Spectrophotometric Determination of Ciprofloxacin in Pharmaceutical Preparations by Charge – Transfer Reaction

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Abstract

A Simple, rapid and sensitive spectrophotometric method is described for quantitative determination of ciprofloxacin. The method is based on the reaction of this drug as an *n*-electron donor with Methylene blue as π-acceptors to produce charge transfer complex . The complex was prepared in KCl– HCl buffer of pH 2 (method A) and in NaOAc – AcOH buffer of pH 5.2 (method B) to give highly coloured complex. The coloured product is quantitated Spectrophotometrically at 665 nm. Optimization of the different experimental conditions is described.The absorbance was found to increase linearly with increase in concentration of Ciprofloxacin which was corroborated by the calculated correlation coefficient values (1, 0.999). Beer's law is obeyed in the concentration ranges 2 – 10 µg /ml of drug for Methylene blue . Various analytical parameters have been evaluated and the validated by statistical data .The proposed method is suitable for quantitative determination of ciprofloxacin in Pharmaceutical Preparations .

Key words Spectrophotometric determination; Ciprofloxacin; charge– transfer complex

Introduction

The quinolones have emerged as one of the most important classes of antibiotics of the past two decades. Ciprofloxacin (CF) [1-cyclopropyl-6-fluoro-1,4dihydro-4-oxo-7-(piperazinyl) quinolone-3-carboxylic acid] (Fig. 1) is a synthetic fluoroquinolone derivative which has demonstrated broad spectrum activity against many pathogenic gram- negative and gram-positive bacteria. Different techniques have been proposed for the determination of the drug. CF has been determined by polarography[1,2,3], adsorptive stripping voltammetry[4,5,6] and high-performance liquid chromatography (HPLC)[7,8]. Extraction spectrophotometric procedures using bromothymol blue[9], tetrachloro-benzoquinone[10], *p*-nitrophenol[11,12] and supracen violet 3B[13] have also been described .CF forms chelates with different metals, enabling thus the photometric[14], fluorimetric[15] and chromatographic determination[16]of the drug .

Spectrophotometric methods reported for the determination of the studied drugs included oxidative coupling with 3-methyl-2-benzothiazolinonehydrazone hydrochloride (MBTH) and cerium (IV) ammonium sulphate[17]and ion-pair complex formation with xanthene dyes such as eosin and merbromine[18] .Also, complexation with iron (III)[19,20] or tris (o-phenanthroline) iron (II) and tris (bipyridyl) iron (II),[21] derivative spectrophotometry of their copper (II) complexes,[22] charge-transfer complexation with π-acceptors such as , 2,3-dichloro-5,6-dicyano-ρ-benzoquinone[23], 7,7,8,8-tetracyanoquinodimethane[24], , tetracyanoethylene [25], the acid-dye[26], bromocresol green[27] and ternary complex formation with eosin and palladium[28] had been widely reported. Methylene blue used in charge transfer reaction by determination of roxithromycin , the reaction between roxithromycin and methylene blue was completed in alcohol-HCl medium at room temperature. The maximum absorption wavelength was at 666nm[29] . The study on the inhibition of choline oxidase by a number of quinoid dyes including meldola blue (MB), nile blue (NB) and methylene blue (MetB) likewise pointed to likely charge transfer type interactions between the dyes and the flavin prosthetic group [30]. The development of novel hybrid material with enhanced photodynamic properties based on methylene blue and CdTe nanocrystals. Absorption spectroscopy, visible photoluminescence spectroscopy and fluorescence lifetime imaging of this system reveal efficient charge transfer between nanocrystals and the methylene blue dye [31]. The photoinduced[32] hydride transfer reaction between methylene blue (MB^+) and leuco crystal violet (CVH) was investigated spectrophotometrically under conditions of direct excitation of MB^+ with steady-illumination of visible light and of photosensitization by benzophenone (BP) and α-nitronaphthalene (NNP) with UV light**.** The hydrogen transfer[33] from dihydro-aromatic hydrocarbons to Methylene Blue, catalyzed by aromatic hydrocarbons was studied, the Methylene Blue being regarded as a model compound of flavin co-enzymes. The reaction was always carried out in the absence of air at 0°C in 1,2-dichloroethane. The absorption peak of Methylene Blue at 659 nm was employed as a measure of the extent of the redox reaction**.** The prompted us to develop simple, sensitive and accurate spectrophotometric methods for the determination of Ciprofloxacin in pure and pharmaceutical formulations . These methods are sensitive and based on the formation of charge – transfer complex of Ciprofloxacin with Methylene Blue in KCl – HCl buffer of pH 2 and NaAOc –AcOH buffer of pH 5.2 .

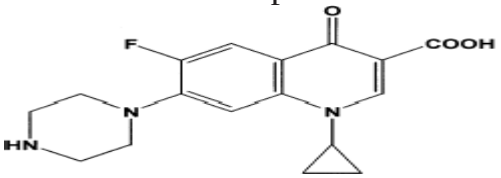


Fig. 1. Structure of ciprofloxacin.

Experimental

Apparatus

1- UV –Visible spectrophotometer Shimadzu 160 Double beam
2- pH meter Kent

Reagent and Chemicals

All chemicals used were of analytical or pharmaceutical grade . Ciprofloxacin was obtained as a gift sample from Kontam Pharmaceutical Co.,China.

Procedure:-

1.Methylene Blue (1.563×10^{-4} M)

An aqueos solution of Methylene Blue (1.563×10^{-4} M) was prepared by dissolving 0.05g of methylene blue (M.Wt= 319.85) dye in water and diluting to 100 ml . One ml of this solution was diluted to 100 ml .

2 . Buffers preparations

Series of buffer solutions of KCl– HCl (pH=1–2) and NaOAc–AcOH (pH=3.6–5.2) were prepared by following the standard method[34].

A . Hydrochloric acid –Potassium Buffer (pH =1–2)

Mix 50 ml of 0.1 M KCl (7.45 g/L) and indicated volume of 0.1 M HCl . Adjust the final volume to 100 ml with distilled water . Adjust the final pH using a sensitive pH meter ,Table(1).

Table (1). Volume and pH required for KCl – HCl Buffer preparation

HCl (ml)	97	64.5	41.5	26.3	16.6	10.6
pH	1	1.2	1.4	1.6	1.8	2

B . Acetate Buffer , (pH = 3.6–5.2)

Mix 0.1 M acetic acid (5.8 ml to 1L)and 0.1M acetate solutions (Sodium acetate anhydrous (8.2 g/L) in the proportions indicated and adjust the final volume to 100 ml with distilled water . Adjust the final pH using a sensitive pH meter ,Table(2) .

Table (2) . Volume and pH required for Na OAc–AcOH Buffer preparation

Acetic acid(ml)	46.3	41	30.5	20	14.8	10.5
Sodium acetate(ml)	3.7	9	19.5	30	35.2	39.5
pH	3.6	4	4.4	4.8	5	5.2

3 .Ciprofloxacin Stock Solution

A stock solution of Ciprofloxacin containing 250 µg/ ml was prepared in distilled water by dissolving 0.025 g of ciprofloxacin in distilled water and made up to 100 ml in a standard flask . The solution is stable at room temperature .

4 . Ciprofloxacin Working Solution

An aliquot solution containing 0-10 µg /ml of Ciprofloxacin was transferred into a series of 10 ml volumetric flasks .A volume of 3 ml of KCl –HCl buffer of pH 2 (for method A) or 3 ml of NaOAc–AcOH buffer of pH 5.2(for method B) were added and mixed. 3 ml of 1.563×10^{-4} M Methylene blue was added to each of the volumetric flasks. The contents were shaken well and left at room temperature for 5 min , the absorbance of the blue colored complexs were measured at 665 nm against the corresponding reagent blank . A calibration graphs was plotted .Fig.(7,8).

5 . Asaay procedure for Ciprofloxacin Tablets

Five tablets of CF were weighed and powdered .The average weight of a tablet was calculated. An amount of the powder equivalent to 200 mg of CF was weighed into a 100 ml volumetric flask containing about 75 ml of distilled water . It was shaken well for about 20 min , filtered through a Whatman filter paper No .40 to remove the insoluble matter and diluted to the mark with distilled water. Further appropriate solution of CF preparations were made by using distilled water. Six different concentrations of each solution of pharmaceutical preparation were analyzed in six replicate by recommended procedure. Table (5, 6) .

Results and discussion

The photolytic study[35] of MB^+ was carried out in acidic and basic aqueous solutions and in the presence of various substances. The optical absorbtion spectra of the MB^+ aqueous solutions over a large concentration range showed significant deviations from the Lambert-Beer's low, a fact which was accounted for by the formation of molecular aggregates (di- and trimers), favoured among other factor by the large polarity of the water molecules [36]. The peak located at 664 nm observed in dilute solutions (10^{-5}–10^{-6} M) is attributable to the monomer as shown in Fig.(2).

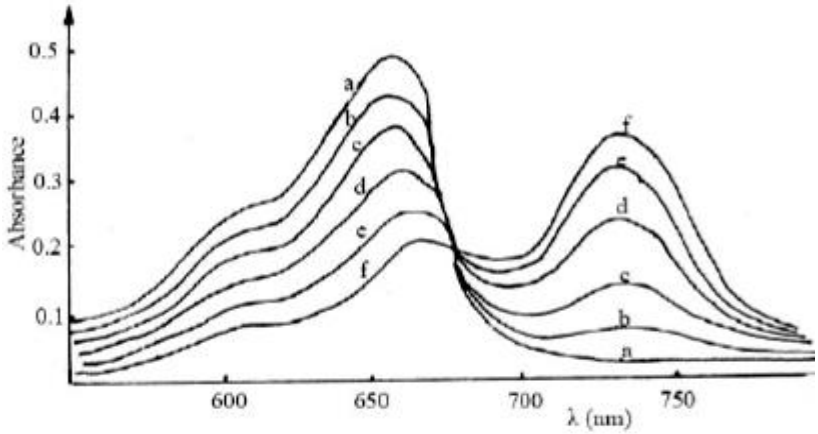


Fig. 2. The absorption spectra of acid solutions of MB^+ ($5 \cdot 10^{-6}$ M):
a) without H_2SO_4 , b) H_2SO_4 0.25 N , c) H_2SO_4 0.36 N , d) H_2SO_4 0.72 N , e) H_2SO_4 13N , f) H_2SO_4 23N.

Increase of the dye's concentration leads to molecular aggregation which is proved by new bands at 610 nm and 570 nm, assigned to the dimer [37] and to the trimer [38] respectively. Moreover, the increase of the solution acidity converts the MB^+ cations into the protonated forms MBH^{2+} and MBH_2^{3+}. Fig. (2) shows the absorption spectra of a neutral and acid dilute solution. One can see the decrease of the 664 nm peak and the increase of the 740 nm peak with the increase of acidity, an effect which was assigned to the protonated forms of the dye. The isobestic point appearing at 680 nm is due to the equilibrium established between these two forms. Among the totally oxidized (MB^+, MBH^{2+}, MBH_2^{3+}), totally reduced (MBH, MBH^{2+}, MBH_3^{2+}) and the partially oxidized forms $MB^{\bullet}$, $MB^{\bullet}H^+$, $MB^{\bullet}H_2^{+2}$, $MB^{\bullet}H_3^{+3}$.The solution spectra of Methylene blue in visible region exhibited two bands at 668 and 609 nm , this bands due to π -π^* transitions. This spectrum was taken using a 1 cm quartz cuvette filled with 10µM solution of Methylene Blue in water, Fig.(4). In Charge – transfer complexs, one species acts as an electron donor and another as an electron acceptor. Transition of an electron from an orbital of the donor species to an orbital of the acceptor can result from absorption of radiation . Ciprofloxacin[39] drug (an electron donor) , Fig.(3), reacts with Methylene Blue[40] (an electron acceptor) Fig.(4), in acidic buffer to give Charge – transfer complexs, Fig. (5,6) , which exhibits absorbance maximum at 665 nm .

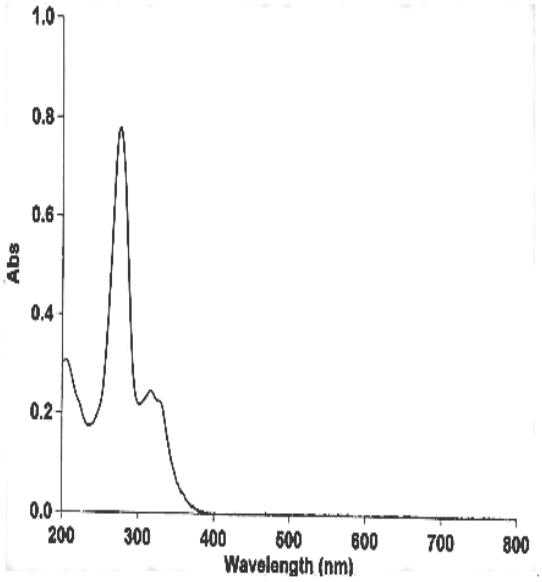


Fig.3. Absorption Spectrum of Ciprofloxacin

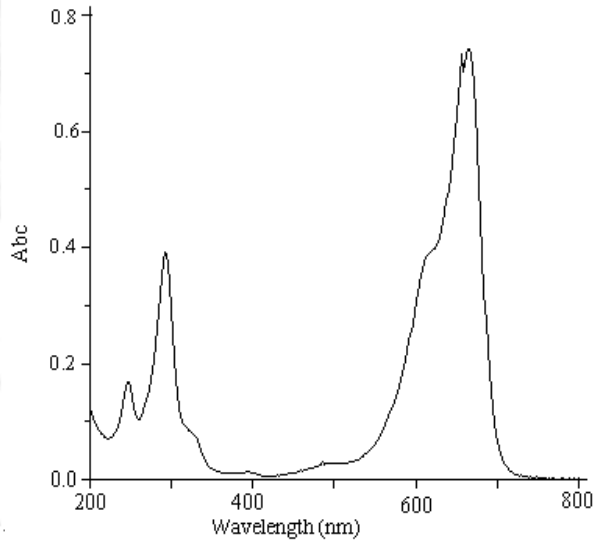


Fig .4. Spectrum of Methylene blue in water

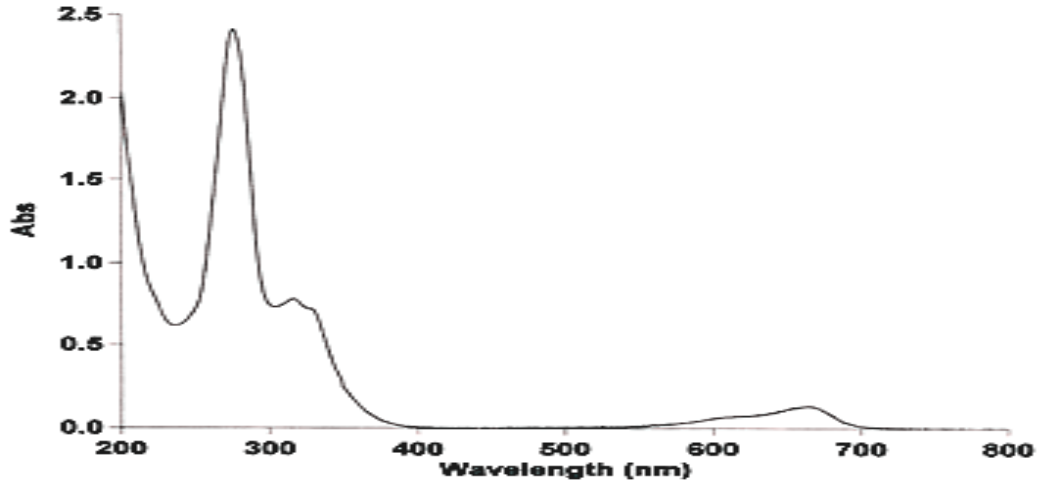


Fig 5 . Absorption Spectrum of Charge Transfer
Complex for Ciprofloxacin and Methylene Blue(method A)

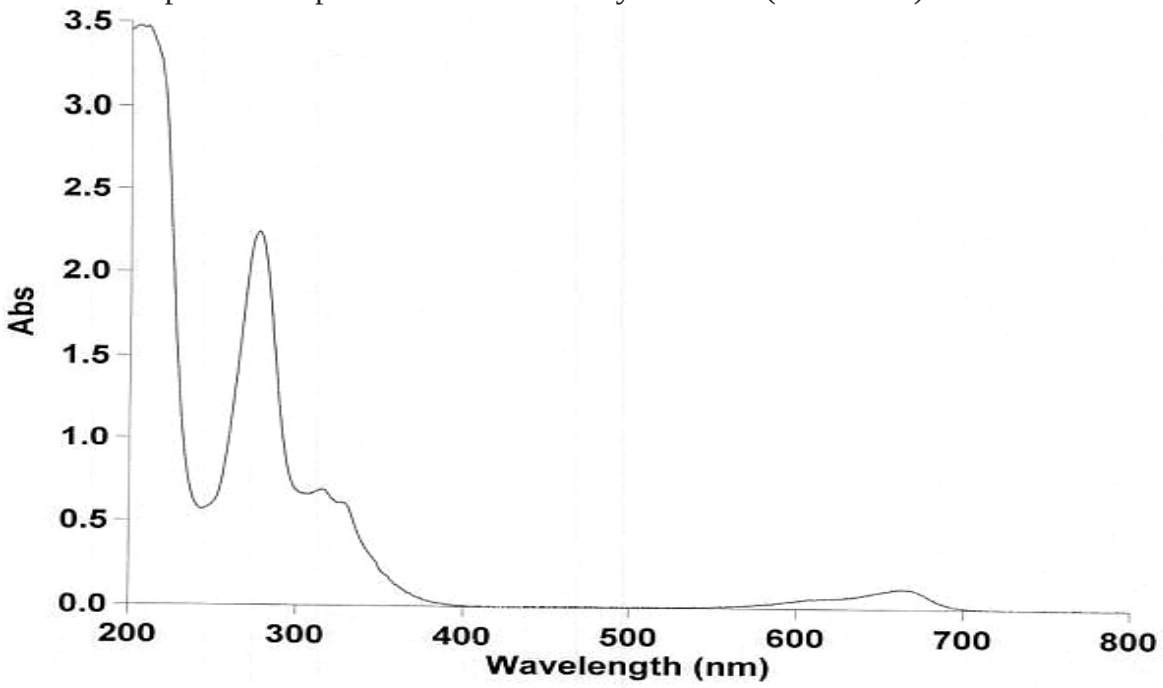


Fig 6 . Absorption Spectrum of Charge Transfer
Complex for Ciprofloxacin and Methylene Blue(method B)

Optimization of Reaction Conditions

Optimum reaction conditions for quantitative determination of charge – transfer complex were established via a number of preliminary experiments . It was observed that the effective formation of complex depends on the type of buffer used and its pH . The effect of pH was studied by formation the colored complexes in the presence of various buffers such as KCl –HCl (pH =1 –2) and NaOAc –AcOH (pH =5.2) . It was noticed that the maximum color intensity and constant absorbance were observed in KCl –HCl of pH 2 and in NaOAc –AcOH buffer of pH 5.2 . Low absorbance values were observed for the buffers having more or less than the above pH values , Table(3) .

Effect of Temperature on the Colored Complex

To study the effect of temperature on reaction yield, we carried out the reaction at 25 , 30 , and 40 C^0 . The obtained results showed that the reaction of drug with methylene blue was completed at room temperature and there was no need heating the reaction solution .The complex was stable for 24 h at room temperature .

Effect of Time on the Coloured Complex

The coloured of complex developed in a few minutes after all the reagents had been added and mixed attained maximum intensity after about 5 min at 25C^0 .The absorbance of complex was read after 1, 5 , 10 ,15 and 20 min and it was found that the absorbance could be read after 1 min as it had achieved the miximum and was constant . The analysis time required from sampling to measurement was about 5 min .

Linearity and Range

Beer's law range , molar absorptivity , regression equation and correlation coefficient determined are given in Table(3). Linear relationships were found between the absorbance at λ_{max} and the concentration of the drug in the range 2–10 µg /ml .Regression analysis of Beer's plots at λ_{max} reveals a good correlation . The graphs show low intercept and is described by the regression equation obtained by the least squares method The correlation coefficient was 1 , 0.999 indicating good linearity , the high molar absorptivities (7.45×10^3 l mol^{-1} cm^{-1} , 9.59×10^3 l mol^{-1} cm^{-1}) of the resulting coloured complex indicate the high sensitivity of the method .

Table(3) Optical Characteristics , Precision and Accuracy Data

Parameter	Proposed methods	
	KCl-HCl	NaOAc –AcOH
λ_{max} (nm)	665	665
Molar absorptivity($lmol^{-1}$ cm^{-1})	7.45×10^3	9.59×10^3
Beer's law (µg/ml)	2–10	2-10
Linear Regression equation,Y^a	$Y = 0.01X +0.1$	$Y=0.0148\ X+0.12$
Slope (*b*)	0.01	0.0148
Intercept(*c*)	0.1	0.12
Correlation Coefficient ,*R*	1	0.999
Limit of quantification (µg/ml)	0.25	0.21

a)$Y= bx+c$, were *x* is the concentration of drug in µg/ml

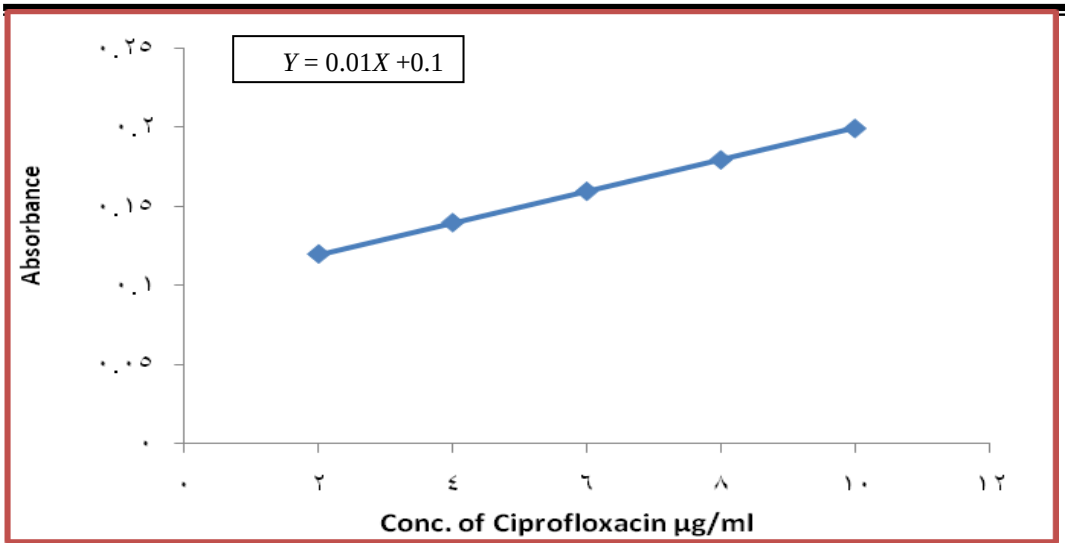


Fig .7. Calibration graph of Complex in KCl – HCl buffer of pH 2

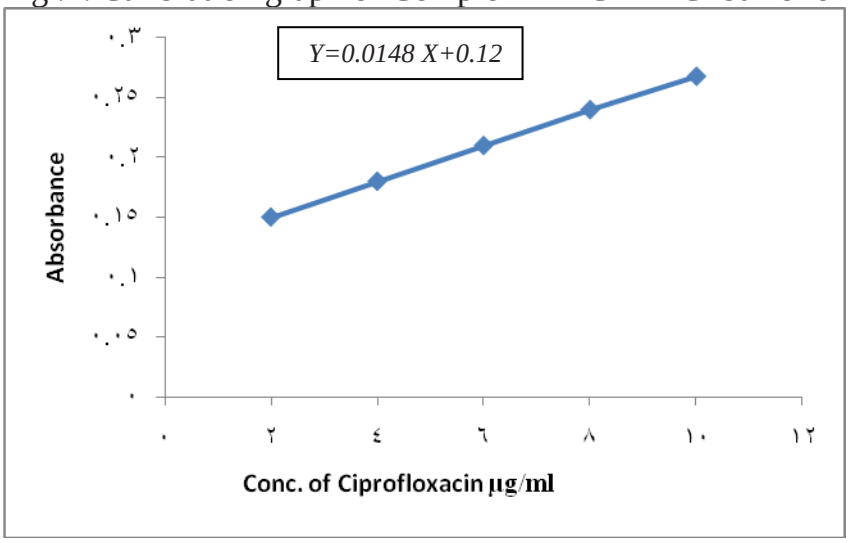


Fig.8 . Calibration graph of Complex in NaOAc –AcOH of pH 5.2

Determination of Stoichiometry of complex :-

Job's method of continuous varations [41] was used to determine the nature of complex . In this method a series of mixtures are prepared which two constituents are present at varying concentrations ,but their sum is held constant .0.025 g/100ml of Ciprofloxacin and 1.563×10^{-4} M of Methylene blue were prepared both in water . To nine 10 ml volumetric flasks 0.5 , 1, 1.5 ,2 , 2.5 , 3 , 3.5 , 4 , and 4.5 ml of Ciprofloxacin solution and then 4.5 , 4 , 3.5 , 3 , 2.5 , 2 , 1.5 , 1 , and 0.5 ml of methylene blue were transferred . 3 ml of KCl– HCl buffer of pH 2 was added mixed well then diluted with water , the reaction was allowed to proceed to equilibrium at least 5 min . the absorbance of the mixtures were measured at 665 nm .Normally , a maximum appear in the curve at a mole fraction corresponding to the complex that forms.The drug to dye stoichiometric ratio was found to be a 1:1 complex Ciprofloxacin :Methylene Blue .Fig.(9) .

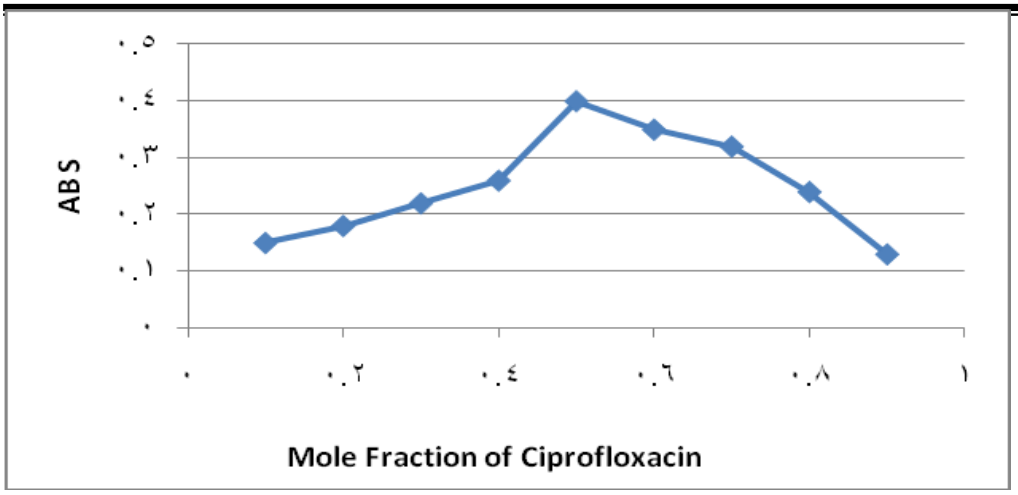


Fig .9 . Determination of Stoichiometry of complex

Equations of Reaction

Study of radical species [35]formed in methylene blue solutions show that the charge density of the unpaired electron is localized on the nitrogen ,Fig.(10).

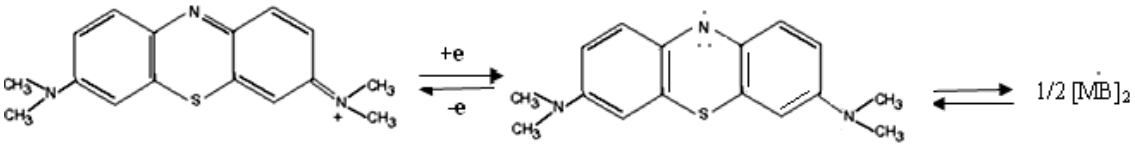
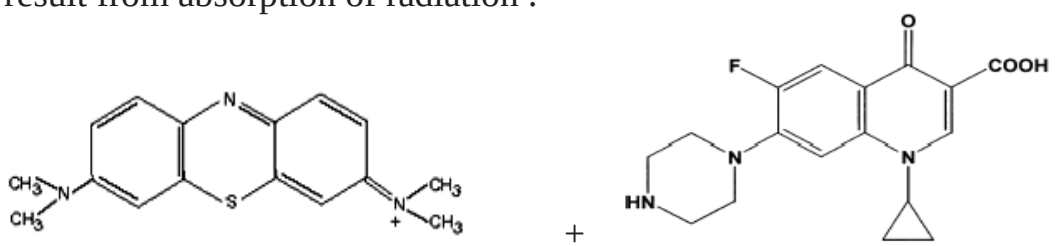


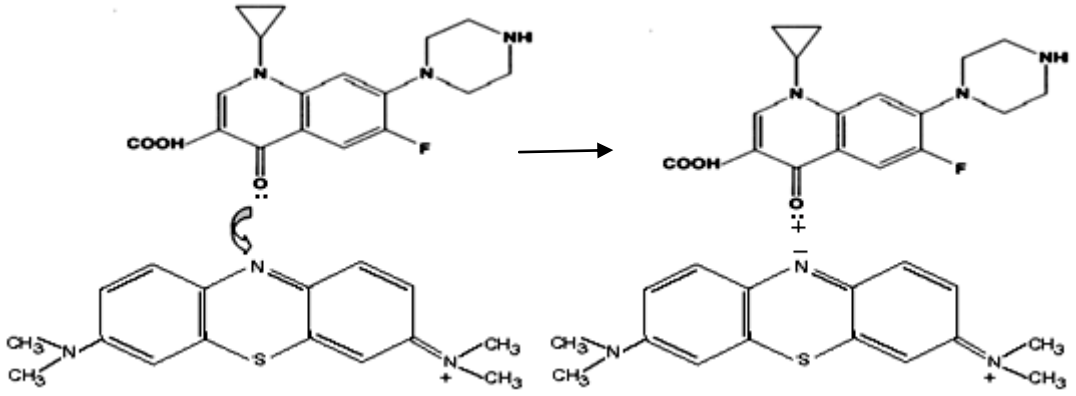
Fig.10. Radical of methylene blue

Ciprofloxacin reacts with Methylene blue in acidic buffer to give Charge – transfer complex ,Fig.(11).Transition of an electron from an orbital of the donor species (Ciprofloxacin) to an orbital of the acceptor (Methylene blue) can result from absorption of radiation .



Methylene blue

Ciprofloxacin



Complex

Fig .11. Reaction Scheme

Quantitation of Ciprofloxacin in pharmaceutical preparation:-

The Ciprofloxacin concentration in each tablet was quantified by measuring the absorbance of the complex for each tablet solution and compare the result with the calibration curve of the standard solutions. Table (4) summarize the result .

Table (4) . RSD% , Recovery , and E% of ciprofloxacin samples. Values are expressed as means (n= 6).

E%	Recovery%	RSD%	S .D	Determind Amount(mg)	Labeled Amount(mg)	Number of Samples
– 0.068	99.932	0.048	0.2404	499.66	500	n= 6

Ruggedness

To ascertain the ruggedness of the method , six replicate determination at different concentration levels of the drugs were carried out . The values of RSD% for different concentrations of drug from the determination are given in Table(5,6) , and indicated that the proposed method has reasonable ruggedness .

Table (5) . Determination of Ciprofloxacin in Pharmaceutical Preparations(method A) .

Recovery%	E%	RSD%	S.D	The proposed method Found(µg/ml)[b]	Ciprofloxacin Taken (µg /ml)[a]	Sample No.
99.33	– 0.666	0.468	0.028	5.96	6	1
100.13	0.133	0.0939	0.0141	15.02	15	2
100.2	0.2000	0.141	0.0353	25.05	25	3
100.22	0.224	0.157	0.0494	31.32	31.25	4
100.34	0.342	0.241	0.0848	35.12	35	5
99.86	– 0.140	0.0988	0.0494	49.93	50	6

a) Calculated with respect to the total weight

b) Found by using KCl-HCl buffer of pH2

Table (6) . Determination of Ciprofloxacin in Pharmaceuticals Preparations (method B).

Recovery	E%	RS%	S.D	The proposed method Found (µg/ml)[d]	Ciprofloxacin Taken (µg /ml)[c]	Sample No.
98.83	–1.166	0.828	0.0494	5.93	6	1
100.66	0.000	0.469	0.0707	15	15	2
103.2	3.200	2.2231	0.565	25.8	25	3
100.128	0.128	0.09	0.0282	31.29	31.25	4
100.51	0.514	0.362	0.127	35.18	35	5
99.94	–0.060	0.0424	0.0212	49.97	50	6

c) Calculated with respect to the total weight

*d)*Found by using NaOAc- AcOH of pH5.2

Conclusion

Unlike the gas chromatographic and HPLC procedures , the spectrophotometer is simple and is not of high cost . The importance lies in the chemical reaction upon which the procedures are based rather than upon the sophistication of the instrument . This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations .The reagents utilized in the proposed methods are cheaper , readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation The method are free from interference by common additives and excipients . The wide applicability of the new procedures for routine quality control is well established by the assay of Ciprofloxacin in pure form and in pharmaceutical preparations .

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تقدير السبروفلوكساين طيفيا في المستحضرات الصيدلانية بواسطة تفاعل انتقال الشحنة

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الخلاصة

تضمن هذا البحث طريقة طيفية جديدة لتقدير العقار الطبي السبروفلوكساين كميا ، تتميز بالحساسية والسرعة والبساطة . تعتمد الطريقة على تفاعل الدواء كواهب للالكترونات مع صبغة المثلين الزرقاء كمستقبل للالكترونات لتكوين معقد انتقال الشحنة Charge transfer complex . تم تحضير المعقد باستخدام المحلول المنظم KCl–HCl عند pH =2 والمحلول المنظم NaOAC –AcOH عند pH=5.2 . شخص المعقد طيفيا باستخدام مطيافية الاشعة فوق البنفسجية – المرئية . تم تقدير المعقد اللوني كميا عند طول موجي665 nm . لوحظ ان امتصاص المعقد يزداد خطيا مع زيادة تركيز السبروفلوكساين بمعامل ارتباط مقداره (1 و 0.999) كما لوحظ ان طريقة التقدير تخضع لقانون بير لامبرت عند تركيز2–10 مايكروغرام / مل . تم الحصول على نتائج مختلف المتغيرات عن طريق الطرق الاحصائية . يمكن تطبيق الطريقة الجديدة لتقديرالسبروفلوكساين كميا في المستحضرات الصيدلانية.