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Spectrophotometric Determination of Chloramphenicol in Pharmaceutical Preparations

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الخلاصة

تم اقتراح طريقة طيفية بسيطة وحساسة لتقدير كميات متناهية في الصغر من الكلورامفينكول. وتعتمد هذه الطريقة على اختزال مجموعة النايترو الموجودة في الكلورامفينكول إلى مجموعة الامينو ثم يقترن الأخير مع كاشف البروميثازين وبوجود ايون السيريوم الرباعي، فتتكون صبغة ملونة تعتمد شدة لونها على كمية الكلورامفينكول الموجودة في المحلول وتمتص عند 606 نانوميتر وبحساسية عالية إذ كانت الإمتصاصية المولارية مساوياً إلى 1.292×10^4 لتر.مول$^{-1}$.سم$^{-1}$ ودلالة ساندل مساوية إلى 0.025 مايكروغرام/سم^2 ووجد أن قانون بير ينطبق على مدى من التركيز ما بين 0.4 إلى 12 جزء/مليون. و كان الخطأ النسبي ما بين −2.73 إلى 0.37 + % والانحراف القياسي النسبي ما بين 3.84± إلى 0.44± %، اعتماداً على مستوى التركيز، وقد طبقت الطريقة بنجاح في تقدير الكلورامفينكول في المستحضرات الصيدلانية (كبسول وقطرة العين ومرهم العين).

Abstract

A spectrophotometric method for the determination of trace amounts of chloramphenicol has been proposed. This method depends upon the reduction of the nitro to amino group, condensation with promethazine reagent in the presence of cerium (IV) ions to form a colored dye which exhibits maximum absorption at 606 nm with a high sensitivity (molar absorptivity is 1.292×10^4 $l.mol^{-1}.cm^{-1}$, and Sandell's sensitivity index of 0.025 $\mu g/cm^2$). Beer' s law is applied within the concentration range of obeyed in 0.4 to 12 ppm with a relative error of -2.73 to + 0.37% and a relative standard deviation of ± 3.84 to ± 0.44%, depending on the concentration level. The method has been successfully applied to the determination of chloramphenicol in pharmaceutical preparations (capsules, eye drop and eye ointment).

Introduction

Chloramphenicol (CAP) (1) is one of the first widely used antibiotics (2).CAP is 2,2-dichloro-N-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-acetamide (Fig. 1) (3).

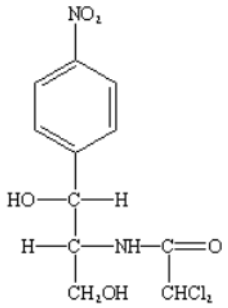


Fig.1. Structure of chloramphenicol

It is a broad spectrum antibiotic which acts by interfering with action of peptidyl transferase after binding to 50S subunit of ribosome and inhibit protein synthesis (3).CAP has been widely used both in medical and veterinary practice, although reasonably safe in domestic animals, however, it is known to exert several side effects in humans such as allergic reactions, gastrointestinal disorders, dose dependent bone narrow depression and grey syndrome in newborns (4). The most serious and potentially lethal effect of chloramphenicol is a plastic anemia (4). Several methods have been reported for the determination of CAP including titration(5),spectrophotometry(2,6-8)biosensor immunoassay (9), gas chromatography (10,11) , liquid chromatography-mass spectrometry(12),liquid chromatography-electrospray ionisation tandem

mass spectrometry (13,14),enzyme-liquid immunosorbent assay (15-17), molecularly imprinted polymer for HPLC (1), chemiluminescences (18), differential-pulse polagraphy (19) and derivative spectrophotometry (20). Among the various methods available for the determination of the drug, spectrophotometry continues to be very popular, because of its simplicity, specificity and low cost. The present investigation deals with the development of a spectrophotometric method based on oxidative-coupling reaction for the assay of chloramphenicol in pharmaceutical preparation.

Experimental

Apparatus

Cintra 5-GBC Scientific Equipment and UV-Visible spectrophotometric. CECIL-CE 1021 digital single beam spectrophotometer with 1.0 cm matched silica and quartz was used for all absorption measurements.

Reagents

Chemicals used are of the highest purity available. A pure CAP was obtained from the State Company for Drug Industries and Medical Appliances (SDI), Samara, Iraq.

Chloramphenicol solution (10000µg ml^{-1}): 0.5g of pure CAP was dissolved in a 20 ml of absolute ethanol and diluted the solution to 50 ml with distilled water.

Reduced chloramphenicol RCAP(500µg ml^{-1}):5 ml of (10000µg ml^{-1}) solution was transferred into a100 ml conical flask, 10 ml of distilled water, 20 ml of hydrochloric acid (11.55N) and 4 g of zinc powder were added. The flask was allowed to stand for 15 minutes and then filled up to the mark with distilled water after filtering the solution.

Reduced chloramphenicol working solution (100µg ml^{-1}): 20 ml of reduced CAP solution (500µg ml^{-1}) was transferred, the solution was brought to pH 7.0 with sodium carbonate solution, filtered, then the filtrate solution diluted to 100 ml in a standard volumetric flask.

Procedures for pharmaceutical preparations

Capsules (BROWN and BURK, (UK) LTD., London)

The contents of 12 capsules (250mg) were weighed and the powder was mixed. The accurately weighed portion of the powder equivalent to one capsule dissolved in 20 ml of distilled water (with warming). The solution was filtered into a 50 ml calibrated flask. To obtain RCAP solution 5 ml of this solution was transferred and react with zinc powder and hydrochloric acid preceded as a procedure described above.

Eye drops (REYERLABS, India)

The contents of five bottles of eye drops (0.5%) was mixed. 10 ml of this solution was transferred into a 50 ml calibrated flask and diluted to the mark with distilled water. 25 ml of this solution was transferred and proceeded as procedure for RCAP described above.

Eye ointment (HOLEN)

The contents of five bottles of eye ointment (0.1%) was mixed. 5 g of this ointment was dissolved in 50 ml of petroleum ether then this solution was extraction using distilling water into 4 portion, each portion contain 50 ml of distilled water. Then filtrated RCAP obtain by using procedure described above.

Promethazine.HCI solution (0.1%): The solution was prepared by dissolving 0.1 g of promethazine.HCl from (SDI) in distilled water and completed to 100 ml in volumetric flask. This solution was store in dark bottle; it is stable for at least one week.

Oxidative reagent solution (0.1%): This solution was prepared by dissolving 0.1 g of Ammonium ceric sulphate dihyrate (ACS) from (BDH) in 100 ml of warm distilled water. This solution was used in the same day.

Formic acid solution (1N): This solution was prepared by dilution 9.6 ml of concentrated formic acid (25.97 N) (Fulka) with distilled water to the mark in 250 ml volumetric flask.

Excipient drugs (1000 ppm): This solution was prepared by dissolving 0.1 g in 100 ml of distilled water.

General procedure and Calibration graph

The aqueous solution of RCAP contains (10-700) µg was transferred to 25 ml calibration flask. A 4 ml of promethazine.HCl reagent (0.1%) solution, 5 ml of formic acid (1N) then 8 ml of oxidative reagent (cerium IV) (0.1%) were added and the volume was made up to the mark with distilled water . The absorbance measured after 15 minute at 606 nm against a blank solution which was prepared in a similar way but without the addition of RCAP . The calibration graph as shown in Fig. (2) was liner over the range of (10-300) µg of RCAP/25 ml (0.4-12 ppm). Higher concentration showed a negative deviation from Beer's law. The apparent molar absorpitivity referred to RCAP 1.292×10^{4} $l.mol^{-1}.cm^{-1}$ and Sandel's sensitivity is equal to 0.025 µg/cm.

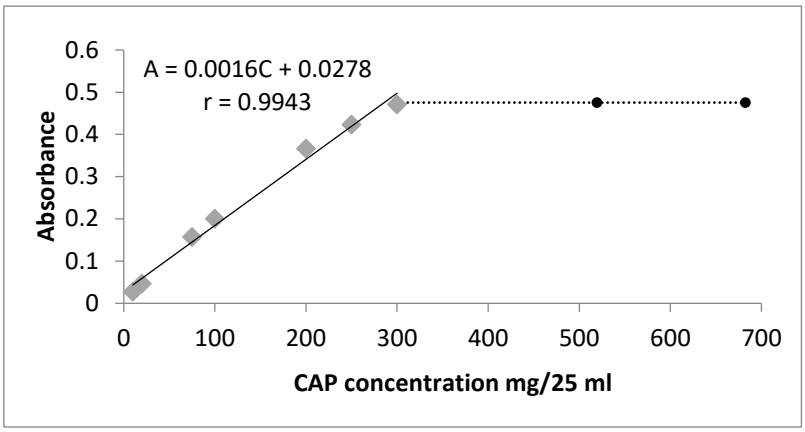


Fig.2Calibration graph for CAP determination using the proposed method

Results and discussion

The effect of various variables on the color development was tested to establish the optimum conditions for determination of CAP by oxidative coupling with promethaizne.HCl reagent.

Principle of the method

The method involves two steps:

Step 1: Oxidation of promethazine.HCl reagent to give a red intermediate.

Step 2: The intermediate couples with reduced CAP to form a blue-green color.

The effect of acids

Effect of different acids has been studied on the intensity of dye; the results are shown in (Table 1). 5 ml of formic acid (1N) has been chosen in the next experiments. Because in formic acid, the reaction becomes less sensitive to media environment than others and the formation of the greenish-blue dye is not affected by acid amount.

Table 1. Effect of different acids on absorbance

Ml of acid	HCl (1N)		H_2SO_4 (1N)		H_3PO_4 (1N)		CH_3COOH (1N)		HCOOH (1N)	
	A*	pH	A	pH	A	pH	A	pH	A	pH
0.5	0.326	1.27	0.323	1.31	0.281	1.51	0.340	1.53	0.330	1.86
1.0	0.327	1.14	0.315	1.22	0.280	1.42	0.344	1.54	0.332	1.85
2.0	0.297	0.95	0.300	1.02	0.270	1.44	0.355	1.52	0.336	1.84
3.0	0.296	0.80	0.282	0.92	0.369	1.40	0.348	1.54	0.324	1.83
5.0	0.287	0.64	0.263	0.77	0.324	1.34	0.360	1.53	0.331	1.82
7.0	0.294	0.50	0.276	0.68	0.330	1.28	0.359	1.53	0.328	1.79
10. 0	0.283	0.36	0.280	0.53	0.339	1.22	0.373	1.50	0.320	1.79

*A = Absorbance of sample

Choice the reagent

Several different reagents (3.1×10^{-3} N) have been studied with 4 ml of each, and also 7 ml of sodium hydroxide (1N) instead of 5 ml acid also have been studied. The results are shown in (Tables 2a, 2b, 2c, 2d).

Table 2a . Test and selection of coupling agent in the presence of 5 ml of 1 M HCl

Coupling agents	Color of		Absorbance of	
	Sample	Blank	Sample	Blank
4-Aminophenol	Light- Brown	Brown	-0.084	0.142
1,10-Phenanthroline	----	----	----	----
p-Phenylenediamine	Light-pink	Light-Brown	-0.045	0.059
Phloroglucinol	----	----	----	----
Promethazine.HCl	Blue-green	Red	0.287	0.051
Thymol	----	----	----	----

Table 2b . Test and selection of coupling agent in the presence of 5 ml of 1 M formic acid

Coupling agents	Colour of		Absorbance of	
	Sample	Blank	Sample	Blank
4-Aminophenol	Light- Brown	Brown	-0.084	0.142
1,10-Phenanthroline	----	----	----	----
p-Phenylenediamine	Light-pink	Light-Brown	-0.045	0.059
Phloroglucinol	----	----	----	----
Promethazine.HCl	Blue-green	Red	0.287	0.051
Thymol	----	----	----	----

Table 2c . Test and selection of coupling agent in the presence of 5 ml of 1 M of phosphoric acid

Coupling agents	Color of		Absorbance of	
	Sample	Blank	Sample	Blank
4-Aminophenol	Light-Brown	Brown	-0.077	0.135
1,10-Phenanthroline	----	----	----	----
p-Phenylenediamine	Light-pink	Light-Brown	-0.057	0.071
Phloroglucinol	----	----	----	----
Promethazine.HCl	Green -Blue	Pink	0.324	0.066
Thymol	----	----	----	----

Table 2d . Test and selection of coupling agent in the presence of 7 ml of sodium hydroxide instead of 5 ml of acid

Coupling agents	Color of		Absorbance of	
	Sample	Blank	Sample	Blank
4-Aminophenol	Brown	Brown	-0.007	0.411
1,10-Phenanthroline	----	----	----	----
p-Phenylenediamine	Brown	Orange	0.164	0.071
Phloroglucinol	Pale-yellow	Pale-yellow	0.010	0.035
Promethazine.HCl	----	----	----	----
Thymol	----	----	----	----

From the results it is clear that promethazine give high results in selectivity and color contrast, its solution is easy to prepare and stable therefore it used for next experiments.

Effect of reagent amount

The effect of reagent amount on sensitivity of method has been studied. A series of solutions contain different volume of promethazine(0.1%) reagent with different amounts of RCAP have been made. The results are shown in (Table 3). 4 ml of the reagent have been chosen from the results obtained because the sensitivity of the reaction is good, the absorbance and correlation coefficient is excellent as well as the absorbance of blank is low.

Table 3. The effect of reagent amount on the absorbance of dye formed

Ml of 0.1% promethazine.HCl solution	Absorbance / µg of CAP present							r_{20-500}	r_{20-200}	r_{40-200}
	20	40	70	100	200	300	500			
0.5	0.045	0.079	0.114	0.130	0.101	0.086	0.057	0.25358	0.52578	0.19590
1.0	0.046	0.086	0.145	0.192	0.240	0.226	0.170	0.54296	0.93931	0.93573
2.0	0.048	0.089	0.152	0.205	0.331	0.350	0.343	0.84519	0.99149	0.99270
3.0	0.047	0.089	0.151	0.206	0.355	0.390	0.369	0.85152	0.99638	0.99722
4.0	0.050	0.092	0.154	0.213	0.372	0.424	0.417	0.88064	0.99762	0.99797
6.0	0.054	0.090	0.150	0.214	0.371	0.419	0.443	0.90623	0.99781	0.99745
8.0	0.043	0.086	0.153	0.210	0.381	0.444	0.471	0.91375	0.99803	0.99847
10.0	0.058	0.089	0.152	0.214	0.370	0.433	0.451	0.90987	0.99775	0.99704

Choice of oxidative reagent

Number of oxidative reagent (1.5×10^{-3} M) in 4 ml has been studied on the absorbance of dye contained; the results are shown in (Table 4).

Table 4. Test and selection of oxidative reagent

Oxidative reagent	Color of		Absorbance of		pH
	Sample	Blank	Sample	Blank	
Ammonium ceric sulphate	Blue-green	Pink	0.350	0.065	1.38
Ammonium metavanadate	v.light blue	Light pink	0.030	0.021	2.51
Potassium dichromate	Light blue	v.light orange	0.132	0.009	2.47
Potassium ferricyanide *	----	----	Turbid	----	----
Potassium iodate	Dark blue	Colourless	0.329	0.009	1.78
Potassium periodate	Dark blue	Colourless	0.466	0.004	2.11
Sodium nitrite	Light pink	Light pink	0.016	0.004	2.76

*The sample became dark turbid blue upon addition of formic acid, while the blank remains yellow.

The results shown in (Table 4) show high absorbance for potassium iodate and potassium periodate consider to the blank which give low absorbance, which are not used because they give unstable products. ACS still used for next experiments.

Effect of oxidative reagent amount

Different volumes of oxidative reagent ACS (0.1%) have been studied on the absorbance of solution contain different volumes of RCAP. Table 5 shows 8 ml was the best therefore it was used for the next experiments.

Effect of surfactant

The effect of several types of surfactants on color intensity of the colored product has been investigated. The results indicate that addition of surfactants give no several effect [increasing the intensity or improving the color contrast ($\Delta\lambda$)], therefore it has not been used in the subsequent experiments.

Table 5 .Effect of oxidative reagent amount

Ml of 0.1% Ce^{+4} solution	Absorbance / µg of CAP present							$r_{20 — 500}$	$r_{20 — 200}$	$r_{40 — 200}$
	20	40	70	100	200	300	500			
0.5	0.036	0.050	0.064	0.067	0.070	0.068	0.059	0.39607	0.80251	0.78796
1.0	0.042	0.078	0.107	0.123	0.129	0.129	0.118	0.58157	0.82270	0.82420
2.0	0.049	0.085	0.145	0.185	0.242	0.247	0.233	0.76983	0.95227	0.95090
3.0	0.046	0.086	0.148	0.213	0.330	0.354	0.353	0.85675	0.98778	0.98723
4.0	0.044	0.086	0.149	0.211	0.370	0.428	0.447	0.90590	0.99715	0.99743
6.0	0.045	0.086	0.149	0.210	0.395	0.521	0.574	0.94669	0.99951	0.99957
8.0	0.050	0.085	0.146	0.204	0.409	0.587	0.744	0.98169	0.99983	0.99996
10.0	0.041	0.081	0.137	0.192	0.396	0.571	0.755	0.98689	0.99976	0.99706

Order of addition

The order of additions of reagent (C, P, O and A) was examined. The results shown in (Table 6) indicated that the order (VII) of addition of reagents was the optimum order due to the high intensity of the formed dye.

Table 6 . Effect of order of addition

Reaction components	Order number	Absorbance	
		Sample	Blank
C + P + O + A*	I	0.402	0.135
C + O +P + A	II	0.401	0.137
C + A + P +O	III	0.410	0.127
C + A + O + P	IV	0.005	0.127
P + O + C + A	V	0.444	0.157
P + A + C + O	VI	0.418	0.154
P + A + O + C	VII	0.451	0.155
O + C + A + P	VIII	0.413	0.147
O + A + C + P	IX	0.409	0.142
O + P + A + C	X	0.427	0.151
A + O + P + C	XI	0.437	0.139

* C= Reduce chloramphenicol , P= Promethazine.HCl (0.1%), O= Ammonium ceric sulphate (0.1%), A= Formic acid (1N)

Effect of time

The effect of time on the development and stability period of the formed colored product was investigated under optimum experiment conditions described before. The formation of colored product being complete after mixing the component of reaction and the absorbance of the colored species remained constant for, at least 45 minute. Fifteen mintutes have been recommended as a formation colored product for the subsequent experiments. (Table 7).

Table 7 .Effect of time on absorption

µg CAP / 25 ml	bsorbance / minute standing time												
	0	5	10	15	20	25	30	35	40	45	50	55	60
20	0.058	0.059	0.053	0.054	0.056	0.055	0.055	0.055	0.057	0.057	0.057	0.058	0.060
50	0.137	0.134	0.131	0.130	0.134	0.129	0.131	0.133	0.135	0.135	0.136	0.140	0.143
100	0.219	0.228	0.233	0.231	0.234	0.230	0.231	0.231	0.234	0.234	0.235	0.237	0.237
200	0.343	0.422	0.434	0.435	0.439	0.439	0.439	0.443	0.443	0.445	0.446	0.447	0.449
300	0.347	0.522	0.558	0.577	0.587	0.595	0.607	0.610	0.617	0.621	0.626	0.628	0.633
500	0.420	0.623	0.673	0.694	0.705	0.716	0.726	0.735	0.744	0.752	0.762	0.771	0.777

Final absorption spectrum

After addition of RCAP to solution contain promethazine reagent, ACS (IV) and formic acid under optimum conditions described before, colored product was formed, shows a maximum absorption at 606 nm in contrast to the reagent blank. The maximum wavelength 606 nm has been used in the subsequent experiments. (Fig. 3).

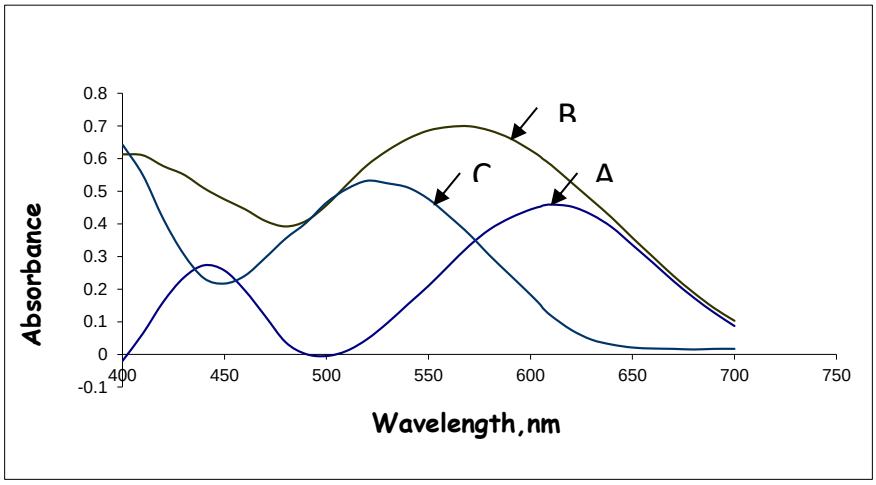


Fig 3. Absorption spectra of 200µg/25 ml of CAP (treated according to the recommended procedure) against (A) blank (B) distilled water and (C) blank measured against distilled water.

Effect of some excipients on the assay of CAP

To test the efficiency and selectivity of the proposed analytical method, a systematic study of excipients that usually present in dosage showed that there was no interference from excipients up to 1000 µg in the present method as shown in (Table 8).

Table 8 . Effect of some excipients on the assay of CAP

Excipient	Recovery % , of 200 µg of CAP per µg of excipient Added			
	100 µg	300 µg	500 µg	1000 µg
Vanillin	98.3	103.2	95.6	100
Glucose	104.7	99.5	101.5	97.0
Dextrose	99.5	98.5	101.7	99.5
Gum acacia	100.3	98.7	100.5	100
Starch	98.5	99.8	101	100.7
Lactose	102.1	98.4	99.5	99.5
CTAB	102.3	101	105.5	107.5
Sucrose	98.8	101.5	98.5	100.3
Tween – 80	99.5	97.0	100.7	98.6
Glycerol	97.8	95.4	99.0	95.9

Accuracy and precision

To check the accuracy and precision of the calibration curve, CAP was determined at three different concentrations. The results (illustrated in Table 9) indicated that the method is satisfactory.

Table 9 . Accuracy and precision of the calibration curve

Amount of CAP taken, µg	Relative error, %*	Relative standard deviation, %*
20	- 2.73	± 3.84
100	+ 2.43	± 4.01
200	+ 0.37	± 0.44

*Average of five determinations.

Nature of Dye

Job's method (21) indicates that the colored product has a composition of 1:2 CAP to promethazine reagent (Fig 4).

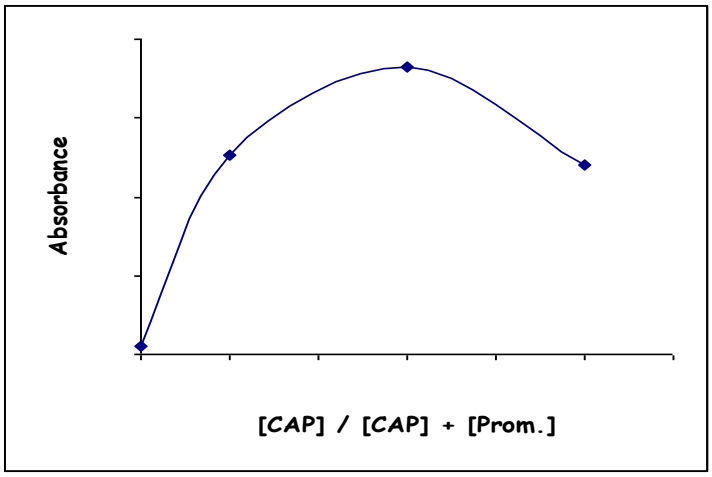
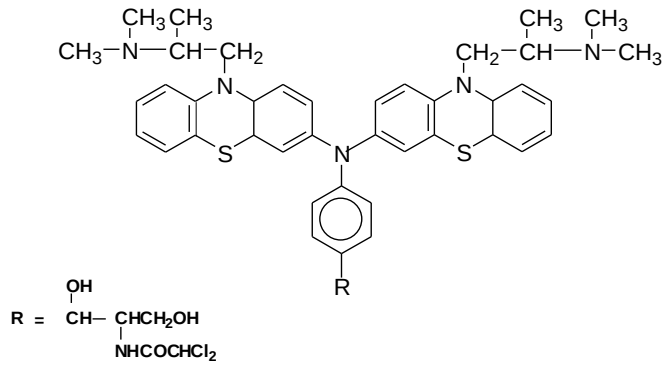


Fig 4 . Job's plot for CAP with promethazine solution

Hence, the colored product may have the following structure.



Effect of organic solvent

Different organic solvents have been used to examine their effects on the dye. The results are shown in (Table 10) .

Table 10. Effect of solvents

Solvents	λ_{max} , nm	ε , l . mol^{-1}. cm^{-1}
Acetic acid	610	1.73×10^4
Acetone	Turbid	----
Dioxane	610	1.85×10^4
DMSO	610	1.78×10^4
Ethanol	610	1.66×10^4
Formic acid	620	0.93×10^4
Iso-butanol	Two layer	----
Methanol	610	1.69×10^4
n-Propanol	610	1.72×10^4
Pyridine	540	1.08×10^4
T.H.F	600	1.59×10^4
Water	606	1.36×10^4

According to its good sensitivity and low cost water has been used for dilution.

Application of the method

The proposed method was successfully applied to determine CAP in its pharmaceutical preparations (capsule, eye drop and eye ointment) (Table11) from different sources. This method was compared with the N-NED (22) method. The results showed that there was no significant difference between the proposed and N-NED method.

Table 11. Analytical applications of determination of CAP in pharmaceutical preparations.

Pharmaceutical preparation	Recovery % *	
	Present method	N-NED method
Capsule	98.5	94.76
Eye drop	100	116.8
Eye ointment	99.2	95.24

*Average of three determinations

Comparison of methods

Table (12) shows the comparison between some of analytical variables for the present method with that of other spectrophotometric methods.

Table 12 . Comparison of the methods

Analytical parameters	Literature method [23]	N-NED [22]	Present method
pH	Acid Medium	Acid Medium	Acid medium
λ_{max} , (nm)	600	550	606
Temperature (C°)	At room Temperature	At room temperature	At room temperature
Development time (min.)	30	10	15
Reagent used	Promethazine.HCI	N-NED	Promethazine.HCI
Stability period (hour)	5	8	45 minute at least
ε , l . mol^{-1} . cm^{-1}	1.87×10^4	----	1.292×10^4
Beer 's law range(ppm)	0.4 – 10	0.8 – 8	0.4 – 12
Average recovery (%)	---	99.6	99.92
RSD (%)	± 2.0	± 1.0	± 3.8 to ± 0.44
Correlation coefficient	0.9981	----	0.9943
Toxicity of reagent *	Safe [a]	Irritant & hygroscopic [b]	Safe [a]
Analytical application	Pharmaceutical preparations	Pharmaceutical preparations	Pharmaceutical preparations

* Aldrich, "Catalog Handbook of Fine Chemicals," Aldrich Chemical Company, Inc., Wisconsin, (1990 – 1991) , pp.(a) : 1101 , (b) : 941

The results indicate that the proposed method is not less efficient than other methods

Conclusion

The determination of chroramphenicol in pharmaceutical preparations has been developed. The method is based on the oxidative-coupling reaction of the drug with promethazine.HCl in the presence of cerium (IV) ion. The blue-green color formed is measured as a function of drug amount. The accuracy of the method has been tested by its application to assay of chloramphenicol in pharmaceutical preparations (capsules, eye drop and eye ointment) and found to be successful.

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