

## Spectrophotometric Method for Determination of Chloramphenicol in Pharmaceutical Preparations using 1,2-Naphthoquinone-4-Sulphonate as a Chromogenic Reagent

Theia'a N. Al-Sabha\* and Bassam A. Rasheed

Chemistry Department, College of Education, Mosul University, Mosul, Iraq

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### 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 condensation reaction of reduced chloramphenicol with sodium 1,2-naphthoquinone-4-sulfonate (NQS) in an alkaline medium to form an orange-red colored Schiff's base of maximum absorption peak ( $\lambda_{\max}$ ) at 480 nm. Under the optimized reaction conditions, Beer's law correlating the absorbance with chloramphenicol concentration was obeyed in the range of 0.8-14  $\mu\text{g ml}^{-1}$ . The molar absorptivity was  $1.02 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ . The limits of detection and quantitation were 0.124 and 0.473  $\mu\text{g ml}^{-1}$ , respectively. The accuracy and precision of the method were satisfactory; the average recovery was 99.54 % and values of relative standard deviations better than 3.5 %. 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, syrup and ointment with good accuracy and precisions. The results obtained by the proposed method are compared with those obtained by the official method.

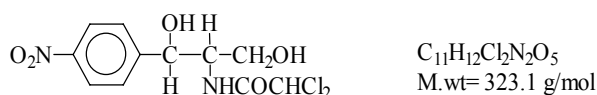
**Keywords:** Spectrophotometry; Chloramphenicol; NQS; Pharmaceutical formulations.

### Introduction

Chloramphenicol [2,2-dichloro-N-[(1R,2R-2-hydroxy-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<sup>[1-3]</sup>. It has been used in veterinary practice for prevention and treatment of many bacterial infections because of its efficiency, easy availability and low cost<sup>[4,5]</sup>. Moreover, Due to its genotoxic effect and severe side effects, such as anemia, leucopenia, agranulocytosis and aplastic 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<sup>[1,6]</sup> various analytical methods have been used for the determination of Chloramphenicol, which include official method<sup>[7,8]</sup>, high-performance liquid chromatography<sup>[9-11]</sup>, gas

\* Corresponding author: e-mail: [dr\\_theiaa@yahoo.co.uk](mailto:dr_theiaa@yahoo.co.uk)

chromatography<sup>[12]</sup>, displacement chromatography<sup>[13]</sup>, ion-selective electrode technique<sup>[14]</sup>, electrogenerated chemiluminescence<sup>[15]</sup>, titrimetry<sup>[16–18]</sup>, electrochemical techniques<sup>[19]</sup>, flow-injection biampereometric method<sup>[20]</sup>, bioluminescence micro method<sup>[21]</sup>, enhanced chemiluminescence method employing an online photochemical reaction<sup>[22]</sup>, atomic absorption spectrometry<sup>[23]</sup>. Many Spectrophotometric methods, depending on reduction of nitro group, have been reported for determination of chloramphenicol using various reagents such as isonicotinic acid hydrazide<sup>[24]</sup>, N-(1-naphthyl)ethylenediamine<sup>[25]</sup>, trisodium pentacyanoaminoferrate<sup>[26]</sup>, Ninhydrin<sup>[27]</sup>, iminodibenzyl, 3-aminophenol and pyrocatechol molybdate<sup>[28]</sup>, orthogonal polynomials<sup>[29]</sup>, ammonium molybdate<sup>[30]</sup> and p-dimethylaminobenzaldehyde<sup>[31]</sup>. However; some of these methods suffer from disadvantages such as low sensitivity and narrow range of determination, tedious and needing extraction, using organic medium, are indirect, and either require a long time for stable color development or exhibit instability of the colored product. The aim of the present work was to provide simple, sensitive, and rapid spectrophotometric method for determining of chloramphenicol in pure form as well as in pharmaceutical preparations.



**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, eye drops and ointment) were kindly provided by state company for Drug Industries and Medical appliance-(SDI) Sammara-Iraq. 1,2-Naphthaquinone-4-sulfonate (NQS) was obtained from MOLEKULA and other chemicals were obtained from Fluka. All solvents were analytical reagent grade and water was distilled.

*Standard solution:* 500 µg ml<sup>-1</sup> reduced chloramphenicol(RCAP) solution was prepared by dissolving of 50 mg of its pure form in 20 ml of distilled water and was reduced using 0.5 g zinc powder and 1 ml of conc. hydrochloric acid and kept for 30 min with

stirring for complete reduction. The reduced solution was filtered and diluted with water to 100 ml in a calibrated flask and kept protected from sun light in ambient bottle.

*Reagent solution:* 0.01 M NQS solution was prepared freshly by dissolving 0.065 g in distilled water and diluted to 25 ml in a calibrated flask. A  $5 \times 10^{-3}$  M solution was prepared by suitable dilution.

*Basic solution:* 0.1 M sodium bicarbonate was prepared by dissolving 0.84 g in distilled water and diluted to 100 ml in a calibrated flask.

#### *Recommended procedures*

Aliquots of the working solution of RCAP ( $0.8\text{--}14 \mu\text{g ml}^{-1}$ ) were transferred into a series of 25 ml calibrated flasks. Then, 2.5 ml of 0.1 M  $\text{NaHCO}_3$  and 1 ml of  $5 \times 10^{-3}$  M NQS were added and the solutions were kept at  $50^\circ\text{C}$  for 15 min in water bath, cooled, and diluted to the mark with distilled water. The absorbance was measured at 480 nm against reagent blank.

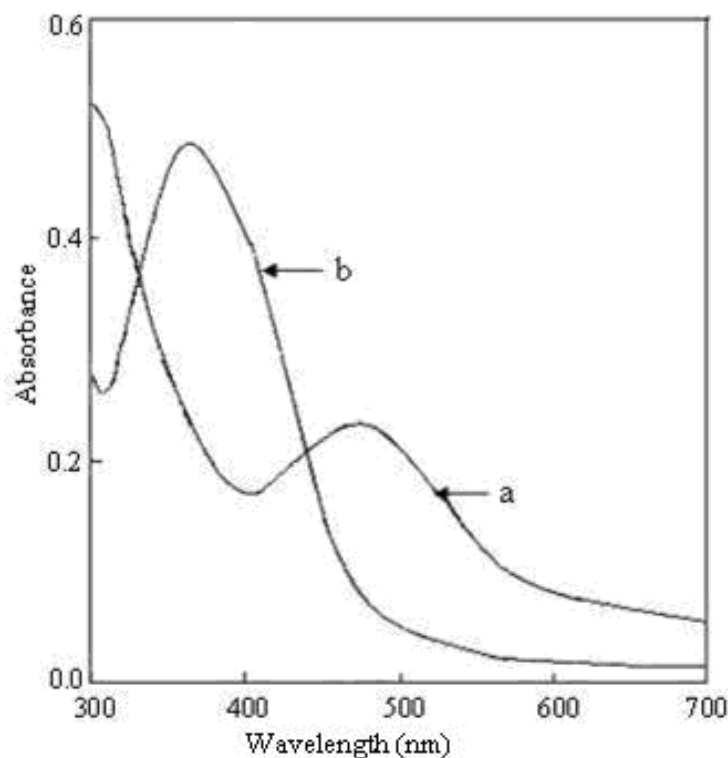
#### *Procedure for chloramphenicol assay in capsules, injection and ointment.*

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, and subjected to the recommended procedure described above for pure chloramphenicol. For injections (0.5% each), a suitable volume was diluted, and the above procedure was followed. For ointment (1%), A portion of the ointment sample equivalent to about 50 mg of chloramphenicol was dissolved in 50 ml of petroleum ether and extracted with the distilled water. It was further extracted with three 25 ml of distilled water. The combined extracts were filtered and made up to 100 ml with distilled water, 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 NQS reagent in the presence of  $\text{NaHCO}_3$  to form an orange-red colored Schiff's base having maximum absorption at 480 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.



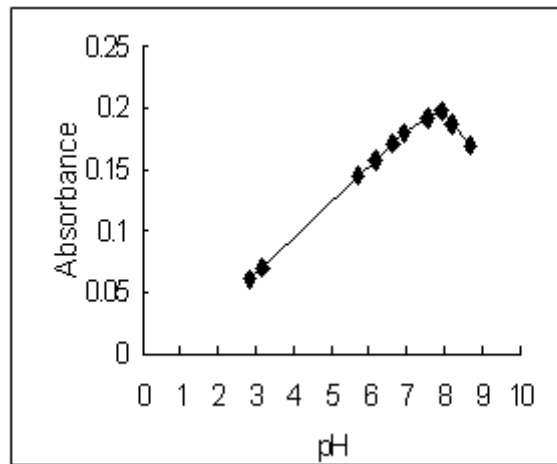
**Figure 1:** Absorption spectra of (a) RCAP ( $8 \mu\text{g ml}^{-1}$ ) product with NQS Reagent ( $5 \times 10^{-3}\text{M}$ ) against reagent blank and (b) reagent blank against distilled water.

#### *Optimization of reaction conditions*

The optimum conditions for the formation of orange-red chromophore were investigated as a function of pH and the type of the buffer, reaction temperature and time and the reagent amount.

#### *Effect of pH, buffer solutions and bases*

The effect of pH was studied in the range 2.84-8.69 using  $\text{NaHCO}_3$  base. The maximum absorbance value was obtained with pH 7.93 in the presence of 2.5 ml  $\text{NaHCO}_3$  (Figure 2). Therefore different buffers of pH 7.93 were prepared by using phosphate and borate buffers to investigate the sensitivity of the product. As shown in Table 1, it was found that these buffers decrease the absorbance, therefore the effect of different bases, such as sodium hydroxide, potassium hydroxide, sodium carbonate, sodium bicarbonate and ammonium hydroxide with 0.1M concentration, on the intensity of the colored product were studied. It was found that sodium carbonate and bicarbonate was suitable to give a good sensitivity for the product with low absorbance value of the blank reagent where as other bases caused a blue shift and gave high absorbance value of blank reagent and ammonium hydroxide show unstable product (Figure 3). However; sodium bicarbonate which gave maximum intensity and stable product was selected in all subsequent experiments.

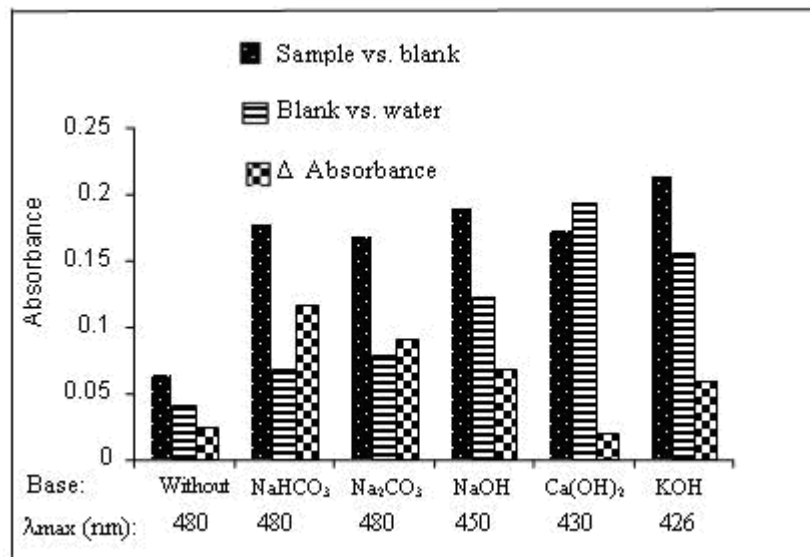


**Figure 2:** Effect of pH on the absorption of 8 µgml<sup>-1</sup> RCAP.

**Table 1:** Effect of buffers on the absorption of 8 µgml<sup>-1</sup> RCAP

Buffer solution (pH 7.93)	$\lambda_{max}$ (nm)	Absorbance	
		Sample vs. blank	Blank vs. water
Without Buffer*	480	0.198	0.035
Na <sub>2</sub> HPO <sub>4</sub> +Citric acid	481	0.118	0.053
KH <sub>2</sub> PO <sub>4</sub> +NaOH	475	0.088	0.056
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> +HCl	485	0.048	0.031
Na <sub>2</sub> HPO <sub>4</sub> +HCl	480	0.128	0.050

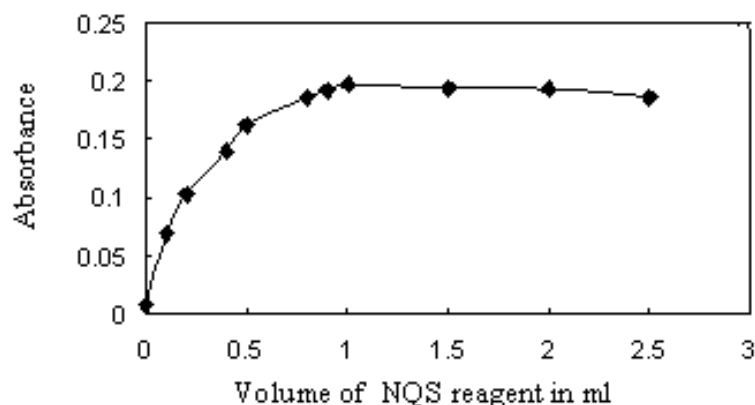
\* In the presence of NaHCO<sub>3</sub>



**Figure 3:** Effect of Different bases on the intensity of 8 µgml<sup>-1</sup> RCAP product with NQS.

#### *Effect of NQS amount*

The optimum amount of reagent was determined by carrying out the reaction with  $8 \mu\text{gml}^{-1}$  RCAP and 0.1-2.5 ml of  $5 \times 10^{-3}$  M NQS solution in the presence of standard amounts of  $\text{NaHCO}_3$  (2.5 ml of 0.1 M) in final volume of 25 ml; as shown in Figure 4, 0.9-2.0 ml NQS solution were gave maximum absorption. However; 1 ml was selected in subsequent experiments.



**Figure 4:** Effect of NQS reagent concentration on absorbance of  $8 \mu\text{gml}^{-1}$  RCAP.

#### *Effect of temperature and time on the color product*

The reaction between RCAP and NQS in the presence of a base was found to be instantaneous. At room temperature ( $23 \pm 2^\circ\text{C}$ ) was found to be very slow, as it required more than 1 hour for completion. Therefore, effect of temperature on the product was studied at different temperatures in the range  $30$ - $60^\circ\text{C}$ ; it was found that the color product show maximum absorbance after 10 min at  $50^\circ\text{C}$  and was stable for a further 30 min. Above  $50^\circ\text{C}$ , the absorbance decreases, indicating dissociation. Hence, 15 min at  $50^\circ\text{C}$  are recommended for the proposed method.

#### *Quantification*

In order to investigate the range in which the colored product adhere to Beer's law, the absorbance of the product was measured at  $\lambda_{\text{max}}$  value after developing the color by following the suggested procedure for a series of solutions containing increasing amounts of chloramphenicol drug. 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 chloramphenicol determined 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 of chloramphenicol ( $0.8$ ,  $4.0$  and  $8.0 \mu\text{g ml}^{-1}$ ) indicated that the method is precise and accurate. Limit of quantitation (LOQ) is determined by taking the

ratio of standard deviation of the blank with respect to water and the slope of calibration curve multiplied by a factor of 10. This means that LOQ is approximately 3.3 times Limit of detection (LOD). Naturally, the LOQ slightly crosses the lower limit of Beer's law range. However, LOD is well below the lower limit of Beer's law range.

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

Parameter	Values of method
Beer's law limits ( $\mu\text{g ml}^{-1}$ )	0.8-14
Molar absorptivity ( $\text{l.mol}^{-1} \cdot \text{cm}^{-1}$ )	$1.019 \times 10^4$
LOD ( $\mu\text{g.ml}^{-1}$ )	0.124
LOQ ( $\mu\text{g.ml}^{-1}$ )	0.473
Average recovery (%)**	99.54
Correlation coefficient	0.9982
Regression equation (Y) *	
Slope, <i>a</i>	0.03154
Intercept, <i>b</i>	0.02893
RSD**	$\leq 3.3$

\*  $Y = aX + b$ , where *X* is the concentration of chloramphenicol in  $\mu\text{g ml}^{-1}$ .

\*\* Average of six determinations.

### Interference

The extent of interferences by some excipients which often accompanied pharmaceutical preparations were studied by measuring the absorbance of solutions containing  $8 \mu\text{g ml}^{-1}$  of chloramphenicol and various amounts of diverse species in a final volume of 25 ml. It was found that the studied excipients do not interfere in the determination of chloramphenicol in its dosage forms. An error of  $\pm 5\%$  in the absorbance reading was considered tolerable. Typical results are given in Table 3.

**Table 3:** Effect of excipients for assay of chloramphenicol

Excipient	Recovery%* of $8 \mu\text{g ml}^{-1}$ chloramphenicol per $\mu\text{g ml}^{-1}$ excipient added			
	4	8	20	40
Glucose	98.93	100.30	100.71	102.49
Lactose	102.40	100.35	101.80	104.30
Starch	100.70	101.77	98.89	96.30
NaCl	97.47	97.83	98.55	102.80
Acacia	101.00	98.19	98.50	97.13
Diphenylamine**	99.27	103.20	103.50	104.60

\* Average of three determinations

\*\* Prepared in ethanol

### Analytical applications

The proposed method was successfully applied to determine chloramphenicol in pharmaceutical 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<sup>[7]</sup> at the 95% confidence level with six degrees of freedom, 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 ( $\mu\text{g}/\text{ml}^{-1}$ )	Recovery <sup>a</sup> (%)	Drug content found (mg)	Average recovery (mg)	Certified value (mg)
Proposed NQS method	Capsule	2	99.00	247.5	252.94 (2.28,3.6) <sup>b</sup>	250
		4	101.13	252.8		
		8	103.40	258.5		
	Eye drops	2	99.60	0.498	0.49 % (1.66,1.26)	0.5 %
		4	95.95	0.479		
		8	98.99	0.494		
Ointment	2	100.17	4.007	3.98 (1.23,2.94)	4.0	
	8	98.66	3.946			
	12	99.85	3.994			
British Pharmacopoeia	Capsule	10	99.60	249	-	250
	Eye drops	10	98.40	0.493	-	0.5%

<sup>a</sup> Average of three determinations.

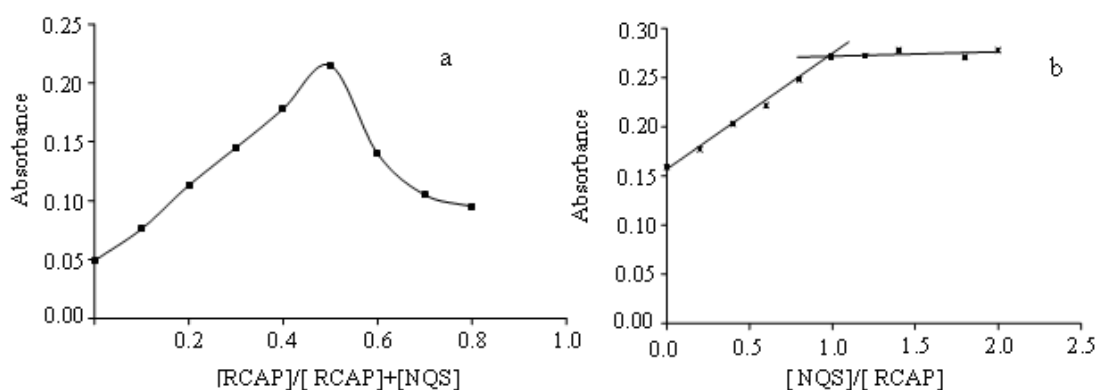
<sup>b</sup> Figures in parenthesis are the calculated values for *t*, and *F*.

**Table 5:** Comparison of spectrophotometric methods for RCAP determination

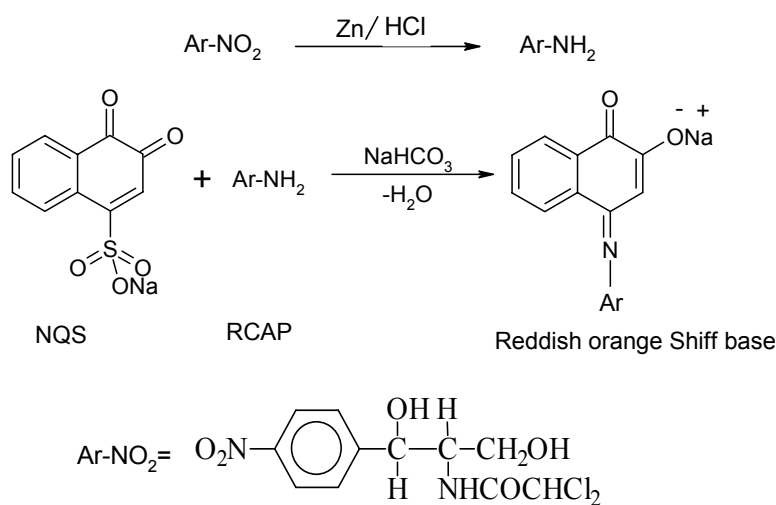
No.	Reagent	$\lambda_{\text{max}}$ (nm)	Linearity ( $\mu\text{g ml}^{-1}$ )	$\epsilon$ (l/Mol.cm)	Remarks	Ref.
1.	Diazotization and coupling with NEDA	550	20 – 200	-	Not sensitive	[25]
2.	Reduction and coupling with trisodium pentacyano amino ferrate	540	4 – 32	-	Freshly prepared reagent required	[26]
3.	Ninhydrin – SnCl <sub>2</sub>	570	10 – 100	$1.7 \times 10^3$	Tedious hydrolysis and heating	[27]
4.	Aminodibenzyl 3-aminophenol sodium molybdate and pyrocatechol	590 470 490	0.25 – 14 0.2 – 10 0.5 – 10	$3.1 \times 10^4$ $3.1 \times 10^4$ $3.2 \times 10^4$	Several reagents, boiling, organic solvent and strong acid medium	[28]
5.	NQS	480	0.8-14	$1.02 \times 10^4$	Proposed method	

### Stoichiometry and reaction mechanism

A characteristic reddish orange colored product of  $\lambda_{\max}$  480 nm is formed when the reduced product of chloramphenicol is allowed to react with NQS in the presence of  $\text{NaHCO}_3$  in aqueous medium. Under the experimental conditions, the light yellow alkaline solution of the o-quinoidal NQS reacts with compound containing one removable hydrogen atom attached to one nitrogen atom, to yield an anionic reddish orange colored paraquinoid imide condensation product. In the present work the stoichiometric ratio of RCAP and NQS was investigated applying the continuous variation (Job's) and mole ratio methods<sup>[32]</sup> using equimolar solutions of each ( $1 \times 10^{-4} \text{M}$ ). As seen in Figure 5, it was found that RCAP forms a product with NQS in the ratio 1:1. The stability constant ( $K_{st}$ ) of the product was determined according to the previous ratio and found  $2.39 \times 10^4 \text{ l. mol}^{-1}$ . However; the probable reaction mechanism based on the reported method<sup>[33]</sup> is given in scheme 2.



**Figure 5:** Continuous variation (a) and mole ratio (b) plots for product of RCAP ( $1 \times 10^{-4} \text{M}$ ) and NQS ( $1 \times 10^{-4} \text{M}$ ) under the optimum conditions.



**Scheme 2:** Probable mechanism for RCAP-NQS product formation

## Conclusion

The proposed method is simple and more sensitive than most of the previously reported spectrophotometric methods. The statistical parameters and the recovery test data indicate the high reproducibility and accuracy of the proposed method. Analysis of authentic samples containing chloramphenicol showed no interference from common additives and auxiliary substances in general. Hence, this method could be considered for the determination of chloramphenicol both in pure form and in pharmaceutical preparations.

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