

## A Novel Spectrophotometric Method for Determination of Chloramphenicol Based On Diazotization Reaction at Room Temperature

Abdul Wafi<sup>1,4,\*</sup>, Ganden Supriyanto<sup>2,3</sup>, and Tjitjik Srie Tjahjandarie<sup>2</sup>

<sup>1</sup>Department of Medical Laboratory Technology, Maharani College of Health Science  
Jl. Simpang Candi Pangung No. 133 Malang, Indonesia

<sup>2</sup>Department of Chemistry, Faculty of Science and Technology, Airlangga University  
Jl. Mulyorejo Kampus C UNAIR Surabaya, Indonesia

<sup>3</sup>Laboratory of Biosensor, Institute of Tropical Disease, Airlangga University  
Jl. Mulyorejo Kampus C UNAIR Surabaya, Indonesia

<sup>4</sup>Department of Pharmacy, Faculty of Science and Technology, Maulana Malik Ibrahim Islamic State University  
Jl. Gajayana No. 50 Malang, Indonesia

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### ABSTRACT

An analytical method for determination of chloramphenicol (CAP) based on the diazotization reaction at room temperature has been developed. The CAP was reduced using zinc powder (Zn) and diazotization reaction was carried out at room temperature in the presence of NaNO<sub>2</sub>, bismuth nitrate pentahydrate (Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O) as catalyst. 2-naphthol was used as coupling agent to form a red-violet solution and the absorbance was measured by UV-Vis spectrophotometer at 554 nm. The optimization of analytical parameters including reducing agent, catalyst, coupling agent and time response were 0.15 g, 0.15 g, 230.67 µg/mL and 8-9 min respectively.

**Keywords:** azo dye; chloramphenicol; diazotization reaction; spectrophotometry

### ABSTRAK

Sebuah metode analitik telah dikembangkan untuk penentuan kloramfenikol (CAP) berdasarkan reaksi diazotasi pada suhu kamar. CAP direduksi dengan menggunakan serbuk seng (Zn) dan reaksi diazotasi dilakukan pada suhu ruang dengan mereaksikan NaNO<sub>2</sub>, bismut (III) nitrat pentahidrat (Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O) sebagai katalis. 2-naftol digunakan sebagai agen pengkopling untuk membentuk senyawa azo yang berwarna merah-ungu dan absorbansi diukur dengan spektrofotometer UV-Vis pada panjang gelombang 554 nm. Optimasi parameter analitik diantaranya agen pereduksi, katalis, agen pengkopling dan waktu respon masing-masing 0,15 g, 0,15 g, 230,67 µg/mL dan 8-9 menit.

**Kata Kunci:** senyawa azo; kloramfenikol; reaksi diazotasi; spektrofotometri

### INTRODUCTION

Chloramphenicol (CAP), is an antibiotic drug. It is broad spectrum antibiotic, isolated from *streptomyces venezuelae*. Chloramphenicol is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. It is widely used because it is inexpensive and readily available. The most serious adverse effect associated with CAP treatment is bone marrow toxicity, which may occur in two distinct forms: bone marrow suppression, which is a direct toxic effect of the drug and is usually reversible, and aplastic anemia, which is idiosyncratic (rare, unpredictable, and unrelated to dose) and generally fatal [1-3]. European Commission, the United States and some other countries have strictly banned

the use of CAP in drug and food product as in eggs, honey, milk and shrimp [4]. The chemical structure of CAP was shown in Fig. 1.

Several analytical methods have been developed for the analysis and determination of CAP, including Chemiluminescence [5], Capillary Zone Electrophoresis [6], Piezoelectric Immunosensor [7], Liquid Chromatography [8], Liquid Chromatography–Mass Spectrometry (LC-MS) [9, 10], Enzyme-Linked Immunosorbent Assay (ELISA) [11], High Performance Liquid Chromatography (HPLC) [12], and Spectrophotometry [13]. In the present work, an analytical protocol for determination of CAP based on diazotization reaction. UV-Vis spectrophotometer was established. The coupling reaction occurred at room temperature in the presence of Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O as

\* Corresponding author.  
Email address : wafi\_ab@yahoo.com

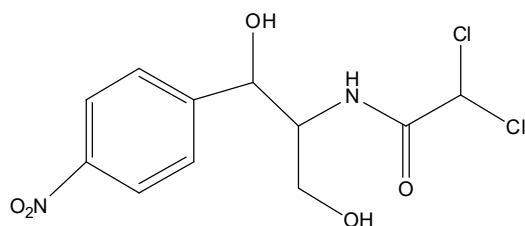


Fig 1. Chemical structure of CAP

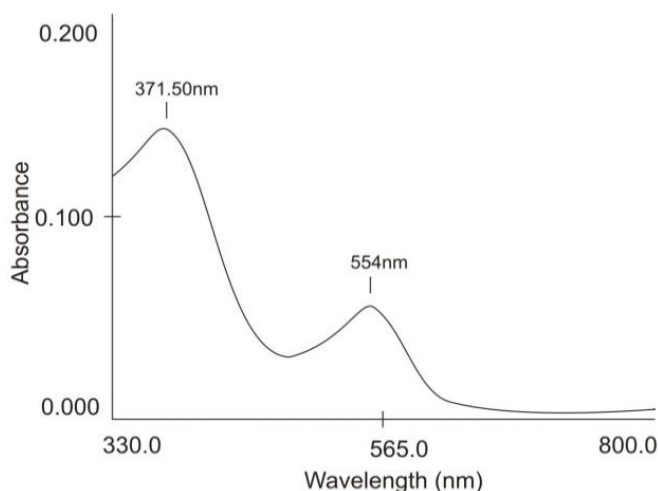


Fig 2. UV-Vis spectra of azo dye

catalyst and 2-naphthol as coupling agent to form azo dye. The analytical parameters including optimization of reducing agent, catalyst, coupling agent and time response for formation of azo dyes had been investigated.

## EXPERIMENTAL SECTION

### Materials

CAP reference standard was purchased from Sigma Aldrich, Singapore.  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  was purchased from Merck, Germany. Ethanol, sodium nitrite, concentrated hydrochloric acid, 2-naphthol and zinc powder were pure analytical grade.

### Instrumentation

The instrumentals were used, including spectrophotometry UV-Vis Shimadzu-1800, Fourier Transform Infra Red (FTIR) Shimadzu-8400S, and glassware.

### Procedure

#### Preparation of reagent

CAP (0.1000 g) was weighed quantitatively and dissolved in ethanol. The solution was transferred to a

100 mL volumetric flask and made up with same solvent to mark.

$\text{NaNO}_2$  (0.5520 g) was weighed quantitatively and dissolved in distilled water. The solution was transferred to a 100 mL volumetric flask and made up with distilled water to mark.

2-naphthol (0.0576 g) was weighed quantitatively and dissolved in 50 mL ethanol. The solution was transferred to a 100 mL volumetric flask and made up with distilled water to mark.

#### Reduction of CAP

A 2.50 mL of CAP solution 969.39  $\mu\text{g}/\text{mL}$  was transferred to a 50 mL glass beaker and added 1 mL distilled water, 1 mL concentrated hydrochloric acid and zinc powder, allowed for 15 min. Then the solution was filtered and transferred quantitatively to a 25 mL volumetric flask and made up with distilled water to mark. The reduced CAP was characterized with FTIR.

#### Preparation of azo dye solution

$\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  (0.15 g) was weighed quantitatively in a 50 mL glass beaker and added 2 mL  $\text{NaNO}_2$  solution, 3 mL of reduced CAP solution. Then, 3 mL of 2-naphthol was added and allowed at room temperature for 8-9 min. The solution was filtered and transferred to a 25 mL volumetric flask, and made up with distilled water to mark. The absorbance of azo dye solution was measured with UV-Vis spectrophotometer at 554 nm.

#### The spectral data of CAP and azo dye

Non-reduced CAP : FTIR (KBr)  $\text{cm}^{-1}$  : 3377, 2935, 2841, 2520, 2145, 1649, 1448, 1408, 1019, 872, 658. Reduced CAP : FTIR (KBr)  $\text{cm}^{-1}$  : 3485, 2922, 2841, 2359, 2065, 1636, 524. Azo dye compound (reduced CAP – 2-naphthol) : UV-Vis Spectrophotometer (Fig. 2) :  $\lambda_{\text{max}}$  (nm) : 371.50 ( $\pi - \pi^*$ , Ar-H), 554 ( $n - \pi^*$ , N=N).

## RESULT AND DISCUSSION

In this study, the spectrophotometric method for determination of CAP based on the diazotization reaction has been performed by reducing of nitro group to amine group on CAP structure using zinc powder. The optimization of zinc powder has been established with various mass of zinc powder. The result of analysis showed that the absorbance increased significantly from 0.05 g to 0.15 g of Zn, but it decreased at 0.20 g of Zn. This is possible to occur side reactions between Zn with 2-naphthol that it interfered the formation of azo compounds. So, The optimum result of CAP reduction was achieved at 0.15 g zinc powder as shown in Fig. 3.

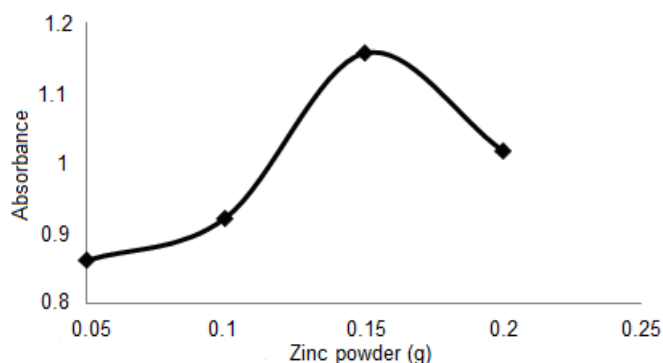


Fig 3. Optimization of CAP reduction by zinc powder

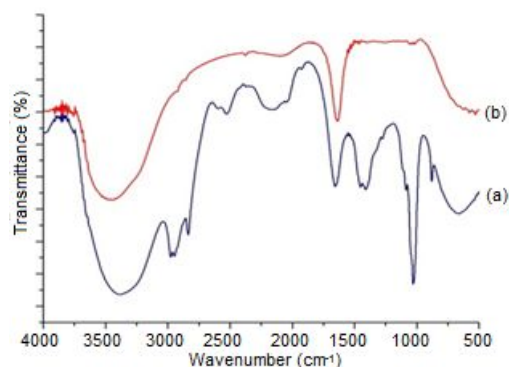


Fig 4. (a) FTIR spectra of non-reduced CAP, and (b) reduced CAP

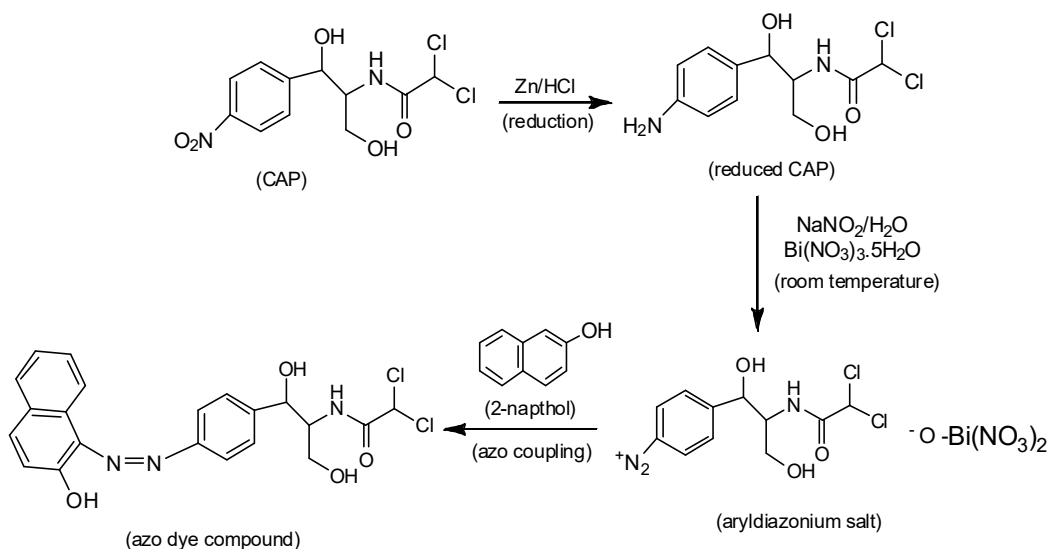


Fig 5. The proposed reaction for formation of azo dye compound [15]

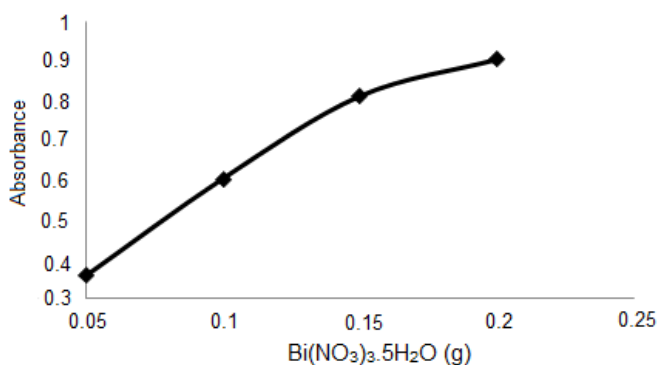


Fig 6. Optimization of catalyst Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O

The spectra of FTIR showed different peak between non-reduced CAP and reduced CAP as shown in Fig. 3. The typical peaks for primary amines were found at 3220-3500 cm<sup>-1</sup> for N-H stretch and at 1617 cm<sup>-1</sup> for N-H bend. However, the Fig. 3 (a) and fig. 3 (b) were difficult to distinguish because the N-H peak overlapped with O-H at 3300-3500 cm<sup>-1</sup> and with C=O stretch at 1640-1700 cm<sup>-1</sup>. The FTIR spectra at 852 cm<sup>-1</sup>

and 1347-1523 cm<sup>-1</sup> were indicated the presence of C-N bonding and NO<sub>2</sub> group respectively as shown in Fig. 4 (a), while in the Fig. 4 (b) those peaks disappear [14]. These different peaks indicated that the nitro group of CAP has been reduced to amine group.

Reduced CAP act as primary aromatic amine for establishing diazonium salt on diazotization reaction. Generally, diazonium salt has poor thermal stability and to avoid decomposition of them, they were handled around 0-5 °C. In this investigation, aryldiazonium salts were synthesized in presence of Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O as catalyst. So that the aryldiazonium salts will be kept stable at room temperature [15].

Generally, reduced CAP, NaNO<sub>2</sub>, and Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O were mixed homogeneously for 1 min. Further, 2-naphthol as coupling agent was added to aryldiazonium salts and mixed homogeneously to form red-violet solution. The absorbance of azo dye solution was measured with UV-Vis spectrophotometer at 554 nm. The proposed reaction for formation of azo dye was shown in Fig. 5.

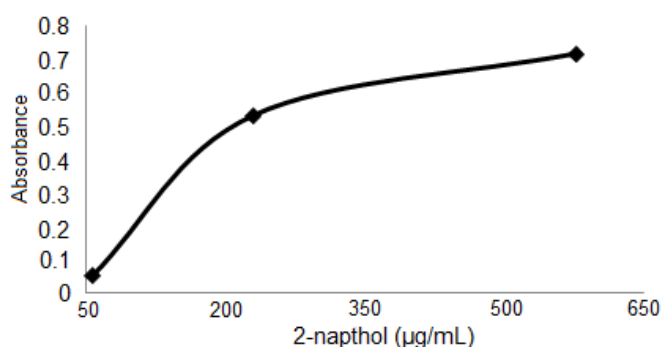


Fig 7. Optimization of coupling agent 2-naphthol

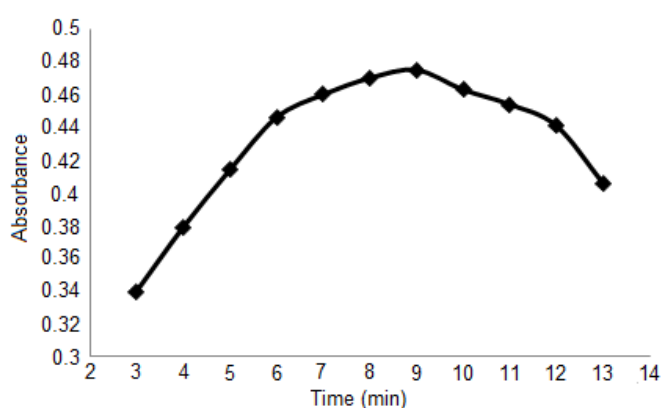


Fig 8. Optimization of response time

The optimization of catalyst and coupling agent showed the increasing of absorbance due to the increasing of mass of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  and concentration of 2-naphthol respectively. Thus, it indicated the increasing of azo dyes. The optimum result of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  and 2-naphthol at 0.15 g and 230.67 µg/mL respectively, as shown in Fig. 6 and Fig. 7. The response time for formation of azo dye compounds has been investigated. In the Fig. 8 was shown the response time optimization of reduced CAP-2-naphthol which showed the increasing of absorbance after 3-9 min but subsequently decreasing because of decomposition of azo dye. The optimum result of response time for formation of azo dye was for 8-9 min. Thus, the proposed method was not taken a long time. So, it is more effective for analysis.

## CONCLUSION

We have successfully demonstrated the spectrophotometric method for determination of CAP based on diazotization reaction at room temperature.

From the present study it can be concluded that this method has simple methodology, easy work-up, short reaction times and low cost.

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