Kinetic Spectrophotometric Methods for the Determination of Chloramphenicol in Pharmaceutical Preparations

Hind S. Al-Ward
Department of Chemistry, College of Sciences, University of Baghdad, Baghdad-Iraq.

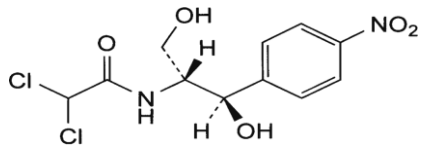
Abstract

Simple and sensitive kinetic methods are described for the determination of chloramphenicol in pure form and pharmaceutical preparations. The methods are based on oxidative – coupling reaction between reduced chloramphenicol (by zinc powder and concentrated hydrochloric acid) with promethazine hydrochloride in the presence of sodium periodate yielding a highly colored product at room temperature, the reaction is followed spectrophotometriclly at λ_{max}= 590 nm. Initial rate and fixed time (at 20 minutes) methods are utilized for concentration determination. The calibration graphs were linear in the concentration ranges (2-20 µg.ml^{-1}) and (0.5-30 µg.ml^{-1}) respectively. The results were validated statistically and checked through recovery studies, and have been applied successfully for the determination of chloramphenicol in commercial dosage forms.

Keywords: chloramphenicol, kinetic spectrophotometry, oxidative–coupling reaction.

Introduction

Chloramphenicol (CAP) is 2,2 dichloro-N-[(1R,2R)-2-hydroxy-1-hydroxymethyl-2-(4-nitrophenyl)ethyl]acetamide, $C_{11}H_{12}Cl_2N_2O_5$, whereas its chemical structure is:



Its molecular weight is 323.1 g mol^{-1}, It is a white, greyish-white or yellowish-white, fine crystalline powder or fine crystals, needles or elongated plates, freely soluble in methanol, ethanol, butanol, ethyl acetate, acetone, and in propylene glycol, slightly soluble in water, and ether, insoluble in benzene, and petroleum ether, it melts at 150.5–151.5°C [1].

Chloramphenicol is a bacteriostatic antimicrobial. It is considered a prototypical broad-spectrum antibiotic, alongside the tetracyclines. Chloramphenicol is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. It is widely used because it is inexpensive and readily available [2]. The most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity, which may occur in two distinct forms: bone marrow suppression, which is a direct toxic effect of the drug and is usually reversible, and aplastic anemia, which is idiosyncratic (rare, unpredictable, and unrelated to dose) and generally fatal. CAP is a non-irritant and is used by local application for the treatment of a variety of infections of the skin, ear and eye including trachoma [3].

Various methods have been reported for the determination of CAP in pharmaceutical preparations, including HPLC [4], LC-Mass spectrometry [5-7], Polarographic [8], electrogenerated chemiluminescence [9], Fluorescent [10], enzymatic method [11], colorimetric and spectrophotometric methods [12-19]. The literature is still poor in analytical procedures based on kinetics, especially for drugs in pharmaceuticals or biological fluids. However, some specific advantages in the application of kinetic methods can be expected such as, selectivity due to the measurement of the evolution of the absorbance with the time of the reaction instead of the measurement of absorbance value. Potassium permanganate has been frequently utilized for kinetic measurements in the field of pharmaceutical analysis. Many pharmaceutical compounds have been determined kinetically through this approach such as tetracycline hydrochloride [20], cephalosporins [21]. A norfloxacine [22] was determined by its reaction with acetaldehyde and 2,3,5,6 – tetrachloro – 1, 4 - benzoquinone to give a colored product. Ketoprofen [23] was determined kinetically by oxidative coupling reaction of the drug with

MBTH reagent in the presence of Ce (IV) in acidic medium. Ramipril has also determined kinetically based on the reaction of the carboxylic group of the drug with a mixture of potassium iodate and potassium iodide and the reaction was followed spectrophotometrically [24]. The aim of the present work was to study the reaction between reduced chloramphenicol and promethazine hydrochloride in the presence of sodium periodate; kinetically in an attempt to evaluate the drug in pharmaceutical preparations. Initial-rate and fixed-time methods were adopted after a full investigation.

Experimental

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV-Visble-260 digital double-beam recording spectrophotometer (Tokyo-Japan), using 1-cm quartz cells.

Reagents:

All chemicals used were of analytical reagent grade. Chloramphenicol standard material was provided from the state company for drug industries and medical appliances (SDI) Sammara-Iraq.

Chloramphenicol (CAP) solution ($500\ \mu g\ ml^{-1}$)= 1.547×10^{-3}M [25].

Prepared by dissolving 0.0500 g of CAP in ethanol transferred into 50 ml volumetric flask, and diluted to the mark with the same solvent. The solution was transferred into a beaker of 125 ml. A 20 ml of distilled water, 20 ml of concentrated hydrochloric acid (11.64 N) and 3 g of zinc powder were added. The beaker was allowed to stand for 15 min at room temperature, then the solution was filtered into 100 ml volumetric flask, washed the residue with distilled water, and diluted to the mark volume with distilled water to obtain 500 $\mu g.ml^{-1}$ of CAP reduced solution. More dilute solutions were prepared daily by appropriate dilution using distilled water.

Promethazine Hydrochloride solution (3.09×10^{-2}M).

Prepared freshly by dissolving 0.9915 gm of pure promethazine hydrochloride in small amount of distilled water then completed to 100 ml with the same solvent.

Sodium periodate solution (3.09×10^{-2}M).

Prepared by dissolving 0.6609 gm with distilled water then completed the volume to 100 ml with the same solvent.

Solutions of pharmaceutical preparations.

1-Capsules samples (Aphenicol / 250 mg Chloramphenicol–Ajanta Pharma limited, India):

The contents of ten capsules were weighed and the powder was mixed. An accurately weighed portion of the powder equivalent to 50 mg of CAP was dissolved in to 30 ml of ethanol. The solution was filtered into a 50 ml volumetric flask, the residue was washed with ethanol and diluted to volume with the same solvent to obtain 1000 $\mu g\ ml^{-1}$ of CAP. This solution was transferred into 125 ml beaker and was reduced as described above.

2-Eye drops samples -10 ml (0.5% chloramphenicol/ 0.005% cetrimide-SDI, Sammara, Iraq):

The contents of three bottles of eye drops were mixed. An aliquot corresponding to 50 mg of CAP (10 ml) was diluted to 50 ml with ethanol in a volumetric flask to obtain 1000 $\mu g.ml^{-1}$ of CAP. This solution was transferred into 125 ml beaker and was reduced as described above.

3-Ointment samples - 5 gm (Betaphenicol sterile ophthalmic / 0.5% chloramphenicol 0.2 % betamethasone - Delta for medicaments, Syria):

The contents of five tubes of ointment were mixed. An accurately weighed amount of ointment equivalent to 50 mg of CAP was extracted three times with 10 ml of ethanol. The solution was filtered into a 50 ml volumetric flask, the residue was washed with ethanol and diluted to volume with the same solvent to obtain 1000 $\mu g\ ml^{-1}$ of CAP. This solution was transferred into 125 ml beaker and was reduced as described above.

Results and Discussion

Preliminary investigations

Throughout the preliminary investigations of oxidative coupling reaction between reduced CAP with promethazine HCl in the presence of sodium periodate to give a soluble purple colour dye that have a maximum absorbance at 590 nm. The absorbance of the colored product was measured versus reagent

blank increases with time and then remains stable for at least 120 min. This was used as a basis for a useful kinetic method for the determination of CAP in pharmaceutical preparations. Initial studies were directed towards the optimization of the experimental conditions in order to establish the optimum conditions necessary for quantitative formation of the product with maximum sensitivity.

Optimization of the experimental conditions

The effect of various variables on the color development was tested to establish the optimum conditions for determination of CAP. In subsequent experiments, 500 μg ml^{-1} of CAP was taken to a 25 ml final volume and the absorbance was measured at room temperature (25°C) for series of solutions by varying one and fixing the other parameters at 590 nm versus reagent blank after 20 min from the beginning of the reaction.

1- Effect of volume of Promethazine HCl (3.09×10^{-2} M).

The effect of volume of the reagent solution was investigated by carrying out the reaction using different volumes of promethazine HCl ranging from (0.5-2.5 ml). The maximum absorbance was obtained upon using 1 ml of (3.09×10^{-2} M) promethazine HCl solution.

2-Effect of volume of Sodium periodate solution (3.09×10^{-2} M).

The effect of volume of the oxidant solution was studied by carrying out the reaction using different volumes of sodium periodate solution ranging from (0.5-2.5 ml). An increase in absorbance was obtained upon using 1.5 ml of oxidizing solution (3.09×10^{-2} M).

3-Effect of order of addition

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

4- Effect of temperature

The effect of temperature on the oxidative coupling reaction study show that the absorbance of the dye remains constant at room temperature (25 C^o) for more than 120 min, and decrease at (0-5C^o). Heating of the mixture of reaction over 45 C^o will precipitate the dye formed with decreasing in absorbance after 10 mins.

Absorption spectra

After obtaining the optimum conditions for the formation of the product, the absorption spectra of the product solution versus reagent blank and reagent blank versus distilled water were recorded within 300 to 700 nm (Fig.(1)). The maximum absorption of the product was found at 590 nm, which was the same as found in the preliminary investigations, and it was used in all subsequent experiments.

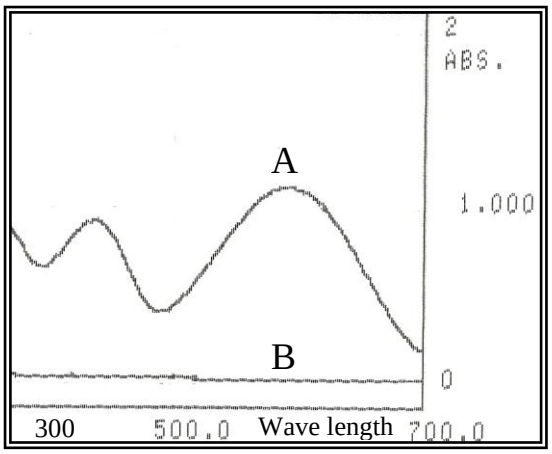


Fig.(1) The absorbance spectra of (A) the colored dye against blank and (B) the blank against distilled water.

Analytical procedure for calibration

In to a series of 25 ml volumetric flask, transfer increasing volumes of standard stock solution (500 μg.ml^{-1}=1.547×10^{-3}M) containing (0.1-1 ml) of reduced CAP to cover the range of the calibration graph (2-20 μg.ml^{-1}) for the initial-rate method and (0.25-1.5 ml) of reduced CAP to cover the range of the calibration graph (0.5-30 μg.ml^{-1}) for the fixed-time method, to this solutions added 1.5 ml of Sodium periodate (3.09×10^{-2} M) shake thoroughly, then 1 ml of (3.09×10^{-2} M) of promethazine HCl was added and the contents were dilute to the mark with distilled water and shake well and transferred to a spectrophotometer cell. The absorbance of the colored product was measured as a function of time (after 5 minutes and after 20 minutes for the two methods respectively), at 590 nm against a reagent blank prepared in the same way but containing no CAP at room temperature (25 C^o). The initial rate of the reaction at different concentration was

obtained from the slop of the tangent to the absorbance time curve as shown in (Fig.(3)), and analytical values of statistical treatments for the calibration graph for the fixed time method was shown in (Fig.(6)).

Stoichiometry of the reaction

The stoichiometry of the reaction, combining ratio between promethazine HCl and CAP, was established by limiting the logarithmic method [26], using two sets of experiments. In the first set, the CAP concentration was varied while keeping a constant promethazine HCl concentration (3.09×10^{-2} M); in the second set, the promethazine HCl concentration was varied while keeping a constant concentration of CAP ($1.547x10^{-3}$M).

A plot of log absorbance versus log [CAP] and log [promethazine HCl] gave straight lines; the values of the slopes were 0.4978 and 0.466, respectively (Fig.(2)). Hence, it is concluded that, the molar reactivity of the reaction is 0.4978 / 0.4462, i.e. the reaction proceeds in the ratio of 1:1 (CAP: promethazine HCl).

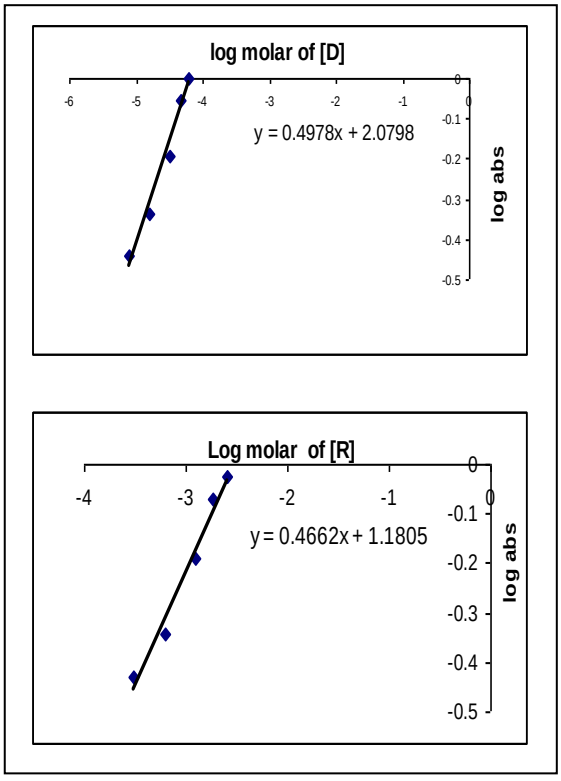
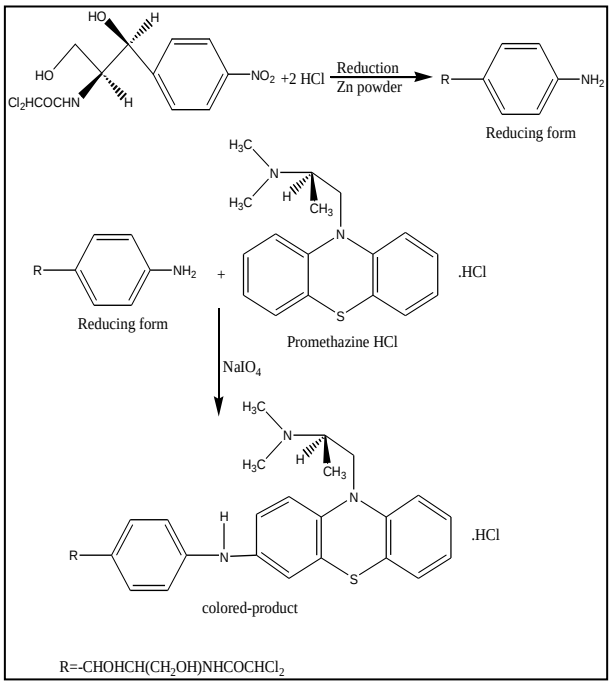


Fig.(2) Limiting logarithmic plots for the molar ratio.

Based on the obtained molar reactivity, A reaction subsequent based on the above results is shown in Scheme (1).



Scheme (1) Reaction scheme for the reaction between chiorawphneeal and promethazine HCl.

Evaluation of the kinetic methods

The quantitation of CAP under the optimized experimental conditions outlined above would result in a pseudo-first order with respect to its concentrations where promethazine HCl, were at least 20 time of the concentration of CAP. However, the rate was directly proportional to CAP concentration in a pseudo-first order equation as follows:

Rate = k' [CAP] ..... (1)

where k' is the pseudo-first order rate constant.

Several experiments were then carried out to obtain CAP concentration from the rate data according to equation (1). Initial rate, fixed time methods [27,28] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

1- Initial rate method

The initial rates of the reaction were determined by measuring the slopes of the initial tangents the absorbance time curves for the first 5 min (Fig.(3)). Furthermore, logarithmic analysis of the reaction rate (R) was plotted against log concentration of the drug (Fig.(4)).

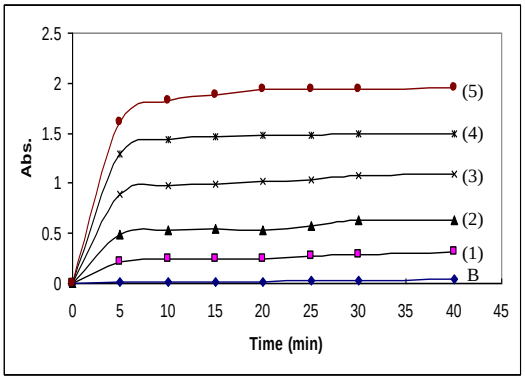


Fig.(3) Absorbance verses time graph showing the dependence of the reaction on CAP concentration. B: blank, (1) 6.19 × 10^{-6} M, (2) 15.6 × 10^{-6} M, (3) 31.2 × 10^{-6} M, (4) 4.68 × 10^{-5} M and (5) 6.24 × 10^{-5} M.

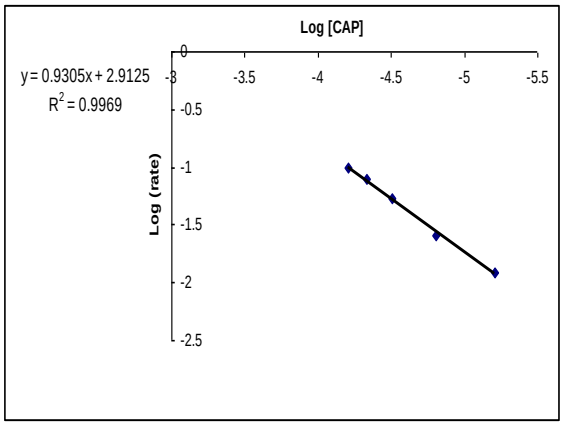


Fig.(4) Log (rate) versus log[CAP] graph.

The rate of reaction was also found to be dependent on CAP concentrations; the rates were followed at room temperature (25°C) with various concentration of CAP in the range of 2–20 µg ml^{-1} keeping the reagent and the oxidant concentrations constant. The reaction rate was found to obey the following equation:

$Rate = k' [CAP]^n$ .....(2)

Where k' is the pseudo-order rate constant and n is the order of the reaction. The rate of the reaction may be estimated by the variable-time method [29] (differential initial rate method) [30] as ΔA / Δt, where A is the absorbance and t is the time in minutes. Taking logarithms of rates and concentration, equation (3) is transformed into:

$Log(rate)=log\Delta A/\Delta t=logk'+nlog[CAP]$.....(3)

Regression of log (rate) versus log [CAP] gave the regression equation:

Log (rate) = 2.915 + 0.9305 log C

Where (r = 0.9969).

Hence k = 822 min^{-1} = 14 sec^{-1} and the reaction is first order (n = 0.9305) with respect to CAP concentration.

The analytical values of statistical treatments for the calibration graphs are summarized in (Table (1)).

Table (1)
analytical values of statistical treatments for the calibration graph of the initial-rate method (at 5 mins.).

Parameters	*value*
Correlation coefficient, r	9.973X10^{-1}
Linearity percentage, r^2%	99.73
Test for a significant correlation ,t^*	23.528
Regression equation	y=0.0835x+0.0545
Slop, b (ml.µg^{-1})	8.35 X 10^{-2}
Intercept, a	5.45 X 10^{-2}
Standard deviation of the residuals, $S_{y/x}$	5.00 X 10^{-2}
Standard deviation of the slop, S_b	3.424 X 10^{-2}
Standard deviation of the intercept, S_a	7.963 X 10^{-3}
Linearity range (ppm)	2-20
Molar absorptivity (l.mol^{-1}.cm^{-1})	2.697 X 10^3
Sandell's sensitivity S (µg.cm^{-2})	1.197 X 10^{-1}
Limit of detection, LOD (µg.ml^{-1})	1.796
Limit of quantification, LOQ (µg.ml^{-1})	5.988

****t-tabulate =4.303 at confidence level 95% and (n – 2) = 3 degrees of freedom***

2- Rate Constant Method

The best way to obtain an average K` value for the reaction, is to plot the log (A) versus time for CAP in the concentration range 2.0 - 20.0 µg.$ml^{-1}$ (6.21× $10^{-6}$ –6.24×$10^{-5}$ M) (Fig.(3)), obtained pseudo first rate constant **K`** corresponding to different CAP concentrations. These **K`** values were calculated from the slops of curves multiplied by -2.303, (Table (2)).

Table (2)
Values of K` calculated from slops of Log A versus t graphs at 590 nm.

[Drug]	*Equation*	*K`/min^{-1}*
6.20 X 10^{-6}	LogA= 0.0040t-0.6695	-9.212x10^{-3}
15.6 X 10^{-6}	LogA= 0.0025t-0.3015	-5.757x10^{-3}
31.2 X 10^{-6}	LogA= 0.0021t-0.0386	-4.836x10^{-3}
4.68 X 10^{-5}	LogA= 0.0011t-0.1473	-2.533x10^{-3}
6.24 X 10^{-5}	LogA= 0.0008t-0.2353	-1.842x10^{-3}

Regression of [CAP] versus K` gave the following equation:
K`=121.82[Drug]–0.0088
Where r = 0.9483 as shown in (Fig.(5)).

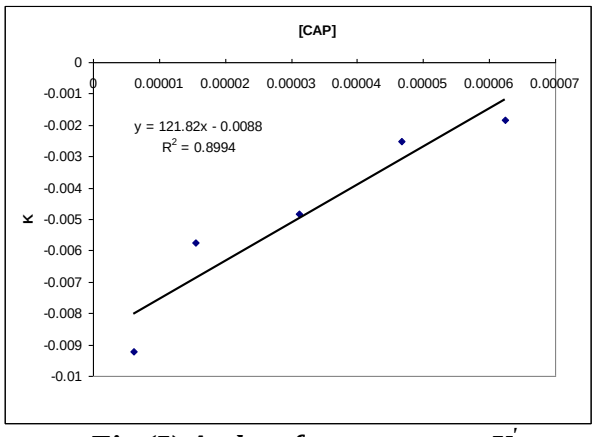


Fig.(5) A plot of rate constant K' versus [CAP].

3-Fixed-time method

At a pre-selected fixed time, Calibration graphs of absorbance versus initial concentration of CAP were established at fixed times of 5, 10, 15, 20, 25, 30, 35, and 40 min with regression equations assembled in (Table (3)).

Table (3)
Regression equations for CAP at different fixed time over range 1.547×10^{-6}to9.285×10^{-5}M at room temperature.

Time (min)	*Regression equation*	*R^2*
5	A=0.0545+0.0835X	0.9947
10	A=0.0553+0.089X	0.9936
15	A=0.0570+0.0927X	0.9968
20	A=0.0650+0.0961X	0.9991
25	A=0.0670+0.095X	0.9974
30	A=0.0852+0.0943X	0.9942
40	A=0.0936+0.0945X	0.9936

It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 20 min, which was therefore, chosen as the most suitable time interval for measurement. After optimizing the reaction conditions, the fixed time method was applied to the determination of CAP in pure form over the range 0.5–30 µg.ml^{-1}, (Fig.(6)), and analytical values of statistical treatments for the calibration graphs are summarized in (Table (4)).

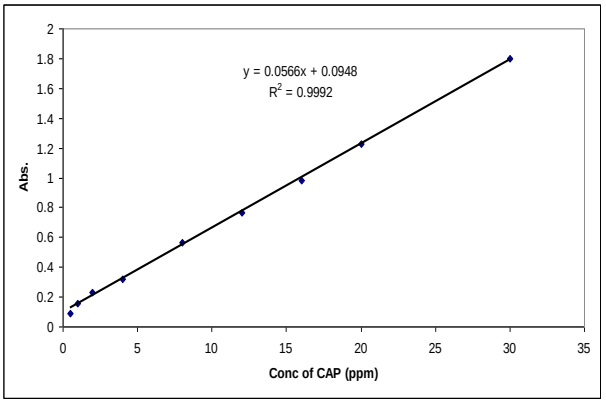


Fig.(6) Calibration graphs of CAP at fixed time 20 min.

Table (4)
Analytical values of statistical treatments for the calibration graph of the fixed time method.

Parameters	*value*
Correlation coefficient, r	9.997 X 10^{-1}
Linearity percentage , r^2%	99.97
Test for a significant correlation, t*	132.245
Regression equation	y=0.0566x+0.0947
Slop, b (ml.µg^{-1})	5.658 X 10^{-2}
Intercept, a	9.466 X 10^{-2}
Standard deviation of the residuals, $S_{y/x}$	8.001 X 10^{-3}
Standard deviation of the slop,S_b	0.02139
Standard deviation of the intercept, S_a	0.009525
Linearity range (ppm)	0.5-30
Molar absorptivity (l.mol^{-1}.cm^{-1})	1.822
Sandell's sensitivity S (µg.cm^{-2})	1.767 X 10^{-2}
Limit of detection, LOD (µg.ml^{-1})	0.4285
Limit of quantification, LOQ (µg.ml^{-1})	1.428

****t-tabulate = 2.447 at confidence level 95% and (n – 2) = 7 degrees of freedom.***

From values of test for a significant correlation (t-calculate > t-tabulate) and linearity percentage (> 95%). These calibration graphs possess excellent linearity.

Accuracy and precision

To determine the accuracy and precision of CAP which was determined in five replacements of three different concentrations. The results shown in (Tables (5, 6)), indicate that a satisfactory precision and accuracy could be obtained with the proposed method.

Table (5)
Accuracy and precision of the initial–rate 0 method.

Concentration of CAP µg.ml^{-1}		Error %	Rec. %	R.S.D %
Present	Found*			
4.00	4.05	0.50	100.50	1.509
12.00	12.28	0.33	100.33	0.415
20.00	19.96	0.20	100.20	0.375

**** for five determinations.***

Table (6)
Accuracy and precision of the fixed-time method.

Concentration of CAP µg.ml^{-1}		Error %	Rec. %	R.S.D %
Present	Found*			
2.00	1.98	1.00	101.00	1.238
8.00	7.93	1.00	101.00	0.851
16.00	16.03	-0.18	99.810	0.424

**** for five determinations.***

Pharmaceutical applications

The initial-rate and fixed-time methods were applied to the determination of CAP in pharmaceutical preparation by the analysis of two different concentrations of pharmaceutical preparations using the analytical procedures. The results are given in (Table (7)) and (Table (8)).

Table (7)
Application of the proposed method of CAP In pharmaceutical preparations by the initial-rate method.

Drug sample	Concentration of CAP (ppm)		Error %	Rec %	R.S.D %
	Present	Found*			
1	5	5.09	-1.80	98.20	1.06
	15	15.25	-1.66	98.33	0.94
2	5	4.95	1.00	101.00	0.77
	15	15.08	-0.53	99.46	0.31
3	5	5.11	-2.20	97.80	2.31
	15	15.19	-1.26	98.73	1.79

****for five determinations***
1- Aphenicol capsule
2- Cetrimide eye drops
3- Betapheni Ointment

Table (8)
Application of the proposed method of CAP In pharmaceutical preparations by the fixed-time method.

Drug sample	Concentration of CAP (ppm)		Error %	Rec. %	R.S.D %
	Present	Found*			
1	5	4.93	1.40	101.40	0.85
	15	14.84	1.06	101.63	0.62
2	5	5.03	-0.60	99.40	1.19
	15	14.89	0.73	100.73	1.01
3	5	5.02	-0.40	99.60	1.31
	15	15.21	-1.40	98.60	0.93

****for five determinations***
1- Aphenicol capsule
2- Cetrimide eye drops
3- Betapheni Ointment

The proposed method was compared successfully with the BP method [1] for both pure CAP and the pharmaceutical preparations for both initial-rate and fixed-time method, good recoveries were obtained as shown in (Table (9)).

Table (9)
Comparison of the proposed methods with standard method.

Drug sample	*Recovery%**		
	Fixed-time method	*Initial-rate method*	*BP method*
Pure CAP	101.00	100.70	100.00
Aphenicol capsule	101.63	98.33	99.53
Cetrimideey e drops	100.73	99.46	100.60
Betapheni Ointment	98.60	98.73	98.67

for five determinations

Conclusions

The proposed methods are showing good sensitivity, and low detection limit. In addition, the proposed procedures show relevant selectivity allowing analysis without separation steps, and providing suitable alternative to the many chromatographic procedures proposed [4-7]. The proposed methods are advantageous when they are compared with colorimetric methods [12-19] in having higher sensitivity. The data given above reveal that the proposed methods are accurate and sensitive with good precision and accuracy. With this method, one can do the analysis with speed at low cost without losing accuracy. The proposed method can be used as alternative method to reported ones for the routine determination of CAP in the pure form and in pharmaceutical preparations depending upon the availability of chemicals and equipment.

References

[1] "British Pharmacopoeia on CD-Rom" The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA). London. 5th., ed., 2007.

[2] Falagas, M. E.; Michalopoulos, A. A.; "Potential of old-generation antibiotics to address current need for new antibiotics"; Expert Rev Anti Infect Ther., 6, 593–600, 2008.

[3] Wilson, A.; Schild, H. O.; Modell, W.; "Applied Pharmacology"; 11th Ed., Churchill Livingstone, London, 1975.

[4] Pan, Y.; Xu, Q.; Kang, X.; Zhang, J.; "Determination of chloramphenicol residues in milk by reversed-phase high performance liquid chromatography with fluorescence detection"; 23, 577-580, 2005.

[5] Storey, j.; Pfenning, A.; Turnipseed, S.; Nandrea, G.; Rebecca, L.; Burns, C.; Madson, M.; "Determination of Chloramphenicol Residues in Shrimp and Crab Tissues by Electrospray Triple Quadrupole LC/MS/MS"; 19, 1-16, 2003.

[6] Jiang, Y.; Zhong, X.; Zhong, T.; Shen, C.Y.; Ding, T.; Chen, H. L.; Wu, B.; Shen, W. J.; "Determination ofchloramphenicol in royal jelly by liquid chromatography/ tandem mass spectrometry", veterinary drug residues, Journal of AOAC International; 47, 3464–3469, 2006.

[7] Teresa, K.; "Determination of Chloramphenicol in Feed Use of High - Performance Liquid Chromatographic - Mass Spectrometry", Branch Institute of Animal Drugs Inspection, 2003.

[8] Summa, A. F.; "Polarographic determination of chloramphenicol preparations", J. Pharm. Sci.; 54, 442-444, 1965.

[9] Lindino C. A.; Bulhões, L.O. S.; "Determination of chloramphenicol in tablets by electrogenerated chemilumine-scence"; J. Braz. Chem. Soc., 15, 876-880, 2004.

[10] Haughland, P.; Kang. R.; Young, H.; Steven, L.; Melner, M.; "Fluorescent chloramphenicol derivatives for determination of chloramphenicol acetyltransferase activity", Molecular Probes, 18, 722- 730, 1991.

[11] Morris, H.C.; Miller, J.; Campbell, L. S.; Hammond, P. M.; Berry, D. J.; Price, C. P.; "A rapid, enzymatic method for the determination of chloramphenicol in serum"; Journal of Antimicrobial Chemotherapy, 22, 935-944, 1988.

[12] Chukwuenweniwe, J. E.; Johnson, S.; Adelusi, S. A.; "An alternative colorimetric method for the determination of chloramphenicol"; Tropical Journal of Pharmaceutical Research, 2, 215-221, 2003.

[13] Wahbi, A. M.; Abdine, H.; Korany, M.A.;El-Yazbi, F. A.; "Spectrophotometric determination of chloramphenicol-sulphacetamide in eye drops"; Pharmazie., 33, 721-722, 1978.

[14] Naik, S.; Nagaraja, P.; Yathirajan, H.; Hemanthakumar; Mohan, H.; "New spectrophotometric methods for the quantitative determination of chloramphenicol in pharmaceuticals" , Pharmaceutical Chemistry Journal, 40, 576-581, 2006.

[15] Shah, R. C.; Raman, P.V.; Shah, B. M.; "Spectrophotometric determination of chloramphenicol and tetracycline hydrochloride in mixtures" Journal of Pharmaceutical Sciences, 52, 167–168, 1963.

[16] Freeman, F. M.; "the colorimetric determination of chloramphenicol", J. Chem. Soc., 81, 298-299, 1956.

[17] Mahrous, M. S.; Abdel-Khalek, M. M.; "Spectrophotometric determination of phenothiazines, tetracyclines and chloramphenicol with sodium cobaltinitrite", Talanta, 30, 792-794, 1983.

[18] Moţ, A.; Soponar, C.; Medvedovici, F.; Sarbu, A.; "Simultaneous Spectro-photometric Determination of Aspirin, Paracetamol, Caffeine, and Chlorphenamine from Pharmaceutical Formulations Using Multivariate Regression-Methods" World Wide Science. org, 2010.

[19] Al-Sabha, T. N.; Rasheed, B. A.;" Spectrophotometric Method for Determination of Chloramphenicol in Pharmaceutical Preparations using 1,2-Naphthoquinone-4-Sulphonate as a Chromogenic Reagent", JJC, 5, 201-210, 2010.

[20] Ahmidaa, N. H. S.; El-Hashemea, F.; El-Enany N.; Belal, F.; "Kinetic spectro-photometric method for the determination of tetracycline hydrochloride in pharmaceutical formulations", Applied Science Research, 1, 1-11, 2009.

[21] Omar, M. A.; Abdelmageed, O. H.; Attia, T. Z.; "Kinatic spectrophotometric determination of certain cephalosporins in pharmaceutical formulations", International Journal of Analytical Chemistry, 5, 12-15, 2009.

[22] Darwish, I. A.; Sultan, M. A.; Al-Arfaji, H. A.; "Novel selective kinetic spectrophotometric method for the determination of norfloxacin in its pharmaceuticals formulations", Talanta, 78, 1383-1388, 2009.

[23] El-Brashy, A.; Eid, M.; Talaat, W.; "Kinetic spectrophotometric method for the determination of Ketoprofen in pharmaceuticals and biological fluids, International Journal of Biomedical Science, 2, 405-412, 2006.

[24] Rahman, N.; Ahmad, Y.; Najmul S.; Azmi, H.; " Kinetic Spectrophotometric Method for the Determination of Ramipril in Pharmaceutical Formulations", AAPS Pharm Sci Tech., 6, 543-551, 2006.

[25] Abd-AlSatar, R. S.; "development of new spectrophotometric methods for determination of some organic drug compounds in pharmaceutical preparations " Ph.D thesis, Baghdad University, 2006.

[26] Roso, j.; "Advanced Physicochemical Experiments", Sir Issac Pitman and Sons, London, 1964.

[27] Weisberger, A.; Friess, S. L.; Lewis, E. S.; "Techniques of OrganicChemistry", vol. 3, Part 1, Interscience, New York, 1953.

[28] Bendito, D. P.; Silva, M.; "Kinetic Methods in Analytical Chemistry", Ellis Horwood, Chichester, 1988.

[29] Yatsimirskii, K. B.; "Kinetic Methods of Analysis" , Pergamon Press, Oxford, 1966.

[30] Laitinen, H. A.; Harris, W. A.; "Chemical Analysis", 2nd Ed., McGraw-Hill, New York, 1975.

الخلاصة

طورت طرق طيفية حركية بسيطة وحساسة لتقدير دواء الكلورامفينيكول بصورته النقية وفي المستحضرات الصيدلانية. اعتمدت الطرق على تفاعل الازدواج التأكسدي للدواء المختزل (بوساطة مسحوق الخارصين وحامض الهيدروكلوريك المركز) مع كاشف هيدروكلوريد البروميثازين بوجود بيريودات الصوديوم كعامل مؤكسد لتكوين صبغة بنفسجية قيست طيفيا" عند الطول الموجي الاعظم= ٥٩٠ نانوميتر. وتم دراسة حركية التفاعل بوساطة طريقتي معدل السرعة الابتدائية والزمن الثابت وثبتت الظروف الفضلى للتفاعل لكلا الطريقتين وكان مدى الخطية لتقدير الدواء بأستخدام طريقة معدل السرعة الابتدائية بين (٢-٢٠ مايكروغرام/مل) وبأستخدام طريقة الزمن الثابت عند ٢٠ دقيقة بين (0.5-30 مايكروغرام/ مل). طبقت الطريقتين بنجاح في تقدير دواء الكلورامفنيكول في المستحضرات الصيدلانية.