Spectrophotometric Method for Determination of Chloramphenicol in Pharmaceutical Preparations using o-Chloranil Reagent

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الخلاصة

تم وصف طريقة طيفية سهلة وحساسة لتقدير الكلورامفينيكول بشكله النقي وفي مستحضراته الصيدلانية. تعتمد الطريقة على التفاعل بين الكلورامفينيكول المختزل والكاشف اورثو- كلورانيل في الوسط المائي القاعدي مكونا معقد الشحنة المنتقلة ذي لون ارجواني يمتلك طيفا امتصاصيا له أقصى امتصاص عند 527 نانوميتر. أمكن تطبيق قانون بير ضمن مدى التراكيز 2-65 مايكروغرام/مللتر في حين كانت الامتصاصية المولارية 3.72×10^3 لتر.مول$^{-1}$سم$^{-1}$. بلغ حد الكشف 0.456 والحد الكمي 1.521 مايكروغرام/مللتر ومعدل نسبة الاسترجاع 100.9% في حين كان ألانحراف القياسي النسبي أفضل من 2.2%. كذلك تم دراسة طبيعة المعقد وميكانيكية التفاعل. طبقت الطريقة بنجاح في تقدير الكلورامفينيكول في مستحضراته الصيدلانية بشكل كبسول وقطرة العين بدقة وتوافق جيدين. كما تم مقارنة الطريقة المقترحة مع الطريقة القياسية في الدستور البريطاني وطرائق طيفية أخرى.

ABSTRACT

A simple, sensitive and rapid spectrophotometric method for the determination of chloramphenicol in pure as well as in dosage form is described. The method is based on the reaction of reduced chloramphenicol with o-chloranil in an aqueous alkaline medium to form a purple colored charge transfer complex of maximum absorption peak at 527 nm. Under the optimized reaction conditions, Beer's law correlating the absorbance with chloramphenicol concentration was obeyed in the range of 2-65 μg ml^{-1} with molar absorptivity 3.72×10^3 L mol^{-1}cm^{-1}. The limits of detection and quantitation were 0.456 and 1.521 μg ml^{-1}, respectively.

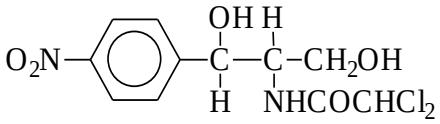
The accuracy and precision of the method were satisfactory; the average recovery was 100.9 % and values of relative standard deviations better than 2.2 %. The stoichiometry of the reaction was studied, and the reaction mechanism was postulated. The proposed method was successfully applied to the determination of chloramphenicol in its pharmaceutical capsule and eye drops with good accuracy and precisions. The results obtained by the proposed method are compared with those obtained by the official method and other reported methods.

Keywords: Spectrophotometry; Chloramphenicol; o-Chloranil; Pharmaceutical preparations.

INTRODUCTION

Chloramphenicol [2,2–dichloro–N- [(1R,2R –2–hydroxyl - 1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl] acetamide], (Scheme 1), is a broad-spectrum antibiotic active against Gram-positive and Gram-negative bacteria. It is produced naturally by the soil bacterium *Streptomyces Venezuelan*, but is presently mainly produced by chemical synthesis [1–3]. It has been used in veterinary practice for prevention and treatment of many bacterial infections because of its efficiency, easy availability and low cost [4,5]. Moreover, Due to its genotoxic effect and severe side effects, such as anemia, leucopenia, agranulocytosis and a plastic anemia in some people, its use is limited to the therapy of serious infections (*e.g.* typhoid fever and meningitis). Furthermore, its use in food production, such as aquaculture farming, has been banned worldwide [1,6]. Various analytical methods have been used for the determination of Chloramphenicol, which include official method [7,8], chromatography [9,10], ion-selective electrode technique [11], chemiluminescence [12,13], titrimetry [14,15], electrochemical techniques [16], flow-injection biamperometric method [17], atomic absorption spectrometry [18]. Many Spectrophotometric methods, depending on reduction of nitro group, have been reported for determination of chloramphenicol using various reagents such as isonicotinic acid hydrazide [19], N-(1-naphthyl)ethylenediamine[20], trisodium pentacyanoaminoferrate [21], Ninhydrin [22], iminodibenzyl, 3-aminophenol, pyrocatechol and molybdate [23], orthogonal polynomials [24], ammonium molybdate [25] and p-dimethylaminobenzaldehyde [26]. However; some of these methods suffer from disadvantages such as low sensitivity and narrow range of determination, tedious and needing extraction, using organic medium, and either require a long time for stable color development or exhibit instability of the colored product. Charge transfer complex formation reactions have been applied for sensitive spectrophotometric

determination of chloramphenicol using p-chloranil [27], p-fluoranil [27] and TCNQ [28] reagents as π-acceptors. The aim of the present work is to provide simple, sensitive, selective and rapid spectrophotometric method for determining of chloramphenicol in pure form as well as in pharmaceutical preparations depending on the charge transfer complex formation reaction using o-chloranil as π-acceptor .



$C_{11}H_{12}Cl_2N_2O_5$
M.wt= 323.1 g/mol

Scheme 1: Chemical structure of chloramphenicol

EXPERIMENTAL

Apparatus

All absorption measurements were made on a Shimadzu UV-210A double - beam spectrophotometer supplied with a digital printer DP80Z and matched 1-cm optical silica cells. Heating of solutions was carried out on a water bath of frost instruments, LTD. The reading of pHs made on a PW 9420 pH meter supplied with an electrode type CE 10-12 pH. Weighing was carried out on a balance type of Mettler H 54 AR.

Chemicals

Chloramphenicol and its pharmaceutical formulations (capsule and eye drops) were kindly provided by state company for Drug Industries and Medical appliance-(SDI) Sammara-Iraq. o-Chloranil was obtained from MOLEKULA and other chemicals were obtained from Fluka and BDH companies. All solvents were analytical reagent grade and water was distilled.

Standard solution: 500 μg ml^{-1} reduced chloramphenicol (RCAP) solution was prepared by dissolving of 50 mg of its pure form in 10 ml of distilled water and was reduced using 4 g zinc powder and 20 ml of conc. hydrochloric acid and kept for 15 min with stirring for complete reduction. The reduced solution was filtered and diluted with water to 100 ml in a calibrated flask, then 5 ml was neutralized with 5 ml of 20% sodium carbonate and diluted with water to 25 ml to obtain 100 μg ml^{-1} and kept protected from sun light in ambient bottle.

Reagent solution: 5×10^{-3} M o-chloranil solution was prepared by dissolving 0.123 g in absolute ethanol and diluted to 25 ml in a calibrated flask with the same solvent.

Basic solutions: 20% sodium bicarbonate and 0.01 M sodium hydroxide were prepared by in distilled water.

Recommended procedure

Aliquots of the working solution of RCAP (2-65 μg ml^{-1}) were transferred into a series of 5 ml calibrated flasks. Then, 0.4ml of 5×10^{-3} o-chloranil and 0.5 ml of 0.01 M NaOH were added and the solutions were diluted to the mark with distilled water and kept at room temperature for 5 min. The absorbance was measured at 527nm against reagent blank.

Procedure for chloramphenicol assay in capsules and eye drops

Ten capsules (250 mg each) were emptied, pulverized, and dissolved in distilled water with vigorous stirring. The solution was diluted to 1 liter. An aliquot equivalent to 50 mg of chloramphenicol was taken and reduced using zinc and HCl. The solution was filtered, neutralized and subjected to the recommended procedure described above for pure chloramphenicol. For eye drops (0.5%), a suitable volume was diluted, and the above procedure was followed.

Results and discussion

Spectral characteristics

The proposed method involves the reduction of chloramphenicol and reaction with o-chloranil reagent in the presence of NaOH to form a purple colored charge transfer complex having maximum absorption at 527 nm. This wavelength was used for all subsequent measurements. The absorption spectra of the reaction product are shown in Figure 1. The corresponding reagent blank have low absorbance at this wavelength.

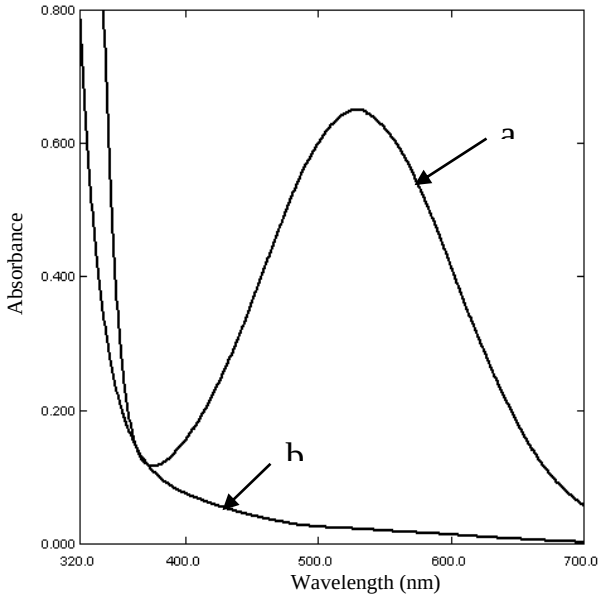


Figure 1: Absorption spectra of (a) RCAP (50 μg ml^{-1}) complex with *o-CA* reagent (5×10^{-3}M) against reagent blank and (b) reagent blank against distilled water.

Optimization of experimental conditions

The optimum conditions for the color development of the *o-CA*-RCAP complex were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of colored species. The following experiments were conducted for this purpose and conditions so obtained were incorporated in general procedure.

Effect of solvent

Different solvents such as methanol, ethanol, acetonitrile, acetone and water as medium for the reaction have been tried in order to achieve maximum sensitivity and complex stability, As shown in Table1. It was found that on using water as solvent for chloramphenicol and absolute ethanol as solvent for *o-CA* in the presence of NaOH and dilution with the water were gave maximum color intensity. Therefore; these systems of solvents are recommended in this method.

Table 1: Effect of solvents on absorbance of *o-CA*-RCAP complex

RCAP (20µ/ml)	o-CA Dissolved in	Dilution by	λ_{max}	Absorbance
Water	Methanol	Water	533.5	0.238
Water	Methanol	Methanol	545	0.228
Water	Ethanol	Water	527	0.274
Water	Ethanol	Ethanol	_	turbid
Water	Acetone	Water	530	0.261
Water	Acetone	Acetone	_	turbid
Water	Acetonitrile	Water	532.5	0.231
Water	Acetonitrile	Acetonitrile	530	0.271

Effect of pH and buffer solutions

The effect of pH on the absorption of the complex was studied using different pH values ranged from 2 to12 by using of 0.01 M HCl and NaOH. It was found that the complex was formed at pH 9.2 by addition of NaOH (Figure 2). Decrease in absorbance was observed through addition of HCl, which may be attributed to the liberation of hydrogen chloride. Therefore different buffers of pH 9.2 was prepared to examine the sensitivity. A negative effect was observed on the color intensity.

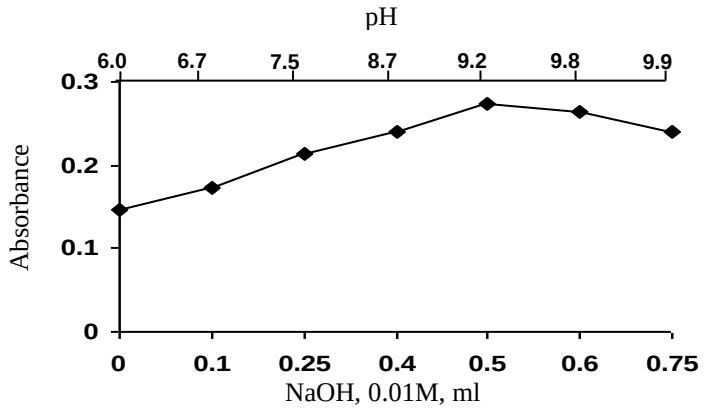


Figure 2: Effect of pH and NaOH amount on the absorptio of 20 µgml^{-1} RCAP complex with *o-CA*

Effect of bases

To obtain high sensitivity for the complex, different bases such as sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate with fixed volume and a concentration of 0.01M were examined by addition to a fixed amount of RCAP. It was found that sodium hydroxide gave maximum color intensity (Figure 3), and the optimum amounts of this base were found to be 0.5 ml which was used in the subsequent experiments.

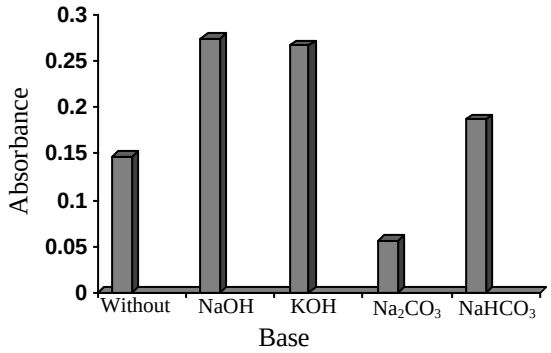


Figure 3 : Effect of Different bases on the intensity of 20 µgml^{-1} RCAP complex with *o-CA*

Effect of o-CA concentration

The effect of changing the *o-CA* concentration on the absorbance of solution containing a fixed amount of RCAP was studied. It was observed that the absorbance increases with increasing *o-CA* concentration and reached maximum on using 0.4 ml of 5×10^{-3}M *o-CA* (Figure 4). Therefore, this volume of this concentration was used in the subsequent work.

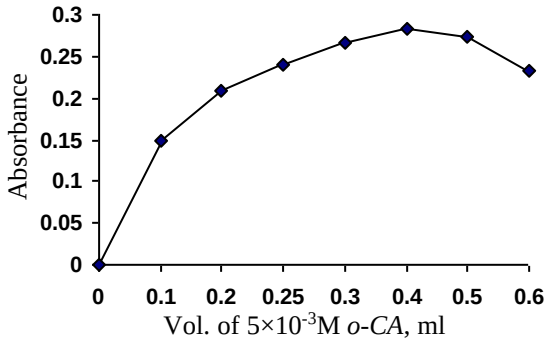


Figure 4: Effect of *o-CA* reagent concentration on absorbance of 20 µgml^{-1} RCAP

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and in thermostatically controlled water-bath at different temperatures. The absorbance was measured at 5 and 10 minutes intervals against reagent blank treated similarly. It was observed that maximum absorbance and stability was obtained at room temperature (25°C). It was found that complex gave maximum absorption after 5 min. and remain constant for more than 3 hrs and the color was fading slowly thereafter. Hence, 5 min at room temperature is recommended for the proposed method.

Effect of surfactant

Effect of various surfactants including sodium dodecyl sulphate (SDS), cetylperydinum chloride (CPC), cetyltrimethylammonium bromide (CTAB), Tween-80 and Triton x-100 were tested. It was found that these surfactants decreased the absorbance of solutions.

Effect of order of addition of reactants

To obtain optimum results the order of addition of reagents should be followed as given under the general procedure, otherwise a loss in color intensity was observed.

QUANTIFICATION

In order to investigate the range in which the colored complex adhere to Beer's law, the absorbance of the complex was measured at 527 nm after developing the color by following the general procedure calibration graph for a series of solutions containing increasing amounts of RCAP. The Beer's law limits and molar absorptivity values were evaluated and given in Table 2, which are indicated that the method is sensitive. The linearity was represented by the regression equation and

the corresponding correlation coefficient for the studied determined drugs by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of six replicates of each three different concentrations

for chloramphenicol indicated that the method is precise and accurate. Limit of detection (LOD) are in the accepted range below the lower limit of Beer's law range.

Parameter	RCAP
Beer's law limits (μg ml^{-1})	2-65
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	3.72×10^3
LOD (μg.ml^{-1})	0.456
LOQ (μg.ml^{-1})	1.521
Average recovery (%)[a]	100.9
Correlation coefficient	0.9989
Regression equation (Y)[b]	
Slope, *a*	0.0127
Intercept, *b*	0.017
RSD [b]	≤ 2.18

Table 2: Summary of optical characteristics and statistical data for the proposed method

[a] Average of six determinations.
[b] $Y = a X + b$, where X is the concentration of drug in μg ml^{-1}.

INTERFERENCE

The extent of interference by some excipients which often accompany pharmaceutical preparations were studied by measuring the absorbance of solutions containing fixed amount of RCAP and various amounts of diverse species in a final volume of 5 ml. It was found that the studied excipients did not interfere seriously (Table 3). Slight positive interference was observed in the presence of large excess of excipients. However; an error of 5.0 % in the absorbance readings was considered tolerable. Typical results are given in table 4.

Table 3: Effect of excipients for assay of chloramphenicol

Exciepient	Recovery % of 20 µg/ml of chloramphenicol per µg/ml Foreign added				
	20	40	100	200	400
Glucose	98.80	100.79	101.38	99.42	102.77
Lactose	99.22	100.57	101.30	102.77	103.74
Arabic Gum	101.97	101.57	104.32	104.45	106.57
Sodium chloride	100.00	100.79	100.95	101.30	103.67
Sucrose	100.22	101.20	98.80	101.20	100.87
Starch	100.79	100.47	100.47	101.20	101.69

STOICHIOMETRY AND STABILITY CONSTANT

The molar ratio of the n-π charge transfer complex formed between the RCAP and *o-CA* reagent was investigated by applying the mole ratio and continuous variation (Job's) methods [29]. The results indicated that complex was formed in the ratio of 1:1 (Figures 5). This finding supports that the n-π CT complex is formed through amino group. The stability constant (K_{st}) of the complex was determined according to the previous ratio and found 4.2×10^4 l. mol^{-1}. However; the probable reaction mechanism based on the reported method [30] is given in scheme 2.

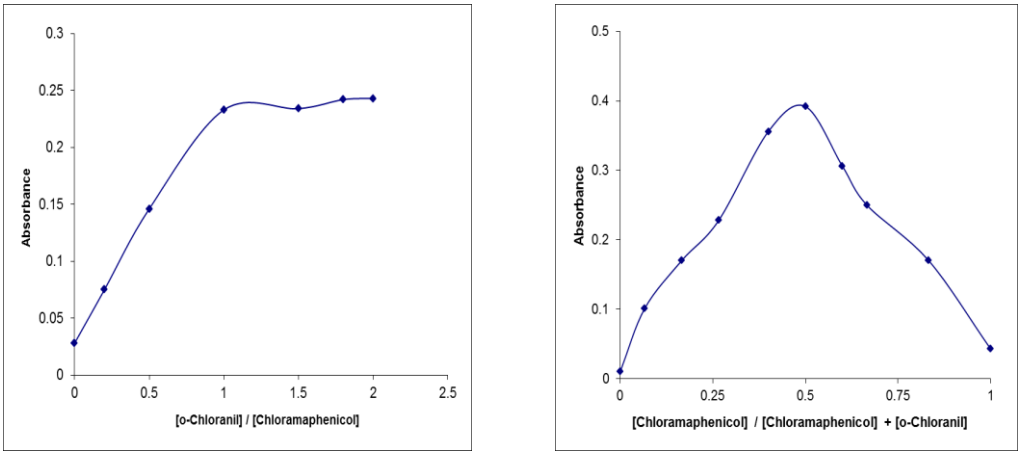
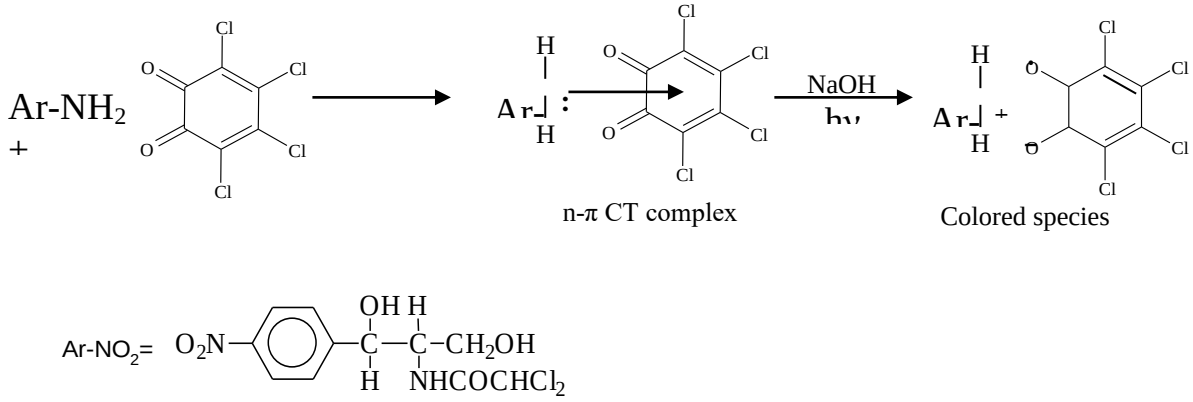


Figure 5: Mole ratio (a) and continuous variations (b) plots for complex of RCAP (1×10^{-3}M) and *o-CA* (1×10^{-3}M) under the optimum conditions.

REACTION MECHANISM

The interaction of RCAP with *o-CA* in ethanol solvent, was a charge-transfer complexation reaction between the RCAP as n-donor drug and *o-CA* as π-acceptor, followed by the formation of a radical anion. Complete electron transfer from the donor to the acceptor moiety took place with the formation of intensely colored radical ions with high molar absorptivity values, (scheme 2)



Scheme 2: Probable mechanism for the reaction of *o*-CA with RCAP

ANALYTICAL APPLICATIONS

The proposed method was successfully applied to determine chloramphenicol in pharmaceutical capsules and eye drops preparations. The obtained results were compared statistically by a Student's *t*-test for accuracy and a variance ratio *F*-test for precision with the official method [7] at the 95% confidence level with six and four degrees of freedom respectively, as cited in Table 4. The results showed that the experimental *t*-test and *F*-test were less than the theoretical value (t=2.45, F=6.39), indicating that there was no significant difference between the proposed method and official method. The proposed method is compared favorably with other reported methods as shown in Table 5.

Table 4: Assay of chloramphenicol in pharmaceutical preparations using the proposed method and comparison with the official method

Procedure applied	Pharmaceutical preparation	Drug amount present ($\mu g \cdot ml^{-1}$)	Recovery[a] (%)	Drug content found (mg)	Average recovery (mg)	Certified value (mg)
Proposed *o-CA* method	Capsule	15 35 50	99.00 101.13 103.40	244.0 255.2 250.5	249.9 (1.97,1.05)[b]	250
	Eye drops	15 35 50	99.60 95.95 98.99	0.515 0.492 0.494	0.50 % (2.12,1.76)	0.5 %
British Pharmacopoeia	Capsule	10	99.2	248	-	250
	Eye drops	10	101.8	0.509	-	0.5%

[a] Average of three determinations.
[b] Figures in parenthesis are the calculated values for *t*, and *F* respectively.

Table 5: Comparison of spectrophotometric methods for RCAP determination

Analytical parameters	Present method o-Chloranil	Literature method		
		p-Chloranil [27]	p-Flouranil [27]	TCNQ [28]
λ_{max} (nm)	527	342	350	464
pH	9.2	9.0	9.0	7.5
Temp. (°C)	25*	40	45	40
Development(min)	5	50	40	20
Stability period(min)	>3 hrs	40	30	25
Beer's law (µg/ml)	2-65	0.4-14	0.2-14	0.15-32
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	3720	12560	14570	27570
Recovery (%)	100.90	98.4	100.72	99.67
RSD(%)	0.91	0.46	0.49	0.91

*Room temperature

CONCLUSION

The proposed method is sensitive (trace amounts can be determined), accurate (average recovery range 100.9%), precise (RSD ≤ 2.18) and simple since it does not need neither temperature control nor solvent extraction step. Analysis of authentic samples containing chloramphenicol showed no interference from common additives and auxiliary substances in general. The statistical analysis is in good agreement with those of the official British Pharmacopoeia and other reported methods. Hence, this method could be considered for the determination of chloramphenicol both in pure form and in pharmaceutical preparations.

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