



Assay of Chloramphenicol in its Pharmaceutical Preparation (eye drop) via Oxidation and Bleaching Color of Neutral Red Dye

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Abstract:

A simple and rapid spectrophotometric method for the determination of chloramphenicol in its pure form and in its pharmaceutical preparation (eye drop) was suggested. The method involved the use of a new reagent, Neutral red (NR) dye. The method depends on the oxidation of reduced chloramphenicol with an excess amount of N-bromosucinimide (NBS) in an acidic medium, and then the remaining amount of (NBS) was used to bleach the color of the NR dye and measure the absorbance of the residual amount of dye at the wavelength 523 nm which is directly proportional to the concentration of chloramphenicol. All factors that affected the two reactions were studied, and these factors include the type and amount of acid used, the amount of oxidizing agent, the reaction medium, the times required for complete oxidation, and the bleaching time, it was recommended to use the optimal conditions. The limits of Beer's law obeyed from 1 to 8 µg/ml of chloramphenicol with a determination coefficient of 0.9992 and the molar absorptivity was $3.5835 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$ and Sandell's sensitivity index was $0.009017 \mu\text{g}.\text{cm}^{-2}$, The value of (LOD) and (LOQ) were $0.02374 \mu\text{g/ml}$, and $0.07914 \mu\text{g/ml}$ respectively. The method has been successfully applied for the determination of chloramphenicol in its pharmaceutical preparation (eye drop).

Keywords: chloramphenicol, N-bromosucinimide, neutral red dye, spectrophotometric

تقدير الكلورامفينيكول في مستحضره الصيدلاني (قطرة العين) عن طريق الأكسدة وقصر لون صبغة الحمراء المتعادلة

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الملخص

تم اقتراح طريقة طيفية بسيطة وسريعة لتقدير الكلورامفينيكول في صورته النقية وفي مستحضره الصيدلاني (قطرة العين). تضمنت الطريقة استخدام كاشف جديد، الصبغة الحمراء المتعادلة. تعتمد الطريقة على أكسدة الكلورامفينيكول المختزل بكمية زائدة من العامل المؤكسد ن-بروموسكسيناميد بوسط حامضي والمتبقي من العامل المؤكسد يعمل على قصر لون صبغة الحمراء المتعادلة ويتم قراءة الامتصاص للون الصبغة المتبقية عند الطول الموجي 523 نانوميتر ويتناسب الامتصاص طردياً مع تركيز الكلورامفينيكول المختزل. تمت دراسة جميع العوامل التي أثرت على التفاعل وتشمل هذه العوامل نوع وكمية الحامض المستخدم، وكمية العامل المؤكسد، ووسط التفاعل، والأوقات اللازمة للأكسدة الكاملة، وزمن القصر وأوصى باستخدام الظروف المثلى. كانت حدود قانون بير من 1 إلى 8 مايكرو غرام/مل من الكلورامفينيكول مع معامل تقدير 0.9992 وكانت الامتصاصية المولارية 3.5835×10^4 لتر/مول. سم وقيمة معامل ساندل للحساسية 0.009017 مايكرو غرام/سم

². وكانت قيمتي حد الكشف وحد التقدير الكمي 0.02374 و0.07914 مايكروغرام:مل بالتتابع. تم تطبيق الطريقة بنجاح لتقدير الكلورامفينيكول في مستحضره الصيدلاني (قطرة العين).

الكلمات المفتاحية: كلورامفينيكول، ن-بروموسكسيناميد، الصبغة الحمراء المتعادلة، قياس طيفي

Introduction

Chloramphenicol is a broad-spectrum antibiotic that has been widely used in the treatment of various bacterial infections. However, its extensive use has raised concerns about its impact on the environment and public health ([1]. However, because it can lead to dangerous infections, particularly in children [2], its use has been discouraged in many developed nations [3] propose using chloramphenicol formulations as eye drops and ointments to treat bacterial conjunctivitis. The following is its chemical structure [4] (Figure 1).

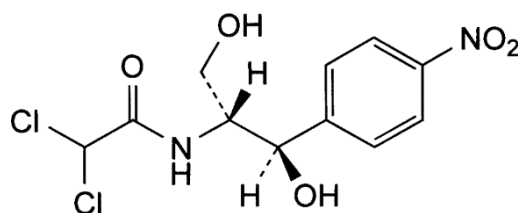


Figure 1: Chemical structure of chloramphenicol.

Numerous techniques and methods were employed in the literature to determine CAPE in both its pure form and formulations: high performance liquid chromatography [5], electrochemical assay [6-8], and hyphenated techniques [9-11]. In addition to using molecularly imprinted polymer in the CAP assay [12], the reduction of the nitro group in CAPE produces an amino group that can be inserted into various spectrophotometric methods [13-16].

This research aims to estimate the levels of chloramphenicol in its formulation (eye drops). On the other hand, the spectrophotometric determination of chloramphenicol as pure and in pharmaceutical preparations is an important aspect of this research.

EXPERIMENTAL

Apparatus used

The measurements were made using a JASCO V-630 double-beam spectrometer. Glass cells with a light path of 1 cm were used. A BEL ENGINEERING sensitive balance was used to complete the weighing operations. The acidity of the solutions was measured using a HANAA PH 211 pH meter.

Chemicals and reagents used

All analytical reagents and chemicals used are of high purity.

Chloramphenicol reductase solution (R-CAP) (500 µg/ml)

This solution was prepared by dissolving 0.0500 g of pure chloramphenicol in 50 mL of ethanol, then placing it in a 125 mL glass beaker and adding 20 mL of distilled water, 20 mL of concentrated hydrochloric acid, and 3 g of zinc powder. The mixture was left for an hour, with intermittent shaking, and then filtered using filter paper, washing the sediment several times with distilled water, placing the filtrate in a 100- mL volumetric flask, completing the volume to the mark with distilled water, and then storing the solution in an opaque bottle.

Reduced chloramphenicol solution (DR-CAP), 100 µg/ml

This solution was prepared by taking 20 ml of (R-CAP) 500 µg/ml solution and then increasing the volume to 100 ml in a volumetric flask.

Neutral Red dye (NR, 100 µg/ml, 0.01%)

A solution of NR was prepared by dissolving 0.0100 g of dye in 100 ml of distilled water in a volumetric flask.

N-bromosuccinimide solution (1x 10⁻³ mol/l)

This solution was prepared by dissolving 0.0177 g of N-bromosuccinimide in 100 ml of distilled water in a volumetric flask.

Hydrochloric acid 1M (approximate)

A solution of 1M hydrochloric acid was prepared by withdrawing (8.6) mL of concentrated acid and adding it to a 100 mL volumetric flask containing an amount of distilled water, then completing the volume to the mark with distilled water.

Pharmaceutical solutions, 100 µg/ml

Eye drop solution from two different companies (Indian and Jordan) The contents of three containers of chloramphenicol (Phenicol) were mixed and 10 mL were taken from the mixture containing 0.5% of pure chloramphenicol, following the approved method of work that includes the reduction of chloramphenicol, and then dilution to prepare 100 µg/ml for each eye drops.

Table 1 includes some of the characteristics of the chemicals used in the research.

Table 1. The characteristics of the chemicals used in the research.

No.	Name of substance	Molecular formula	Molecular weight (g/mol)	Company
1	Chloramphenicol	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₅	323.13	Samarra – Iraq
2	N-Bromosuccinimide	C ₄ H ₄ BrNO ₂	177.98	Fluka
3	Neutral Red	C ₁₅ H ₁₇ N ₄ .HCl	288.78	British drug houses ltd
4	Hydrochloric Acid	HCl	36.45	Gainland chemical company

Method

Suggested method and standard curve

The standard curve for the determination of chloramphenicol (CAP) was prepared by adding increasing amounts of (100 µg/ml) reduced chloramphenicol (R-CAP) solution from 1 to 8 µg/ml to a set of 10 ml volumetric flasks, 0.5 ml of 1M hydrochloric acid, was added then adding 1mL of NBS(1x10⁻³M), then after 20 minutes 1 ml of NR solution (0.01%) was added then dilution to the mark using distilled water after standing for 5 minutes. The absorbance of various solutions was measured against the blank solution at wavelength 523 nm and Figure 2 represents the standard curve for the determination of chloramphenicol, which follows Beer's law from 1 to 8 µg/ml and the values of the molar absorptivity, Sandell's index LOD and LOQ are 3.5835 x10⁴ l/mol.cm, 0.009017 ,0.02374, and 0.07914 respectively.

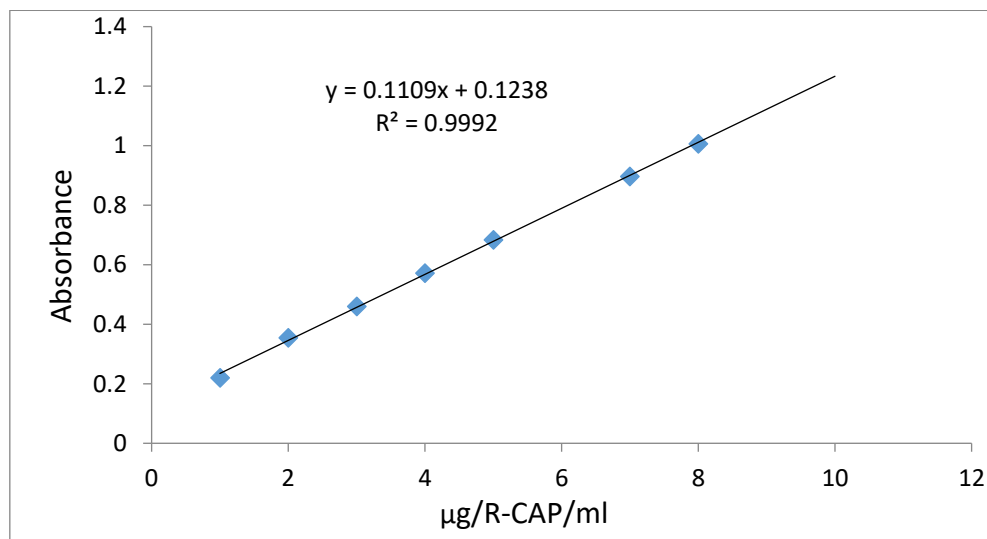
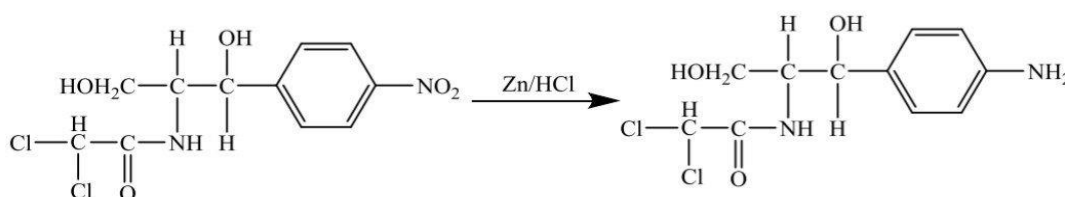


Figure 2. Standard curve for estimating CAP according to the proposed method.

Results And Discussion

Method principle

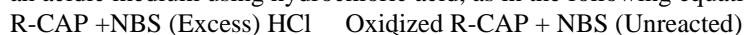
The first step included the reduction of CAP:



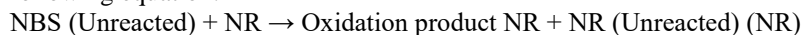
CAP

R-CAP

The second step is the oxidation of R-CAP using an excess of the oxidizing agent N-bromosuccinimide (NBS) in an acidic medium using hydrochloric acid, as in the following equation:



Then, the unreacted NBS is reacted with the dye NR in an acidic medium using hydrochloric acid, so that the color of the dye is bleached, and the color of the dye is measured at the highest wavelength of 523, as in the following equation:



Dye spectrum

The spectrum of the dye was taken to determine the maximum wavelength (λ_{max}) that will be used in subsequent measurements by taking 1 mL of the dye, adding 0.5 mL of 1M hydrochloric acid, completing the volume with distilled water to 10 ml, and taking the spectrum against the blank solution, as in Figure 3.

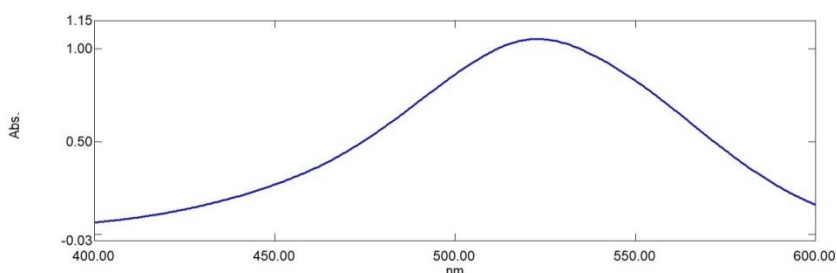


Figure 3. Absorption spectrum of Neutral red dye.

Figure 3 shows that the highest absorption of NR dye is at wavelength 523 nm, and this was adopted in subsequent experiments.

Effect of dye amount

Different volumes of NR dye were studied, ranging from 0.1-1.5 ml, and the absorbance of the dye was measured at wavelength 523nm against the blank solution as shown in Figure 4

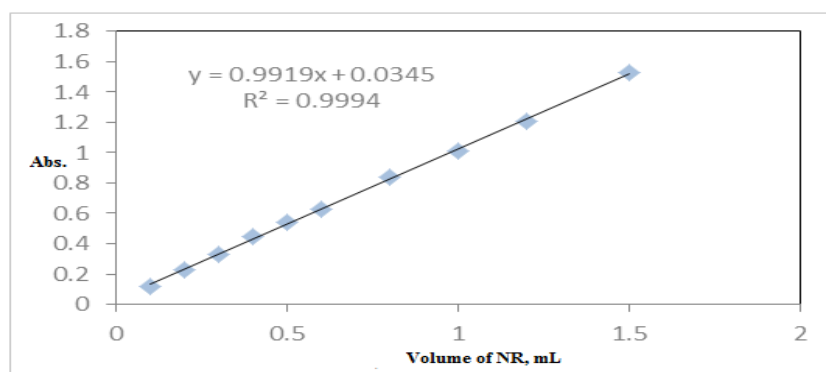


Figure 4. Standard curve for Neutral red dye.

From Figure 4, it is clear that linearity continues to 1.5 ml with a determination factor of 0.9994 and 1 ml of dye was chosen to give it acceptable absorbance and fall within the standard curve.

Selection of an oxidizing agent

The effect of oxidizing agents on the NR dye was studied by adding several oxidizing agents. From the oxidizing agent to a set of volumetric flasks of 10 ml capacity contains 1 ml of dye and 0.5 ml of 1M hydrochloric acid. Then the volume is supplemented with distilled water to the mark and the absorbance is measured at wavelength 523 nm versus the blank solution. Figure 5 shows that the oxidizing agent NBS gives the best bleaching process. Therefore, it was chosen in subsequent experiments.

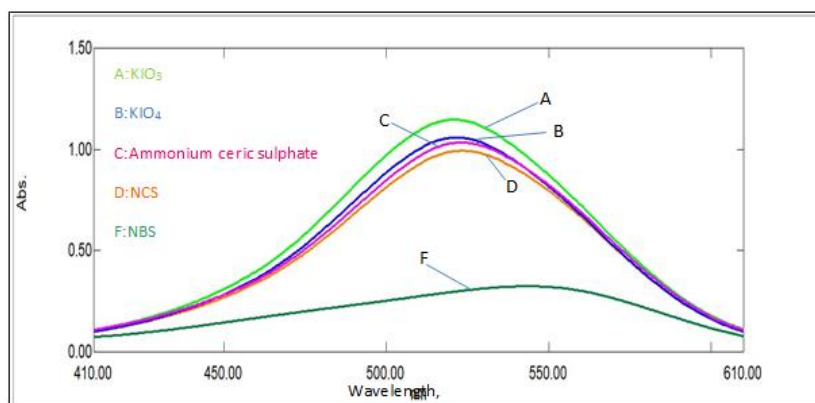


Figure 5. The effect of oxidizing agents on the shortening of the color of NR dye.

Study of choosing the amount of oxidizing agent

Different volumes of NBS solution were added as an oxidizing agent at a concentration of 0.001 M and the extent of its effect on the absorbance of the color of the NR dye was studied, without adding the chloramphenicol solution.

Figure 6 shows that the optimal amount of the oxidizing agent needed to bleach the color of NR dye is 0.5 mL so it was approved in subsequent experiments.

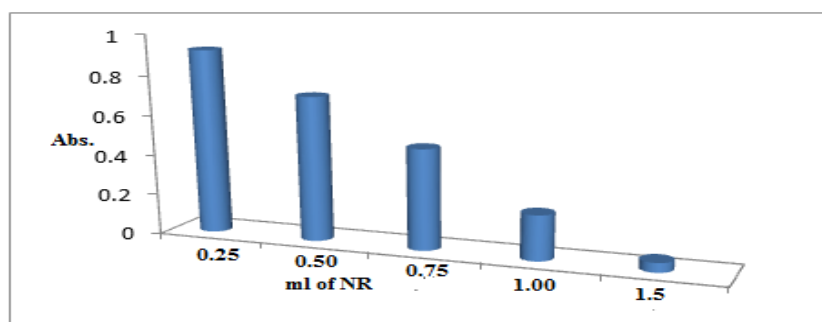


Figure 6. Choosing the optimal amount of oxidizing agent NBS.

Figure 6 shows that the optimal amount of the oxidizing agent needed to bleach the NR dye is 0.5 mL so it was approved in subsequent experiments.

Effect of acid type

The effect of different types of acids on the oxidation of R-CAP was studied by adding 0.5 ml of different acids at a concentration of 1M to 0.5 ml of R-CAP and 1.5 ml of NBS, waiting for ten minutes, then adding 1 ml of NR dye, then left for another 10 minutes, after which the volume is completed to the mark with distilled water, and the absorbance is measured at wavelength 523 nm as shown in Table 2.

Table 2. Choosing the appropriate acid for the process of oxidizing CAP and bleaching the color of the dye.

Type of acid	Absorbance	pH
HCl	0.5459	1.86
H ₂ SO ₄	0.4870	2.04
CH ₃ COOH	0.5068	2.32
HNO ₃	0.4918	2.08

Hydrochloric acid was chosen to give it the highest absorbance, which indicates that the largest amount of R-CAP suffered oxidation.

Effect of acid quantity

The effect of the amount of hydrochloric acid required to complete the chloramphenicol oxidation process was studied, as shown in Table 3.

Table 3. Choose the best volume of hydrochloric acid to perform the oxidation process.

ml of HCl (1M)	Absorbance	pH
0.25	0.5111	2.03
0.5	0.5424	1.91
0.75	0.5243	1.87
1.0	0.4840	1.84
1.25	0.4574	1.68

Table 3 shows that the volume of 0.5 ml of hydrochloric acid gave the highest absorbance of the unreacted dye, which indicates that the largest amount of chloramphenicol suffered oxidation, so it was adopted in subsequent experiments.

The effect of the addition sequence on the reaction components

Several experiments were conducted by changing the addition sequences of the reaction components to obtain the best oxidation process and the results are shown in Table 4.

Table 4. Effect of addition sequences on chloramphenicol oxidation.

Reaction component	Order number	Absorbance
S + H + OX + Dye	I	0.5374
S + OX + H + Dye	II	0.5247
S + Dye + OX + H	III	0.1570
S + Dye + H + OX	IV	0.0473

S= (R-CAP) , H= (Hydrochloric acid), OX = (N-bromosuccinimide) , Dye = (Neutral Red)
From the results shown in Table 4, it was found that the sequence I followed in the previous experiments gave the highest absorbance of the remaining dye, which indicates that the largest amount of R-CAP was oxidized.

Oxidation and bleaching times

The time required to oxidize R-CAP solution (100 µg/mL) was studied by adding 0.5 ml of chloramphenicol to a group of 10 mL volumetric flasks, then adding 0.5 ml of 1M hydrochloric acid and 1.5 ml of NBS, then leaving the solutions for a different period of time (2-30 min.) Then 1 ml of NR dye was added and the solutions were left for a varying period of time, after which the absorbance was measured against the blank solution at wavelength 523nm as shown in Table 5.

Table 5. Choose the chloramphenicol oxidation time and the dye bleaching time.

Temperature (C°)	Absorbance/ min standing				
	5	10	15	20	30
0-5	0.5423	0.5525	0.5411	0.5570	0.5030
RT (23 ±1 C°)	0.6796	0.6428	0.6530	0.6884	0.6868
40	0.9017	0.9202	0.9220	0.9572	0.9280
50	0.9505	0.9752	0.9810	0.9391	0.9370

The results shown in Table 5 show that the best time for chloramphenicol oxidation is 20 minutes and the best time for dye color reduction is 5 minutes. Therefore, it was adopted in subsequent experiments.

The effect of temperature and time on the oxidation of chloramphenicol

The effect of temperature on the oxidation of R-CAP was studied, and it was noted that a temperature of 50°C gives the best oxidation of chloramphenicol, because it gives the highest absorbance of the remaining dye, which indicates that most of the oxidizing agent was consumed in the oxidation of chloramphenicol, and there is a good stability in the absorbance of the remaining dye, but room temperature was used for easy measurement. Absorption, as shown in Table 6.

Table 6: Effect of temperature and time on chloramphenicol oxidation.

Temperature (c°)	Absorbance/ min standing				
	5	10	15	20	30
0-5	0.5423	0.5525	0.5411	0.5570	0.5030
RT (23 ±1°)	0.6796	0.6428	0.6530	0.6884	0.6868
40	0.9017	0.9202	0.9220	0.9572	0.9280
50	0.9505	0.9752	0.9810	0.9391	0.9370

Effect of time on the absorbance of residual dye color's

The effect of time on the stability of the remaining dye was studied by adding two different concentrations of 5 and 7 µg of R-CAP per 10 ml, and the absorbance of the un-reacted dye against the blank solution was measured at wavelength 523 nm. (Table 7).

Table 7. The effect of time on the absorption of the remaining NR dye.

Absorbance / min	CAP µg /ml	
	5	7
Immediately	0.6796	0.8742
5	0.6790	0.8716
10	0.6795	0.8701
15	0.6779	0.8714
20	0.6769	0.8718
25	0.6758	0.8709
30	9.6748	0.8701
35	0.6738	0.8716
40	0.6731	0.8715
45	0.6724	0.8716
50	0.6722	0.8719
55	0.6716	0.8718
60	0.6712	0.8725
After one hour	0.6699	0.8938

The results shown in Table 7 indicate that the absorbance values remain constant for an hour for the two different concentrations.

Effect of organic solvents

Many organic solvents were used in addition to water to dilute to the mark, and the results indicate that propanol and ethanol gave the highest absorption than water, but we continued to use water in subsequent experiments because it is safe, available, and cheap, Figure 7 and Table 8.

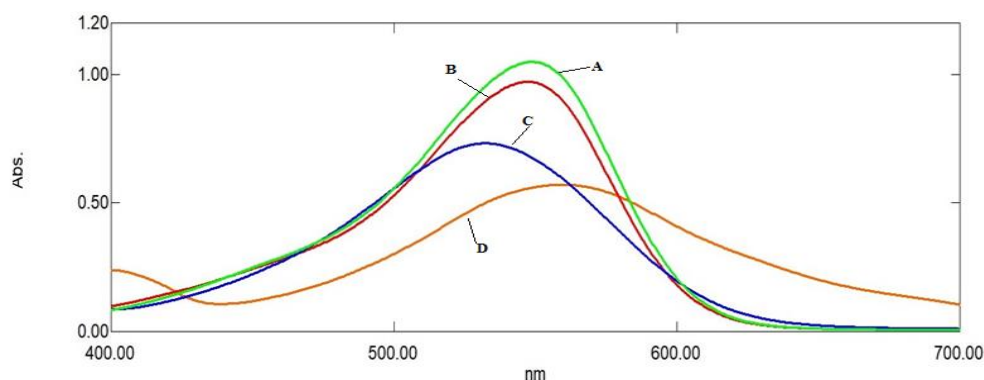


Figure 7. Effect of a number of organic solvents on the absorption spectrum of the remaining NR dye.

Table 8. The results of using various solvents in dilution.

	Solvents	Abs.	λ_{\max} (nm)	ϵ l/mol.cm
A	Ethanol	0.972	547	6.28×10^4
B	Propanol	1.049	548	6.72×10^4
C	Water	0.713	523	4.60×10^4
D	Acetic acid	0.571	558	3.69×10^4

Final absorption spectrum

When 0.5 ml of hydrochloric acid is added to 0.5 ml of R-CAP ($100 \mu\text{g/mL}$) in a 10 mL volumetric flask, followed by the addition of 1.5 ml of the oxidizing agent NBS, then the solutions are left for 20 minutes, after which 1 ml of NR dye is added according to the method. After 5 minutes, dilution of the solutions to the mark with distilled water was done, then measure the spectrum of the remaining NR dye, which gives the highest absorption at wavelength 523 nm (Figure 8)

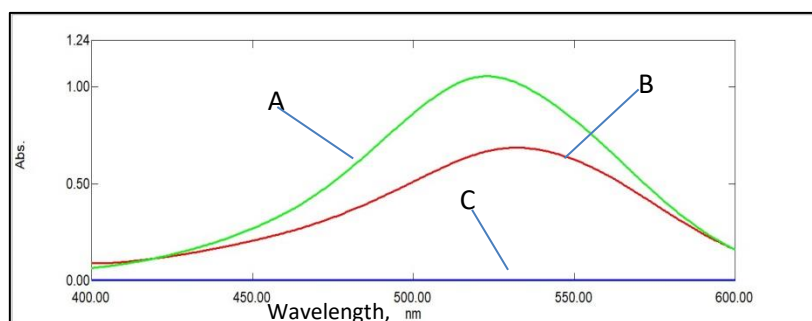


Figure 8. Absorption spectrum of (A) Neutral red dye (B) Neutral red dye remaining after oxidation of $100 \mu\text{g}$ of chloramphenicol and (C) blank solution versus distilled water.

Application part

The results shown in Table 9 demonstrated the success of the method in determining chloramphenicol in pharmaceutical preparations (eye drops) for two different companies, as the recovery percentage reached 100.69% for the Jordanian company and 99.42 for the Indian company.

Table 9. The results of the application part.

Pharmaceutical Preparation	μg CAP measured /ml	Recovery* %	RSD%	RE%	Drug content, %	t_{exp}
PHENICOL Eye drop (Jordan) 0.5 %	5.050	101.00	0.0671	1.00	0.505	0.145
	7.026	100.38	0.0155	0.38	0.502	0.475
PHENICOL Eye drop (India) 0.5 %	4.990	99.80	1.4979	-0.20	0.499	0.636
	6.934	99.05	0.0064	-0.95	0.495	1.610

*Average of 4 determinations.

The results of the method were proven successful in determining chloramphenicol in its pharmaceutical preparation (eye drops) for two different companies, as the recovery % reached 100.69% for the Jordanian company and 99.842% for the Indian company, in addition to the calculated text values being less than the tabulated t value, which indicates the reliability and accuracy of the method.

Comparison with other methods

A comparison was made of the most prominent analytical variables of the currently developed method with the same variables in other spectroscopic methods (Table 10).

Table 10. Comparison of the methods.

Parameters	Suggested method	Literature method [17]	Literature method [18]
Type of reaction	Oxidation- bleaching colour	Diazotization reaction	Oxidative coupling
Reagent used	Neutral red	3-Aminophenol	Pyrocatechol
Wavelength, nm	523	470	500
C) Temperature (R.T	20-50	R.T
Beer's law (µg/ml)	1-8	0.2-10	1.0-22
Molar absorptivity (l.mol⁻¹.cm⁻¹)	3.5835×10 ⁴	3.1 ×10 ⁴	4.26 ×10³

It is clear from the comparison results recorded in Table 10 that the proposed method is no less important than the two methods used in the comparison.

Conclusion

A simple, sensitive, and rapid method has been proposed for the determination of chloramphenicol in its pure form and in its pharmaceutical preparations, by oxidizing it with the oxidizing agent NBS, then the unreacted oxidizing agent bleaching the color of the Neutral red dye in an acidic medium and measuring at wavelength 523nm the color of the remaining Neutral red dye, where the recovery and relative deviation values did not exceed 99.54% and 2.697%, respectively. The method was applied to pharmaceutical preparations in drop form and for two different companies.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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