



A novel spectrophotometric method for determination of chloramphenicol based on diazotization reaction at room temperature

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ABSTRACT

A novel spectrophotometric method for determination of chloramphenicol (CAP) based on the diazotization reaction at room temperature has been developed. The CAP was reduced using zinc powder and diazotization reaction was carried out at room temperature in the presence of NaNO_2 , bismuth nitrate pentahydrate 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 analytical parameters including optimization of reducing agent, catalyst, coupling agent and time response for formation of azo dye compounds had been investigated.

Keywords: Azo dye, chloramphenicol, diazotization reaction, spectrophotometric method.

INTRODUCTION

Chloramphenicol (CAP) is an antibiotic drug. It is a broad spectrum antibiotic and isolated from *streptomycesvenenzuelae*. Chloramphenicol is effective against a wide variety of gram-positive and gram-negative bacteria, including most anaerobic organisms. It is widely used because of its inexpensive and readily available nature. 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 [1, 2]. European Commission, the United States and some other countries have strictly banned the use of CAP in drug and food products as in eggs, honey, milk and shrimp [3]. The chemical structure of CAP is shown in Fig. 1.

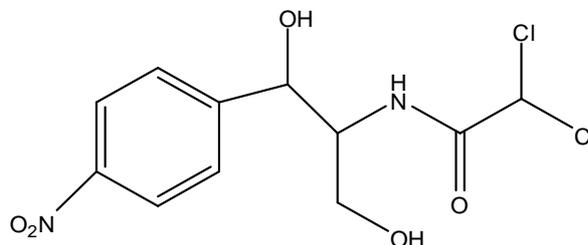


Fig. 1. Chemical structure of CAP

Several analytical methods have been developed for the analysis and determination of CAP including Liquid Chromatography [4], Liquid Chromatography–Mass Spectrometry (LC-MS) [5,6], Enzyme-Linked Immunosorbent Assay (ELISA) [7], High Performance Liquid Chromatography (HPLC) [8], and Spectrophotometry [9]. In the present work, an analytical protocol for determination of CAP based on diazotization reaction. UV-Vis spectrophotometer was established. The coupling reaction was occurred at room temperature in the presence of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ as 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

Reagents

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

Instrumentals

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

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 diluent to mark.
- 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 minutes. 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 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 minutes. 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.

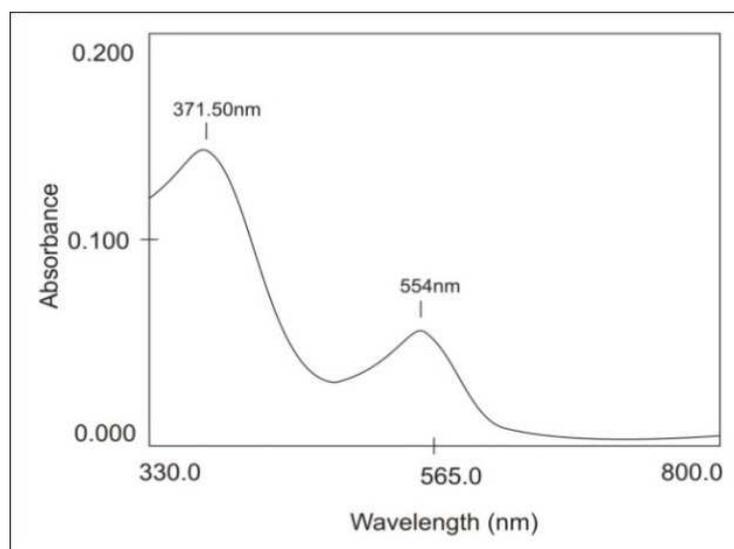


Fig. 2. UV-Vis spectra of azo dye

The spectral data of CAP and azo dye

Non-reduced CAP : FTIR (KBr) cm^{-1} : 3377, 2935, 2841, 2520, 2145, 1649, 1448, 1408, 1019, 872, 658. Reduced CAP : FTIR (KBr) cm^{-1} : 3485, 2922, 2841, 2359, 2065, 1636, 524.

Azo dye compound (reduced CAP – 2-naphthol) : UV-Vis Spectrophotometer (fig.2) : λ_{max} (nm) : 371.50 ($\pi - \pi^*$, Ar-H), 554 (n - π^* , N=N).

RESULTS AND DISCUSSION

In this work, the spectrophotometric method for determination of CAP based on the diazotization reaction has been performed by reducing of nitro group to amine group on chemical structure of CAP 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 was possible to occur side reactions between Zn with 2-naphthol that it interfered the formation of azo compound. So, The optimum result of CAP reduction was achieved at 0.15 g zinc powder as shown in fig. 3.

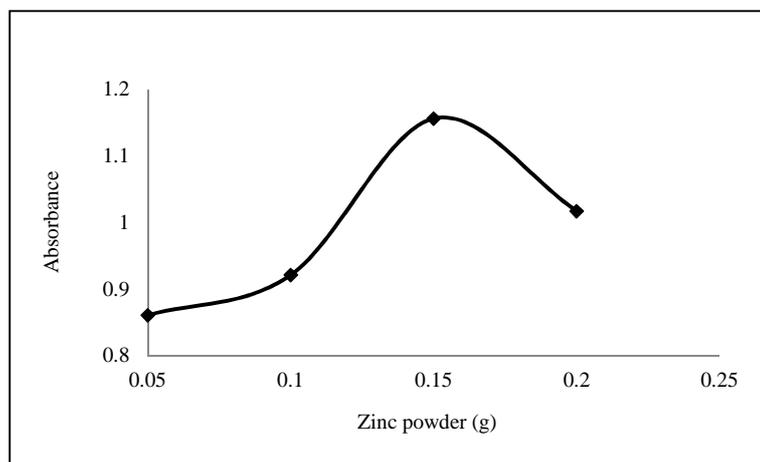


Fig. 3. Optimization of CAP reduction by zinc powder

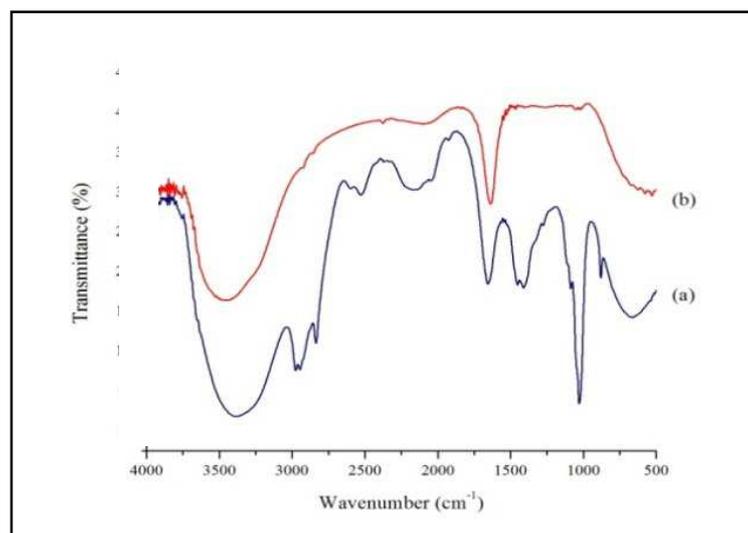


Fig. 4. (a) FTIR spectra of non-reduced CAP, and (b) reduced CAP

The spectra of FTIR showed different peak between non-reduced CAP and reduced CAP as shown in fig. 4. The typical peaks for primary amine was found at 3220-3500 cm⁻¹ for N-H stretch and at 1617 cm⁻¹ for N-H bend. However, the fig. 4 (a) and fig. 4 (b) were difficult to distinguish because the N-H peak overlapped with O-H at 3300-3500 cm⁻¹ and with C=O stretch at 1640-1700 cm⁻¹. The FTIR spectra at 852 cm⁻¹ and 1347-1523 cm⁻¹ were indicated the C-N bonding of NO₂ and NO₂ group respectively as shown in fig. 4 (a), while in the fig. 4 (b) those peaks disappear [10]. 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.

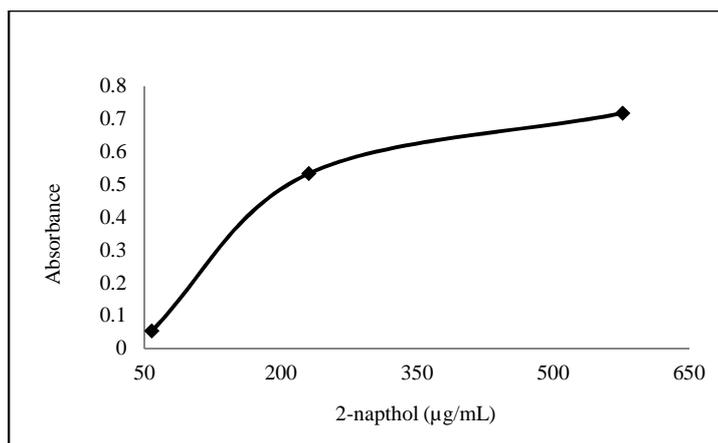


Fig. 7. Optimization of coupling agent 2-naphthol

The response time for formation of azo dye compound has been investigated. In the Fig. 8 was shown the response time optimization of reduced CAP-2-naphthol which is shown the increasing of absorbance after 3-9 minutes but subsequently decreasing because of decomposition of azo dye. The optimum result of response time for formation of azo dye was for 8-9 minutes. Thus, the proposed method was not taken a long time. So, it is more effective for analysis.

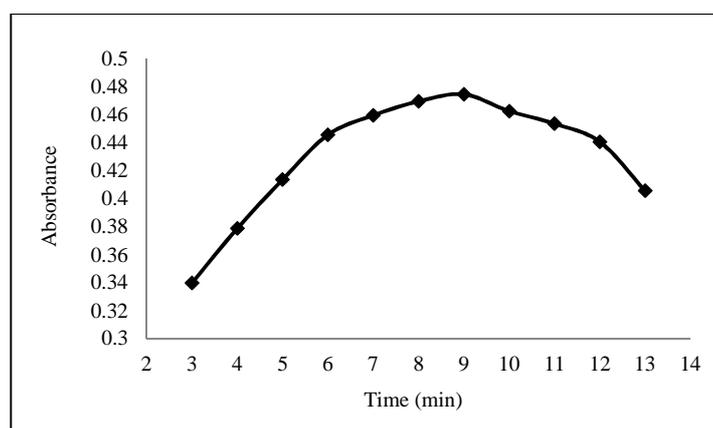


Fig. 8. Optimization of response time

CONCLUSION

We have successfully demonstrated the spectrophotometric method for determination of CAP based on diazotization reaction at room temperature. From the present work, it can be concluded that this method has simple methodology, easy work-up, short reaction times and low cost.

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