid and Zn powder during the reduction step, the nitro group in chloramphenicol will be converted into a primary amine group. Zn powder can reduce nitro groups to primary amine groups in acidic conditions [18]. The Nitro group in chloramphenicol is reduced to the nitroso group and is followed by the reductive addition of two hydrogen atoms to form the hydroxylamine. The last step is water elimination to chloramphenicol's formed primary amine group.

The second step is diazotization to formed diazonium salts. Diazonium salts are formed by the reaction of nitrites with primary aromatic amines under acidic conditions. The reaction of reduced chloramphenicol with concentrated HCl as an acid agent and $NaNO_2$ produces nitric acid (HONO) and water. Because it produces a stable diazonium salt, HCl is utilized as an acid donor. Furthermore, ammonium sulfamate is added to remove excess nitric acid, and water molecules are lost along with the formation of the diazonium salt [19]. The removal of excess nitric acid is necessary because it can damage the formation of color in azo compounds such as reaction oxidation of coupling components. The reaction to form diazonium salts must be carried out at low temperatures, around 0 – 5 ºC, because it has low stability at high temperatures If the diazonium salts are unstable, can be possibility of losing nitrogen [20]. The diazotization reaction

is influenced by several things. Namely, the temperature must be low, from 0 - 5 ºC, carried out in an acidic environment, and the reaction speed because the formation of diazonium salts tends to be slow, so it is necessary to shake it [21]. Figure 3 showed the mechanism of the diazotization.

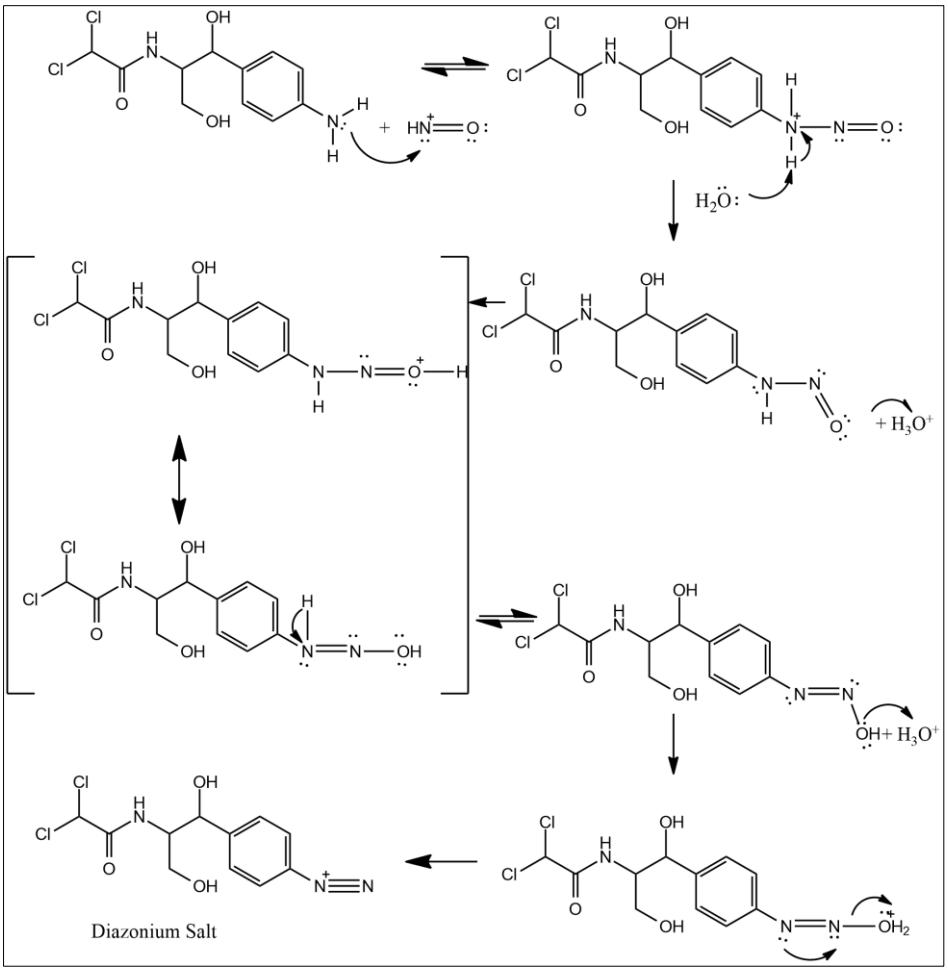

Figure 3 Diazonium Salt Formation [18]

The mechanism of diazonium salt is, first, the formation of nitrosonium ions. Sodium nitrite is protonated with a strong acid such as HCl to produce nitric acid. Then, nitric acid forms nitrosonium ions through protonation of OH and resultant water loss. HCl is used because it can convert nitric acid into electrophile NO^+, the nitrosonium ion. The next step is the formation of the diazonium ion, which also requires acid. The primary amine group will react with the nitrosonium ions to give N-nitrosamine. When the nitrosonium ion reacts with a primary amine group, its positive charge shifts on the nitrogen of the primary amine group as nitrogen attached with aromatic amine gives its lone pair of electrons to the nitrosonium ion. As a result, a nitrogen-nitrogen bond is formed between the primary amine group and nitrosonium ions. Now deprotonation takes place, which gives N-nitrosamine as a product. In excess acid, N-nitrosamine can be converted into a diazohydroxide by protonation and deprotonation. The final step is forming the nitrogen-nitrogen triple bond accompanied by the expulsion of water to produce diazonium salt [22].

The last step is formation of azo compound. azo compounds are formed by reacting diazonium salts with coupling compounds, in this case, *N-(-1-Naphthyl)ethylenediamine dihydrochloride* (NEDA), which has an ortho or para-directed group and concentrated HCl under acidic conditions to produce purple azo compounds. The electrophile of the diazonium salt reacts with a NEDA coupling containing an NH_2-containing electron-releasing group. The diazonium salt's terminal nitrogen attacks the para position of the NEDA. The use of NEDA compounds because can be used in acidic conditions and is commonly used to analyze samples containing primary amine groups [23]. Figure 4 showed the mechanism of azo compound formation.

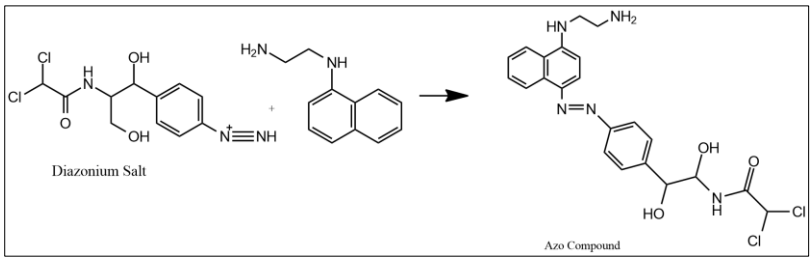

Figure 4 Azo Compound Formation [12]

This research determined the optimal concentration of NEDA as a coupling agent for chloramphenicol analysis. The variations concentration of NEDA coupling is 0.1%, 0.3%, and 0.5%. To determine the absorbance of each NEDA concentration using chloramphenicol at 0.4 – 1 ppm. Table 1 showed the resulting absorbance.

Table 1 Comparison absorbance of various concentrations of NEDA

Concentration (ppm)	NEDA 0.1%	NEDA 0.3%	NEDA 0.5%
0.4	0.057	0.059	0.062
0.6	0.083	0.088	0.085
0.8	0.116	0.111	0.112
1	0.145	0.145	0.15

In the regression equation y = ax + b, the value of a is the slope that indicates the sensitivity of a method, while the value of b is the intercept [24]. Figure 5 showed the linear curve for variations in the concentration of NEDA 0,1% to the absorbance and obtained the regression coefficient (R^2) 0,9981 with the line equation y = 0.1485x – 0.0037 . The value of the sensitivity is 0, 1485.

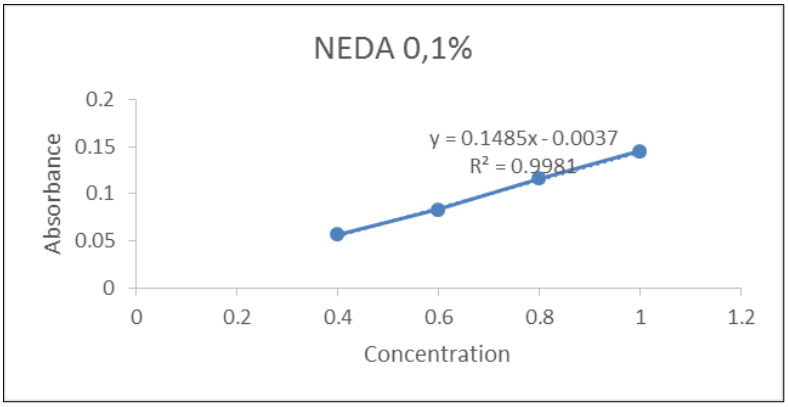

Figure 5 Curve between concentration and absorbance of NEDA 0,1%

Figure 6 showed the linier curve for variations in the concentration of NEDA 0,3% to the absorbance and obtained the regression coefficient (R2) 0,9948 with the line equation y = 0.1405x + 0,0024. The value of the sensitivity is 0,1405.

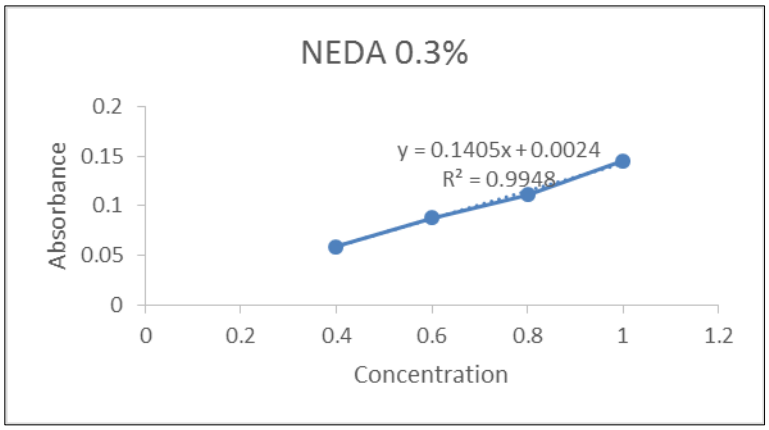

Figure 6 Curve between concentration and absorbance of NEDA 0,3%

Figure 7 showed the linier curve for variations in the concentration of NEDA 0,5% to the absorbance and obtained the regression coefficient (R^2) 0,9863 with the line equation y = 0.1455x + 0,0004. The value of the sensitivity is 0,1455.

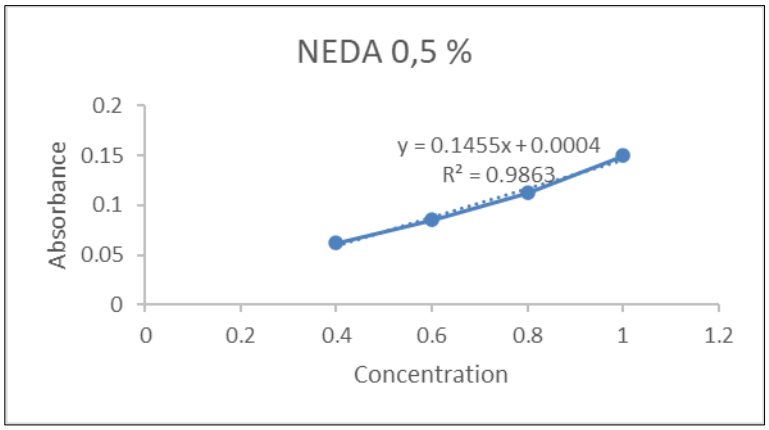

Figure 7 Curve between concentration and absorbance of NEDA 0,5%

The regression coefficient (R^2) and regression equation for the three NEDA concentrations are compared in Table 2. Based on the value of the regression coefficient (R^2) and the slope at the three concentrations of NEDA, it can be conducted that a concentration of 0,1% gives the highest regression coefficient (R^2) and sensitivity value. Therefore, the concentration of the NEDA coupling compound used is 0.1%.

Table 2 Comparison of correlation coefficient (R2) and regression equation of various concentrations of NEDA

NEDA Concentration (%)	Regression Coefficient (R^2)	Regression Equation
0.10	0.9981	y = 0.1485x – 0.0037
0.30	0.9948	y = 0.1405x + 0.0024
0.50	0.9863	y = 0.1455x + 0.0004

Azo compounds were measured by UV-Vis spectrophotometer at a wavelength of 565 nm. The working principle of the UV-Vis spectrophotometric instrument used to analyze chloramphenicol is based on the interaction between electromagnetic radiation and matter, which can be molecules, ions, or atoms. Electronic absorption spectra result from the interaction of molecules having chromophore groups and electromagnetic radiation in the ultraviolet and visible regions [25]. A chromophore group is a functional group with an ultraviolet or visible light absorption spectrum. In addition, there is also an auxochrome group, a functional group that gives absorption intensity in the UV region. Typically, the auxochrome is bound to the chromophore and does not absorb radiation by itself. Examples of chromophore groups include C=C, C=O, N=O, and N=N, while auxochrome groups include OH, NH_2, and CH_3 [26].

3.2. Determination of the Detection Limit of Chloramphenicol

This research is to determine the validation parameters using chloramphenicol concentrations of 0.14 - 1.4 ppm. Validation parameters, such as the determination of linearity, *Limit of Detection* (LOD), and *Limit of Quantitation* (LOQ), can be used to evaluate the performance of the analytical method used in this research. Table 3 show*ed* the resulting absorbance of chloramphenicol concentrations

Table 3 The absorbance of chloramphenicol concentrations

Concentration (ppm)	Absorbance
0.14	0.0178
0.28	0.0367
0.42	0.0563
0.7	0.092
0.98	0.1375
1.4	0.1968

Through the calibration curve used to determine the response of an analytical method, linearity can be determined. The equation y = ax+b and the correlation coefficient (R^2) are derived from the calibration curve. y represents the method's response, the slope, and b is the intercept. The regression equation y = 0.1425x - 0.0036 with a regression coefficient (R^2) of 0.999 is derived from Figure 4. The slope of 0.1425 indicates that the calibration curve demonstrates a linear relationship between concentration and absorbance with a sensitivity value of 0.1425. In addition, the calibration curve with the following formula can be used to determine LOD and LOQ [27] :

$$\text{Standard deviation } (S_{y/x}) = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}} \quad (1)$$

$$\text{Limit of Detection (LOD)} = y_B + 3S_B \quad (2)$$

$$\text{Limit of Quantitation (LOQ)} = y_B + 10S_B \quad (3)$$

Description
y_B = intercept
S_B = standard deviation
The following is the calibration curve for chloramphenicol analysis:

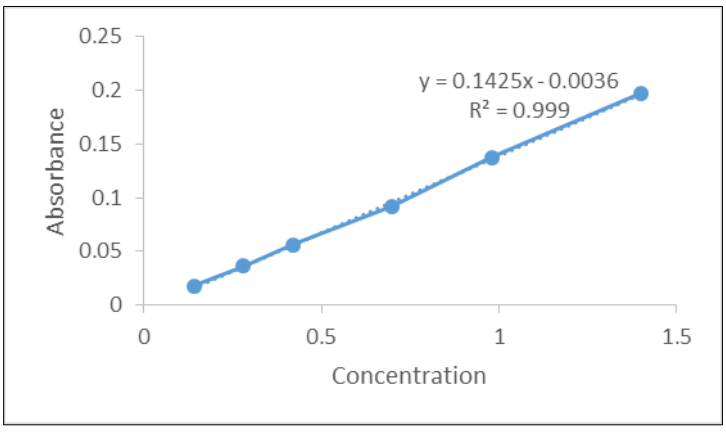

Figure 8 Calibration curve by UV-Vis spectrophotometer

The limit of detection value (LOD) was used to determine the smallest concentration of chloramphenicol that still produced a significant photometric reaction response. Meanwhile, the limit of quantitation value (LOQ) indicates the lowest concentration of analyte contained in a sample that can be quantified. Consequently, the limit of detection (LOD) is 0.0498 µg/mL and the quantitation value is 0.1660 µg/mL.

4. Conclusion

Based on UV-Vis spectrophotometry photometric reactions, the optimum concentration of *N-(-1-Naphthyl) ethylenediamine dihydrochloride* (NEDA) was 0,1%. The limit of detection (LOD) and the limit of quantitation (LOQ) were 0.0498 µg/mL and 0.1660 µg/mL, the sensitivity value is 0.1425, and the calibration curve's correlation coefficient (R^2) was 0.999.

Compliance with ethical standards

Acknowledgments

Author are very grateful to analytical laboratory of Chemistry Department of Universitas Negeri Surabaya, Indonesia for providing the facilities to carry out this research work.

Disclosure of conflict of interest

The authors declared no potential conflicts of interest, financial interest, or personal relationships with respect to the research, authorship, and/or publication of this article.

References

[1] M. Plachetka, M. Krawiec, J. Zakrzewska-Czerwi Nska, and M. Wola Nski, "AdpA positively regulates morphological differentiation and chloramphenicol biosynthesis in Streptomyces venezuelae," *Microbiol. Spectr.*, vol. 9, no. 3, 2021.

[2] Z. Khoshbin, A. Verdian, M. R. Housaindokht, M. Izadyar, and Z. Rouhbakhsh, "Aptasensors as the future of antibiotics test kits-a case study of the aptamer application in the chloramphenicol detection," *Biosens. Bioelectron.*, vol. 122, pp. 263–283, Dec. 2018, doi: 10.1016/J.BIOS.2018.09.060.

[3] T. E. Commission, "On reference points for action for non-allowed pharmacologically active substances present in food of animal origin and repealing Decision 2005/34/EC," 2019. https://www.legislation.gov.uk/eur/2019/1871/2020-01-31

[4] Agropustaka.id, "Ini Materi Siaran Pers YLKI Tentang Bakteri Resisten Antibiotik Pada Daging Ayam," 2021. [Online]. Available: https://www.agropustaka.id/kabar/ini-materi-siaran-pers-ylki-tentang-bakteri-resisten-antibiotik-pada-daging-ayam/

[5] P. Moudgil, J. S. Bedi, R. S. Aulakh, J. P. S. Gill, and A. Kumar, "Validation of HPLC Multi-residue Method for Determination of Fluoroquinolones, Tetracycline, Sulphonamides and Chloramphenicol Residues in Bovine Milk," *Food Anal. Methods*, vol. 12, no. 2, pp. 338–346, Feb. 2019, doi: 10.1007/s12161-018-1365-0.

[6] E. Karageorgou, S. Christoforidou, M. Ioannidou, E. Psomas, and G. Samouris, "Detection of β-Lactams and Chloramphenicol Residues in Raw Milk-Development and Application of an HPLC-DAD Method in Comparison with Microbial Inhibition Assays," *mdpi*, vol. 7, no. 82, 2018, doi: 10.3390/foods7060082.

[7] B. T. Veach, R. Anglin, T. K. Mudalige, and P. J. Barnes, "Quantitation and confirmation of chloramphenicol, florfenicol, and nitrofuran metabolites in honey using LC-MS/MS," *J. AOAC Int.*, vol. 101, no. 3, pp. 897–903, 2018, doi: 10.5740/jaoacint.17-0262.

[8] H. Kikuchi, T. Sakai, R. Teshima, S. Nemoto, and H. Akiyama, "Total determination of chloramphenicol residues in foods by liquid chromatography-tandem mass spectrometry," *Food Chem.*, vol. 230, pp. 589–593, 2017, doi: 10.1016/j.foodchem.2017.03.071.

[9] G. G. Rimkus, T. Huth, and D. Harms, "Screening of stereoisomeric chloramphenicol residues in honey by ELISA and CHARM ® II test-the potential risk of systematically false-compliant (false negative) results," 2019, doi: 10.1080/19440049.2019.1682685.

[10] M. I. Chughtai, U. Maqbool, M. Iqbal, M. S. Shah, and T. Fodey, "Development of in-house ELISA for detection of chloramphenicol in bovine milk with subsequent confirmatory analysis by LC-MS/MS," *J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes*, vol. 52, no. 12, pp. 871–879, 2017, doi: 10.1080/03601234.2017.1361771.

[11] B. Dong *et al.*, "Magnetic assisted fluorescence immunoassay for sensitive chloramphenicol detection using carbon dots@CaCO3 nanocomposites," *J. Hazard. Mater.*, vol. 402, p. 123942, Jan. 2021, doi: 10.1016/J.JHAZMAT.2020.123942.

[12] R. Sharma, H. Krishna, and K. S. M. S. Raghavarao, "Metal Ion–Enhanced Quantification of Chloramphenicol in Milk Using Imipramine Hydrochloride as Diazo-Coupling Agent," *Food Anal. Methods*, vol. 13, no. 12, pp. 2321–2329, 2020, doi: 10.1007/s12161-020-01837-w.

[13] K. A. Scott *et al.*, "A Structural Analysis of the FDA Green Book-Approved Veterinary Drugs and Roles in Human Medicine," *J. Med. Chem.*, vol. 63, no. 24, pp. 15449–15482, 2020, doi: 10.1021/acs.jmedchem.0c01502.

[14] I. Ivanišević, S. Milardović, and P. Kassal, "Recent advances in (Bio)chemical sensors for food safety and quality based on silver nanomaterials," *Food Technol. Biotechnol.*, vol. 59, no. 2, pp. 216–237, 2021, doi: 10.17113/ftb.59.02.21.6912.

[15] K. S. Hussein, A. M. K. Ahmed, and F. Y. Mohammed, "Tikrit Journal of Pure Science," *Tikrit J. Pure Sci.*, vol. 23, no. 9, pp. 1813–1662, 2018, [Online]. Available: http://dx.doi.org/10.25130/tjps.23.2018.141

[16] A. Wafi, G. Supriyanto, and T. S. Tjahjandarie, "Method Validation of Chloramphenicol Analysis in the Shrimp Based on Diazotization Reaction," *Indones. J. Pharm. Sci. Technol.*, vol. 7, no. 2, pp. 1–7, 2020, doi: 10.24198/ijpst.v7i2.26520.

[17] A. Ratnaningsih, "Sintesis Nitrosalisil Alkohol dari Komponen Utama Minyak Gandapura melalui Reaksi Nitrasi serta Uji Aktivitas Antioksidan," Universitas Brawijaya, 2017. [Online]. Available: http://repository.ub.ac.id/id/eprint/155179

[18] M. M. Sianita, "Pengembangan Metode Analisis Kloramfenikol Dalam Akuakultur Secara Fotometrik Menggunakan Molecular Imprinted Polymer (Mip) Sebagai Adsorben," Universitas Airlangga, 2018. [Online]. Available: http://lib.unair.ac.id

[19] M. Fan *et al.*, "Highly sensitive detection of tryptophan (Trp) in serum based on diazo-reaction coupling with Surface-Enhanced Raman Scattering and colorimetric assay," *Anal. Chim. Acta*, vol. 1119, pp. 52–59, 2020, doi: 10.1016/j.aca.2020.04.039.

[20] C. Schotten, "Investigating Multistep Continuous Flow Processes Using Diazonium Salts," pp. 1–259, 2018.

[21] S. Niu *et al.*, "Synthesis of 4-aminobenzenesulfonic acid functionalized carbon catalyst through diazonium salt reduction for biodiesel production," *Energy Convers. Manag.*, vol. 173, pp. 753–762, Oct. 2018, doi: 10.1016/J.ENCONMAN.2018.08.004.

[22] S. S. Kamble and G. S. Shankarling, "Room temperature diazotization and coupling reaction using a DES-ethanol system: A green approach towards the synthesis of monoazo pigments," *Chem. Commun.*, vol. 55, no. 42, pp. 5970–5973, 2019, doi: 10.1039/c9cc01114e.

[23] E. Han, M. Zhang, Y. Pan, and J. Cai, "Electrochemical Self-Assembled Gold Nanoparticle SERS Substrate Coupled with Diazotization for Sensitive Detection of Nitrite," 2022, doi: 10.3390/ma15082809.

[24] K. Kuntari, T. Aprianto, R. H. Noor, and B. Baruji, "Verifikasi Metode Penentuan Asetosal Dalam Obat Sakit Kepala Dengan Metode Spektrofotometri Uv," *JST (Jurnal Sains dan Teknol.*, vol. 6, no. 1, pp. 31–40, 2017, doi: 10.23887/jst-undiksha.v6i1.9398.

[25] S. Prasad *et al.*, "Near UV-Visible Electronic Absorption Originating from Charged Amino Acids in a Monomeric Protein," *Chem. Sci.*, vol. 8.8, pp. 5416–5433, 2017, [Online]. Available: www.rcsb.org

[26] B. Chaudhary and T.-E. Violet, "ISSN NO : 0022-1945 CHEMISTRY OF SYNTHETIC DYES : A REVIEW Volume XII , Issue XI , November / 2020 Page No : 390 Journal of Interdisciplinary Cycle Research ISSN NO : 0022-1945 Page No : 391," vol. XII, no. 390, pp. 390–396, 2020.

[27] J. N. Miller and J. C. Miller, Statistics and Chemometrics for Analytical Chemistry, 6th ed. Pearson Education, 2018.