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Spectrophotometric Assay of Chloramphenicol in Dosage via Dioazotisation and Coupling with 2,5-Dimethyl phenol

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Abstract:
In view of the importance of the medicinal compound Chloramphenicol (CAP)under current study and its uses in the treatment of many pathological conditions. An easy and accurate spectrophotometric method was proposed for the determination of the drug compound in its free form and in its pharmaceutical preparations. The method is based on reducing the nitro group in the Chloramphenicol to the amine and then diazotization of the amine group using nitroso acid prepared simultaneously inside the solution using acidic sodium nitrite. Then coupling of the diazonum salt of CAP with 2,5-dimethyl phenol in a basic medium to give a colored product (Azo dye) The azo dye gives the highest absorption at 453 nm, the Beer's law applied from 1 to 13 µgCAP /ml the method is characterized by a good accuracy and precision, as it reached the values of -2.42 to 0.54% and not more than 1.3050% respectively. Also, the method was highly sensitive and acceptable through the values of molar absorption and Sandell's significance, and their values were $2.775x10^4$l/.mol.cm. and 0.0116 µg/cm^2 sequentially. The research contained the application of the proposed method in estimating CAP in its pharmaceutical formulation (eye drops) with clinically acceptable results
od gave accepted analytical results.

Keywords: Chloramphenicol**,** Reduction**,** Diazotization, 2,5-Dimethyl Phenol, Eye Drops

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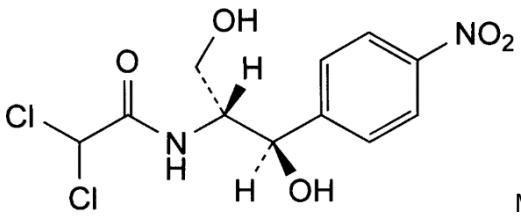
Introduction

Chloramphenicol is used to inhibit of most gram- negative and gram- positive bacteria [1]. CAP is easy availability and not expensive, is a brand of bacteriostatic antibiotic CAP has been used in the treatment of many types of bacterial contaminations. Due to toxicity and side effects at high concentrations, it is forbidden to use it in the food industry in European Union since 2010 [2]. CAP has the following chemical structure [3] (Figure 1)



M. Wt.=323.1g/mol.

Figure 1: The chemical structure of CAP.

Various techniques or methods have been published in the assay of CAP in its dosage forms: electrochemical determination [4-6], hyphenated techniques [7-9] and high-performance liquid chromatography [10]. The spectrophotometric methods were almost based on reducing the nitro group then various reactions were used [11-15], and also molecularly imprinted polymer was used in the assay of CAP [16].

The present work included reduction of CAP then diazotization of reduced- CAP (R-CAP) and coupling DR-CAP with a suitable coupling agent to form colored azo dye that can be followed spectrally.

Experimental Work

Apparatus

JASCO-360 spectrophotometer and Cells of glass and with a light path of 10 mm were used for all spectral measurements and absorbance readings. The pH was measured using a BP3001 pH meter and a BEL-sensitive balance was used to carry out the required weighing operations.

Reagents and solutions

The reagents used in this research were of a high degree of purity and the drug in its pure form will be taken from the State Company for Pharmaceutical Industry Samarra – Iraq.

2,5-Dimethyl phenol (1×10^{-2}M) solution: This solution was prepared by dissolving 0.1220 g of pure 2,5-dimethylphenol (supplied by Fluka company) in a quantity of distilled water and heated for 5 minutes at a temperature of 70-80 °C and then complete the volume to the mark of a 100-ml volumetric flask, and this solution is daily prepared.

2,3-Dihydroxy naphthalene(1×10^{-2}M) solution: This solution was prepared by dissolving 0.160 g of pure 2,3-dihydroxy naphthalene (supplied by Fluka company) in 5ml of ethanol then completing the volume to the mark of a 100-ml volumetric flask with distilled water.

Sodium hydroxide solution 1 M: This solution was prepared by diluting the concentrated ampoule solution (Fluka company) with distilled water to 1000 ml using a volumetric flask, and kept in a plastic container.

Chloramphenicol reducing solution (R-CAP, 500 µg /ml): This solution was prepared by dissolving 0.0500 g of pure chloramphenicol in 50 ml of ethanol, then placed in a 125 ml glass beaker and added to it 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 shaking occasionally, and then the filtration was done using filter paper washing the precipitate many times with distilled water and collecting the filtrate in a volume flask of 100 ml and completing the volume to the mark with distilled water and then save the solution in an opaque container.

Chloramphenicol diazotization reduction solution (DR-CAP, 100 µg/ml)

This solution was daily prepared by taking 10 ml of (R-CAP) solution (500 µg / ml) and adding 10 ml of sodium nitrite solution at a concentration of (1.52×10^{-3} M) with shaking for 10 minutes and then completing the volume to 50 ml in a volumetric flask**.**

Pharmaceutical solutions (100 µg/ml)

Eye Drops solution for different originators (Jordanian, Indian)

The content of three containers of Phenicol eyes drop (0.5%) was mixed and 10 ml of this mixture, which contains 0.0500g of pure chloramphenicol, was taken and following the approved method of

action that includes reducing chloramphenicol and then diazotized it to obtain the diazonium chloramphenicol solution, which is equivalent to 100 µg/ml of chloramphenicol.

RESULTS AND DISCUSSION

Primary experiment

To a 10 ml volumetric flask add 1 ml of DR-CAP and 2 ml of 2,5-dimethylphenol (1x10-2 M) and 1 ml of sodium hydroxide (1M) then diluted with distilled water to the mark. and the absorption spectrum against a reagent blank was taken. The maximum absorbance at 453 nm was recommended in the next experiments.

Optimal conditions

The optimum conditions that affect the absorption of the resulting azo dye were studied in the following experiments. These conditions included:

Selection of the coupling reagent

Solutions of two reagents (2 ml at 1×10^{-2} M) were tested in the process of azo dye formation via coupling with DR-CAP in a basic medium (1 ml of 1 M NaOH) and the results are shown in Table 1.

Table 1: Selection of coupling reagent.

Reagent($1*10^{-2}$M)	Absorbance (A)	λ_{max} (nm)
2,3-dihydroxy naphthalene	0.4366	472
2,5-dimethyl phenol	0.9100	453

From the above results, it was found that the reagent 2,5-dimethyl phenol in a basic medium gives the highest absorption of the product formed at wavelength 453, so it was recommended for use in subsequent experiments.

Effect of reagent amount

To study the optimum volume of the reagent taking different concentrations of DR-CAP to give a high absorbance of the formed azo dye, different volumes from 1 to 2.5ml were added, the result indicated that the volume of 2 ml was optimal, Table 2.

Table 2: The optimal amount of 2,5-dimethyl phenol.

Coupling agent (ml)	Absorbance of µg DR-CAP /ml				R^2
	2.5	5.0	10.0	12.5	
1	0.2378	0.3627	0.6689	0.8646	0.9948
1.5	0.2717	0.4177	0.7452	0.9959	0.9899
2	**0.3057**	**0.4676**	**0.9309**	**1.1618**	**0.9964**
2.5	0520.3	1960.4	0.8615	1.0911	0.9969

Effect of bases

Different volumes of bases after completing the addition of the reaction components and before dilution were added, and the practical results showed that the reaction needs a strong base as weak bases give turbid solutions and the base sodium hydroxide gives close absorption of potassium hydroxide and sodium hydroxide was selected in subsequent experiments (see results in Table 3).

Table 3: The effect of bases.

Type of Base used (1M)	Absorbance	pH
NaOH	0.9264	12.07
KOH	0.9171	12.09
Na_2CO_3	Turbid	-------
$NaHCO_3$	Turbid	-------

Amount of sodium hydroxide

Different volumes of sodium hydroxide solution(1 M) were studied, from the results in Table 4 show 1 ml of sodium hydroxide gives the highest absorbance so it was selected in subsequent experiments.

Table 4: The optimal amount of sodium hydroxide.

NaOH (1M), 1ml	Absorbance of DR-CAP µg/ml		
	10	12.5	15
0.5	0.9040	0.4063	0.2870
1.0	**0.9303**	**1.1336**	**1.4259**
1.5	0.8899	1.0394	1.2971
2.0	0.8788	1.0317	1.2417

Effect of addition sequences

To make sure that an additional sequence was added, different sequences were added and the best sequence was the one that was used in the preliminary study., do it another way a loss in absorbance so it fixed in the next experiments.

Absorption spectrum

The absorption spectrum was taken for the colored product formed from the reaction of 100 µg of DR-CAP with the 2,5-dimethylphenol in the presence of sodium hydroxide. The formation of a colored azo dye gives the highest absorption at the wavelength of 453 nm (Figure 2), so 453 nm was fixed in all the next measurements.

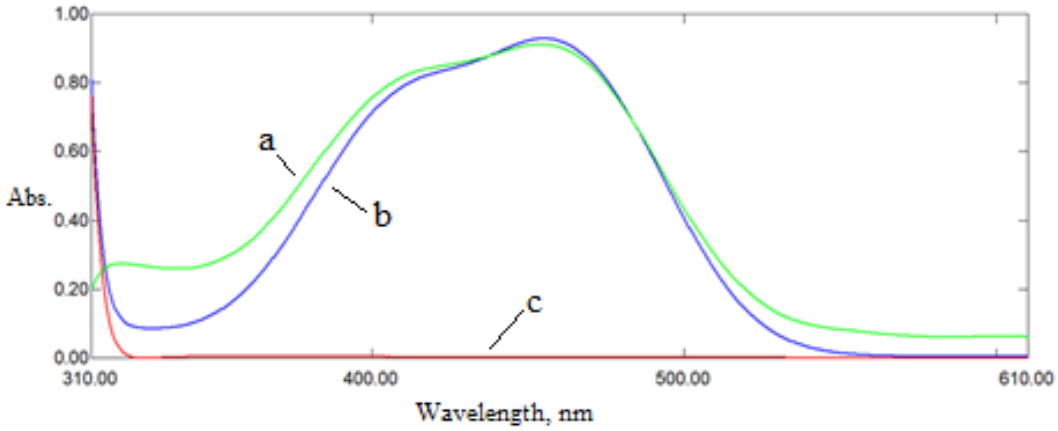


Figure 2. Absorption spectra in (a) 100µg DR-CAP solution against distilled water (b) 100 µg DR-CAP versus reagent blank and (c)reagent blank versus H_2O.

Calibration Graph

A linear calibration graph for chloramphenicol assay via the suggested method (Figure.3) is obtained using the optimum conditions described in the above experiments, demonstrating that Beer's law is obeyed over the concentration range of 1-13 µg/ml with a determination coefficient of 0.9989 and. The yellow-orange product had a molar absorptivity of 2.775×10^4 l/.mol.cm. and 0.0116 µg/cm^2.

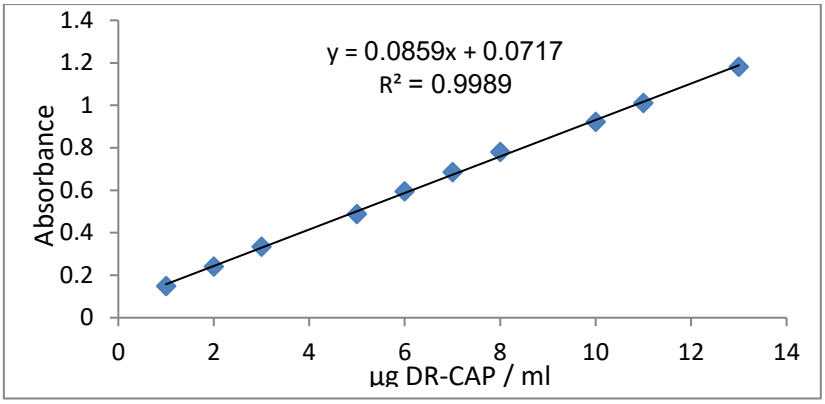


Figure 3. Calibration graph for determination of chloramphenicol.

The stoichiometry of the formed azo dye

The stoichiometry of the reaction between chloramphenicol and 2,5-dimethyl phenol was investigated by applying the Job's and Mole ratio method using equal concentration solutions (3.09×10^{-4} M) of DR-CAP and 2,5-dimethyl phenol [2,5-DMP]. Figure 4, indicated that the ratio of formed azo dye is 1:1 DR-CAP to 2,5-dimethyl phenol (Figure 4).

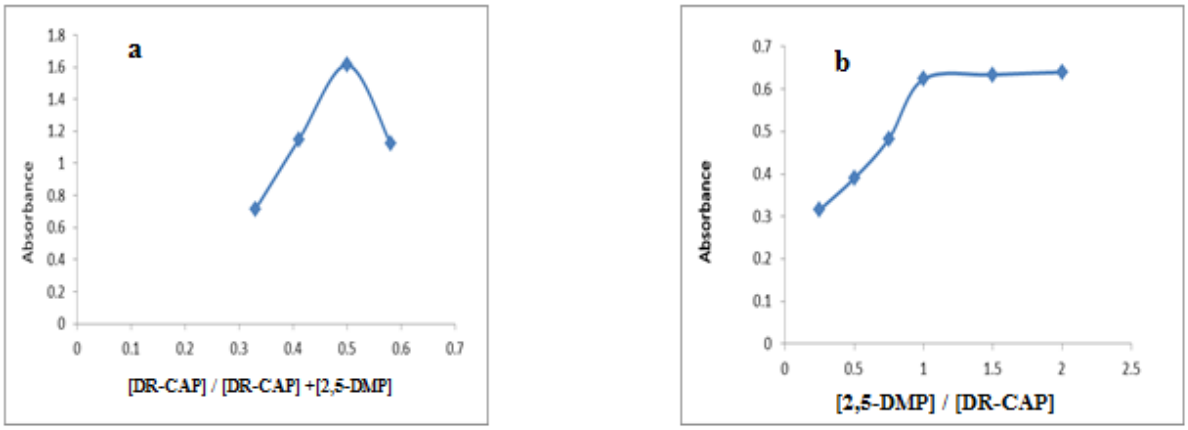
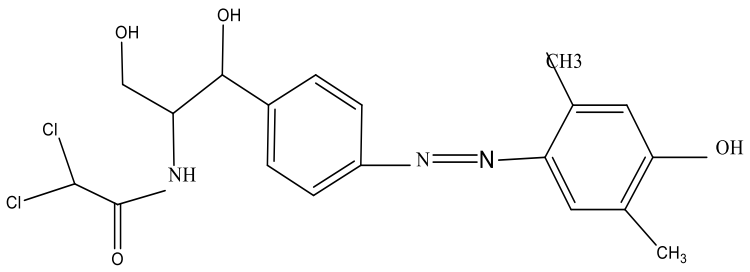


Figure 4: (a) Job's method plot (b) molar ratio method plot of DR-CAP - 2,5-DMP in the presence of sodium hydroxide.

From the results in Figure 4, we conclude that the chemical structure of the formed azo dye between the DR-CAP and the reagent 2,5-DMP in a basic medium is as follows:



Yellow-orange dye

Analytical application

Chloramphenicol was estimated in its pharmaceutical preparations (eye drops) for two different originators, and the results are shown in Table 5.

Table 5: Results of application of the suggested method.

Pharmaceutical preparation	g CAPμ present in10ml	g CAPμ measured in 10ml	Rec.%	Average Rec*. %	Drug content %	RSD%	RE%
PHENICOL 0.5%Eye Drop (Jordan)	50	48.79	97.58	98.52	0.492	0.7108	-2.42
	100	99.46	99.46			0.6963	-0.14
PHENICOL 0.5%Eye Drop (India)	50	50.27	100.54	99.62	0.498	0.5363	0.54
	100	98.70	98.70			1.3050	-1.3

- Average of three determinations.

The results fixed in Table 5, indicated that the average recovery for chloramphenicol drop analysis for Jordan was 98.52% and for India was 99.62%, which indicates that the method is efficient and accurate in the determination of pharmaceutical products.

Conclusions

For the determination of trace quantities of chloramphenicol, a simple, fast, exact, and practical spectrophotometric method has been established, based on reducing the nitro group in the compound to the amino group using zinc powder and concentrated hydrochloric acid and then diazotizing it and coupling with the reagent 2,5-dimethyl phenol in a basic medium using sodium hydroxide solution to give the highest absorption of the azo dye formed at a wavelength of 453 nm. The method was successfully applied in the estimation of chloramphenicol in pharmaceutical preparations (eye drops) from two different companies.

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