RESEARCH ARTICLE

Leishman's Dye as a Novel Reagent in Spectrophotometric Determination of Chloramphenicol

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ABSTRACT

By reviewing the literature, there is no indication concerning the use of Leishman's dye in evaluating drug compounds by dye-color bleaching; hence, it is the first attempt to use Leishman's dye as a novel reagent in the estimation of chloramphenicol (CAP) by an indirect spectrophotometric method in bulk and in its pharmaceutical preparations. The method includes the use of a great amount of N-bromosuccinamide (NBS) in the acidic medium as an oxidizing agent of the drug under investigation (CAP), and then using the residual of NBS for Leishman's dye color bleaching. The absorbance has been measured at 622 nm (the maximum absorption of Leishman's dye). A linear relationship was obtained for the Beer's law with the concentration ranges from 10 to 250 µg/10 mL with acceptable values of molar absorptivity 0.58×10^4 $L.mol^{-1}.cm^{-1}$ and 0.055 $\mu g.cm^{-2}$ of Sandell's sensitivity index, which mean a high sensitivity. An approved estimation of CAP in its various pharmaceutical formulations was found.

Keywords: Bleaching the color, Chloramphenicol, Indirect determination**,** Leishman's dye, N-bromosuccinamide.

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INTRODUCTION

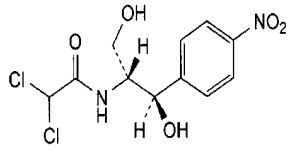
The CAP is a broad spectrum antibiotic. It is isolated from cultures of streptomyces, and it is effective against a wide multiplicity of gram-positive and gram-negative bacteria,[1] it is broadly used because it is inexpensive and readily available.[2] The chemical name of CAP is 2,2-dichloro-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide. It is white or yellowish-white, fine powder (crystalline), somewhat soluble in water, generously soluble in alcohol, and in propylene glycol, and has the following structure (Scheme 1).[3]

The CAP as an ophthalmic dosage with matrix combination such as, hydroxy propyl cellulose and sodium alginate or gel hydroxypropyl methylcellulose, and carbomer to give sustained release of CAP as a treatment for eye's infection.[4,5]

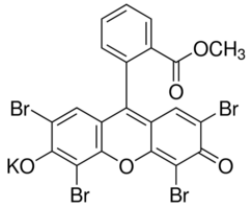
Leishman's stain is used for staining smears; Leishman's stain has a dark blue color; in 1900, Leishman was presented as an Assistant Professor of Pathology in the Army Medical School, and suggested a method for staining blood for malaria and other parasites, which is considered as a modification of the previously presented method by Romanowsky using a compound consisted of methylene blue and eosin (Scheme 2).[6]

To determine the investigated drug, many methods were used previously included: high-performance liquid chromatography (HPLC) which is one of the most powerful and versatile tools for the quantitative determination of CAP[7-9] also liquid chromatography-MS-MS,[10] liquid chromatography. -ESI-MS-MS,[11] liquid chromatography-MS,[12] and GS-MS methods,[13] in addition, other analytical methods included flow injection,[14,15] voltammetry,[16] chemiluminescence[17] atomic absorption spectrometry,[18] and spectrophotometric methods[19-26] were also mentioned.

It is for the first time that a description of a simple, accurate, and precise visible spectrophotometric method for estimating



Scheme 1: CAP structure, M. wt = 323.13 g/mol



Scheme 2: Eosin methylene blue (Leishman's dye, EMB)

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CAP in bulk and in various drugs is presented, the method based on the oxidation of CAP by NBS, then bleaching the color of EMB by the unreacted NBS. The suggested method has been proved successful for estimating CAP in drug formulation.

EXPERIMENTAL

Apparatus

A JASCOV - 630 UV/v spectrophotometer (Japan) with 1 cm matched quartz cells were used for all measurements.

Reagents

All chemicals and reagents used in the present investigation were of analytical-reagents grade.

Chloramphenicol (CAP) Solution 100 µg.mL^{-1}

To prepare 100 µg.mL^{-1} solution, 0.01 gram of CAP was dissolved in 100 mL ethanol in a volumetric flask.

The Working Solution of Leishman Dye, 1.7 × 10^{-3}M

Dissolving 0.15 gram in 50 mL methanol in a dark conical flask which then left for 24 minutes on a magnetic heater (with magnetic bare), after filtration into a 50 mL volumetric flask, it diluted to 50 mL with methanol to reach the mark, to 25 mL a volumetric flask, only 1 mL was taken and diluted to the mark with a distilled water to prepare the working solution.

NCS Solution, 3.2 × 10^{-4} M

An accurate weight equals to 0.0028 gram of NBS (fluke) dissolved in 100 mL distilled water in a volumetric flask.

Hydrochloric Acid Solution, 1 M

Hydrochloric acid solution, 1 M is prepared by using an appropriate volume of concentrated hydrochloric acid (8.6 mL) diluted to 100 mL with distilled water in a volumetric flask.

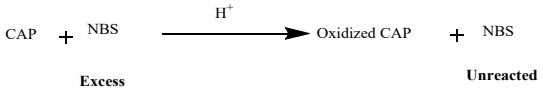
Analytical Procedure

Increasing volumes of CAP solution (100 µg.mL^{-1}) were transferred into a sequence of 10 mL volumetric flasks to cover the range of the calibration graph 10 to 250 µg.10 mL^{-1}, then 1 mL of HCl (1 M) and 3 mL of NBS (3.14 × 10^{-4} M) were added, then the solutions were left at the room temperature (25˚C) for 5 minutes, finally adding 2 mL of EMB dye, after 10 minutes, distilled water was used in diluting the flasks to the mark. The absorbance was measured at 662 nm against the blank.

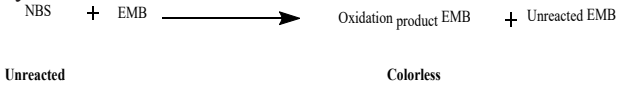
RESULTS AND DISCUSSION

The method comprised of two steps:

- The first step included oxidation of CAP by adding an excess of NBS.



- The second step included bleaching the color of EMB dye by unreacted NBS.



The absorbance measured at 662 nm, which increased linearly with the increasing of CAP concentration. All parameters affected the oxidation of CAP, and the color bleaching of EMP have been studied.

The Optimum Amount of EMP Dye

The change of volumes from 0.25 to 2 mL of EMB dye solution has been engaged, and the volume diluted to 10 mL with distilled water in a volumetric flask. The results indicated that 2 mL of 0.0017 M of EMB was found to be a useful amount for the reaction. It gave the highest intensity with a high determination coefficient (Figure 1).

The Effect of Acids Types and their Amounts

The effect of the acids and their amounts used for the oxidation of CAP was studied, the results obtained by adding various types and volumes of acids (HCl, HNO_3, H_2SO_4, and CH_3COOH), which showed that 1mL of 1 M HCl gave high absorbance with the highest color stability, so that it has been recommended in the subsequent experiments.

The Effect of Temperature

The experiment had been done at room temperature (25°C) gave the highest absorbance. At the temperatures (15, 25, 30, and 40°C), a decrease in absorbance occurred; therefore, all the subsequent measurements were done at room temperature.

The Effect of the Oxidant Reagents

Figure 2 shows that NBS was, NCS and KIO_4 had also been tested, but none gave real advantages compared to NBS as a useful oxidizing agent.

The Effect of the Oxidant Amount

0.5 to 5 mL of 3.14 × 10^{-4} M of NBS has been added to fixed amounts (2 mL) of EMP dye (without CAP). Figure 3 shows that

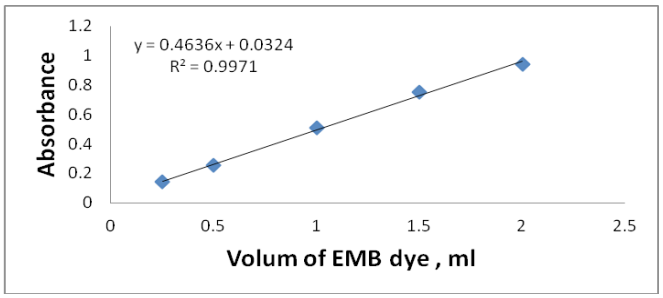


Figure 1: The effect of EMB amount on absorbance

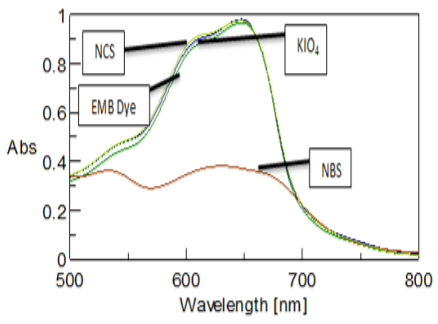


Figure 2: The effect of the oxidant on bleaching the dye

Table 1: The effect of time on oxidation and bleaching the color of EMB dye

	Standing time before dilution (min)						
Standing time of oxidation (min)	*After dilution*	*5*	*10*	*15*	*20*	*25*	*30*
Immediately	0.169	0.172	0.176	0.168	0.169	0.164	0.166
5	0.337	0.348	0.353	0.346	0.341	0.335	0.328
10	0.321	0.334	0.328	0.332	0.318	0.313	0.309
15	0.318	0.320	0.322	0.321	0.313	0.307	0.303
20	0.306	0.308	0.310	0.309	0.305	0.302	0.299
25	0.302	0.304	0.306	0.303	0.301	0.298	0.295

3 mL of NBS solution was sufficient to obtain a maximum of the dye-color bleaching of EMB, 3 mL of NBS solution was recommended for the subsequent experiments.

The effect of optimum time on both the oxidation of CAP by NBS and for color-bleaching of EMB required has been studied. The results showed that the order should be followed as given under the analytical procedure, and the standing time of 5 minutes was necessary for completing oxidation of CAP, and 10 minutes was necessary for color- bleaching of the EMB dye (Table 1).

Calibration Curve

By following the general procedure, a linear relationship has been obtained concerning the absorbance and the concentration of CAP within the range 10 to 250 µg/10 mL (1–25 µg.mL^{-1}), with an acceptable value of determination coefficient (R^2) (Figure 4).

Method Validation

Table 2 shows the various analytical parameters extracted from the suggested method.

Determination of CAP in Pharmaceutical Formulations

The validity of the suggested method for indirect spectrophotometric determination of CAP was checked by the analysis of CAP in different pharmaceutical formulations. The results illustrated in Table 3 indicated valuable accuracy (RE% from -2.9 to 1.4%) and precision (≤1.52%).

Evaluating the Results for the Proposed Method

In order to know the reliability of the proposed method, the content of the CAP in three of its pharmaceutical preparations was estimated by the proposed method and the standard method.[3] The dual t test equation was used. Table 4 demonstrates that the calculated or experimental t exp-value is less than the tabulated value at a confidence level of 95% and for eight determinations, indicating that the method is documented, and there was no significant difference between the two methods.

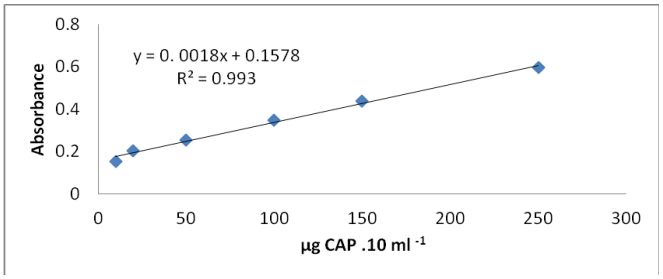


Figure 3: The effect of the oxidant amount

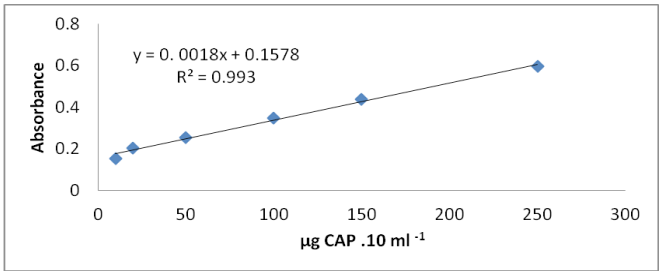


Figure 4: Calibration curve of the CAP determination

Table 2: Analytical parameters of the method

Parameter	*Value*
λ_{max} (nm)	662
Beer's law range	(10–250) µg/10 mL
Molar absorptivity	0.58 × 10^4 L.mol^{-1}.cm^{-1}
Sandell's sensitivity	0.055 µg.cm^{-2}
Stability	60 minutes
Temp. (°C)	25

Table 3: The results of application

Pharma. formula	µg *CAP present/10 mL*	µg *CAP measured/10 mL*	*Rec.* %*	*RSD %*	*RE %*
Phenicol eye drop (Jordon)	50	48.8	97.6	1.01	-2.4
	100	97.1	97.1	1.00	-2.9
CAP-sodium succinate equivalent 1-gram base powder vial (India)	50	50.3	100.6	1.52	0.6
	100	98.6	98.6	0.85	1.40
Chloramphenicol capsules BP 250 mg (India)	50	50.3	100.6	1.27	0.6
	100	98.8	98.8	0.53	1.2

*Average of five determinations

Table 4: Comparison of CAP estimation in pharmaceutical preparations with the proposed and standard method

	Recovery (%)*		
Pharmaceutical preparations	*Present method*	*Standard method*	*T exp.*
Phenicol eye drop (Jordan)	100.1	97.6	2.15
Chloramphenicol capsules BP 250 mg (India)	97.7	102.7	1.99
Chloramphenicol sodium succinate equivalent 1 gram base powder vial (India)	96.8	101	1.90

*Average for five determinations.

Table 5: The comparison of the methods

Analytical parameter	*Present method*	*Literature method*[26]	*Literature method*[19]
Reagent	Leishman's dye	Thymol	NQS
Temperature (°C)	R.T	R.T	R.T
λ_{max} (nm)	662	459	480
Beer's law range (ppm)	1–25	1–12.5	0.8–14
Molar absorptivity ($L.mole^{-1}.cm^{-1}$)	0.58×10^4	2.268×10^4	1.02×10^4
Color of the product	Violet	Yellow	Yellow-orange
Application of the method	Pharmaceutical preparations	Pharmaceutical preparations	Pharmaceutical preparations

Comparison of the Methods

Analytical variables of the proposed method were compared with the same in other spectrophotometric methods used for CAP estimation. Although the results in Table 5 show that the two methods demonstrated in the table are more sensitive, the sensitivity of the proposed method is analytically acceptable.

CONCLUSION

The research presented, new, simple, accurate, and sensitive indirect spectrophotometric method for estimating CAP using NBS as an oxidant agent, and the unreacted NBS bleaching the color of EMB. The method has a prosperous application part for CAP estimation in various pharmacy formulations.

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