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Binding Constant: Fluorescence Quenching of Ciprofloxacin with Fe(III) and Zn(II)

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Absorbance and fluorescence spectral pattern of ciprofloxacin in absence and presence of Fe(III) and Zn(II) has been studied at room temperature and under physiological condition. The fluorescence spectra of the drug in presence of the different quantities and different concentrations of the Fe(III) and Zn(II) showed the quenching of ciprofloxacin. It was observed that with increasing quantity of the quencher the emission intensity decreases with negligible variation in the peak position. The absorption spectra of the drug at different pH exhibits two isosbestic points at 320 and 350 nm indicating the presence of three chemical species in solution. The ratio of the two reacting components, the drug and the metal ions was determined by absorption and fluorescence spectrophotometrically.

Key Words: Ciprofloxacin, HCl, Fluorescence quenching, Binding constant, Stability constant.

INTRODUCTION

Ciprofloxacin is [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-quinolone-3-carboxylic acid] one of the fluoroquinolones used as broad spectrum drug against gram positive and gram negative bacteria. It has been found that the activity of quinolone drugs is completely lost if consumed with antacids containing aluminum or magnesium because the drug is neutralized. At higher pH precipitation occurs and the drug is made unavailable for absorption[1-4]. The mechanism of interaction of quinolone with metal ions is based on the chelation of the metal with the carbonyl and carboxyl groups of the drug (Fig. 1). The antibacterial activity of the drug has also been found to be reduced manifold after chelation[5] of the metal ions *via* these groups.

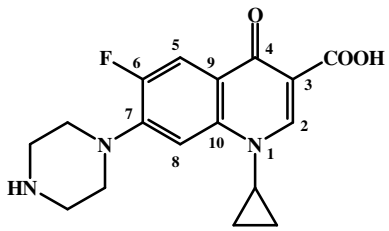

Fig. 1. Structure of ciprofloxacin

The interaction of fluoroquinolones with metal ions has attracted considerable interest not only for the development of analytical techniques but also to provide information about the mechanism of action of the pharmaceutical preparation[6]. Since the metal ions cause fluorescence quenching of the drug, spectrofluorimetric method for quantitative determination of the quinolone type drugs has been developed[7,8] besides titrimetric[9] spectrophotometeric[10], electrochemical[11], electrophoretic[12] and chromatographic[13] techniques.

The maximum solubility of the ciprofloxacin has been reported at 37 °C, which also happens to be the normal temperature of human body although the interaction of drug with metal ions has been studied in a wide range of temperature between 25 -60 °C[14,15]. Since we have to *mimic* the biological system under physiological condition we have studied, the interaction of Fe(III) and Zn(II) with ciprofloxacin at room temperature using absorption and fluorescence emission spectrophotometry at pH 3.98. The stability of the Fe(III) and Zn(II) complexes has been calculated by spectrophotometric and fluorescence emission spectrophotometric techniques. The fluorescence spectroscopy has been widely used to monitor the molecular interaction because of its high sensitivity, reproducibility and relatively easy use. Since no detailed fluorescence study on the binding interaction of ciprofloxacin with Fe(III) and Zn(II) has been done so for, a through investigation was therefore made using this technique. Such interactions between ciprofloxacin and these metal ions can cause fluorescence quenching. Therefore, valuable information's such as binding mechanism, binding constant and binding sites can be obtained using fluorescence quenching study of ciprofloxacin by these metal cations. In addition the thermodynamic parameters of the process were also proposed in this work.

EXPERIMENTAL

Fluorescence emission spectra were scanned using a Hitachi-F-2500 FL-spectrophotometer. The absorption spectra were obtained with Elico-SL-169 double beam UV-Vis spectrophotometer. All potentiometric measurements were carried out with Elico-LI-120 pH meter.

Ciprofloxacin was purchased from Windlas Biotech. Ltd.(India). All solvents and chemicals were of analytical grade. Double distilled water was used throughout. Sodium hydroxide, $Fe(NO_3)_3.9H_2O$ (Merck Ltd., Mumbai, India) $ZnCl_2$ (anhydrous) (SDH Pvt. Ltd. India) and hydrochloric acid (Ranbaxy fine chem. Ltd., India) were used as received.

Preparation of solutions: The stock solution of ciprofloxacin HCl (5×10^{-2} M) prepared in 1×10^{-2} M HCl was stored at 4 °C and those of the metal salts (1×10^{-2} M) were prepared in double distilled water, respectively. All working solutions were prepared by dilution with double distilled water.

Spectrophotometeric methods: Solutions of equimolar concentration (1×10^{-4} M) of ciprofloxacin HCl and metal ions were prepared. The pH of the drug was adjusted between 2.12 to 10.50 by adding sodium hydroxide and hydrochloric acid

(1×10^{-1} M to 1×10^{-2} M). The absorption spectra were recorded in the range 230-360 nm. The ratio of metal to ciprofloxacin.HCl was determined by Job's method. The linearity of ciprofloxacin HCl was found in the range 3.5×10^{-5}-7.5×10^{-4} mg/mL and the correlation factor (R^2) 0.9629.

Fluorescence spectrophotometeric methods: Solution of the ciprofloxacin (5×10^{-6} M) and those of metal ions (1×10^{-6} M to 10.5×10^{-6} M) were prepared. To prepare dilute solutions, an aliquot of stock solution was placed in a 10 mL volumetric flask and made up to the mark with distilled water. Spectra were recorded immediately after sample preparation in the optimum wavelength range 300-600 nm at optimum excitation wavelength of 315 nm. For calibration curve an aliquot of stock solution (5×10^{-7}-1×10^{-5} mg/mL) was prepared which showed linearity with correlation factor (R^2) 0.9739.

RESULTS AND DISCUSSION

Absorption studies: The absorption spectrum of ciprofloxacin run at room temperature and at constant pH 3.98 displayed a strong peak at 272 nm and a weak absorption at 315 nm (Fig. 2). When the UV spectra of the drug were run at varying pH, at room temperature and, under physiological condition, the strong peak was shifted to lower wavelength while the broad peak was shifted to higher wavelength. The decrease in the position and intensity of the first peak is attributed to the extent of ionization of the carboxylic group while the increase in the intensity and height of the peak at 315 nm occurs as a consequence of protonation of the piperazinyl nitrogen on 7-carbon atom. Two isosbestic points appearing at 320 and 350 nm indicated the presence of three chemical species in solution (Fig. 3) similar to those observed by Bark and Barrell[14].

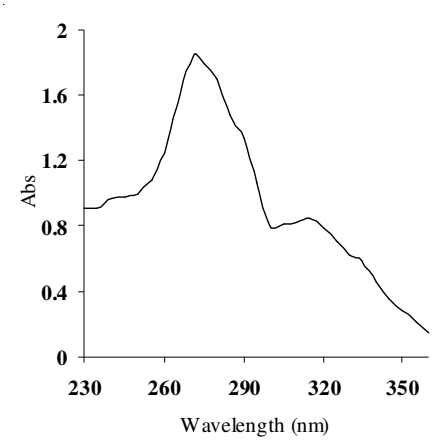

Fig .2. Absorption spectrum of ciprofloxacin (1×10^{-4} M) at pH 3.98

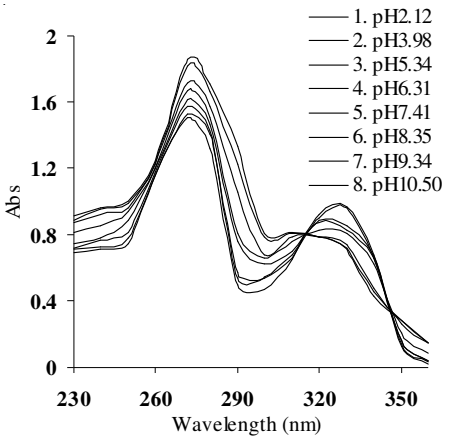

Fig. 3. Absorption spectra of ciprofloxacin (1×10^{-4} M) at different pH (2.12-10.50) at 25 ºC

The apparent ionization constant (pKa') of the drug was calculated (6.871) by the following equation.

$$pKa' = pH + \log \{(A_I - A_M)/(A - A_M)\} \quad (1)$$

where, A_I = Absorbance of drug in basic medium, A_M = Absorbance of drug in acidic medium, A = Absorbance of drug in aqueous medium. The pure drug has maximum solubility at pH 5 although it increases in the presence of Fe(II) and Fe(III) ions[16].

The stability constant of the complexes were calculated by the continuous variation method using the following equation:

$$K = \frac{A / A_{ex} C_X}{(C_M - A / A_{ex} C_X)(C_L - nA / A_{ex} C_X)^n} \quad (2)$$

where, K is the stability constant of the metal chelate formed in solution, M = metal, L = ligand, n = X/(1-X) where X is the mole fraction of the ligand at maximum absorption. A/A_{ex} is the ratio of the observed absorbance to that indicated by the tangent for the same wavelength. C_X, C_M and C_L are the limiting concentration, metal ion concentration and the ligand concentrations, respectively.

The continuous variation curves are shown in Fig. 4. The ratio of ciprofloxacin: metal, is 2:1, which is quite obvious. The fairly large value of log k of the two complexes (Table-1) suggests that they are pretty stable in acidic medium only. The drug under this condition must be acting as ionophore since the solubility of the drug is maximum in presence of Fe(III). However, precipitation occurs when the solution becomes alkaline[17,18].

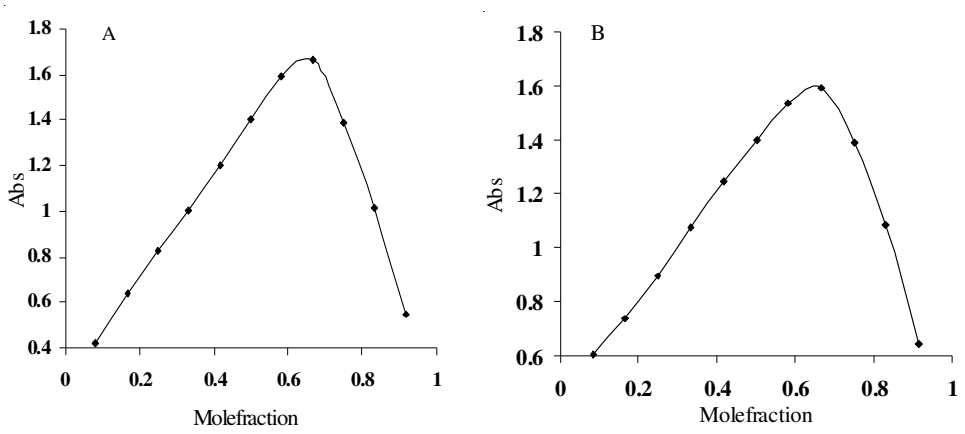

Fig. 4. Continuous variation curves of equimolar solutions of ciprofloxacin and (A) Fe(III) and (B) Zn(II) at 25 ºC

Fluorescence study: The absorption spectrum of the drug is markedly different from its emission spectrum, which is attributed to different molecular geometries (Fig. 5) in ground and excited states. The piperazinyl group of ciprofloxacin acts as

TABLE-1
STABILITY CONSTANT (log K AND -ΔG OF THE FORMED CHELATES AT 25 ºC BY ABSORPTION STUDY

Metal	log K	ΔG (kJ mol^{-1})
Fe(III)	9.378	-53.509
Zn(II)	9.381	-53.526

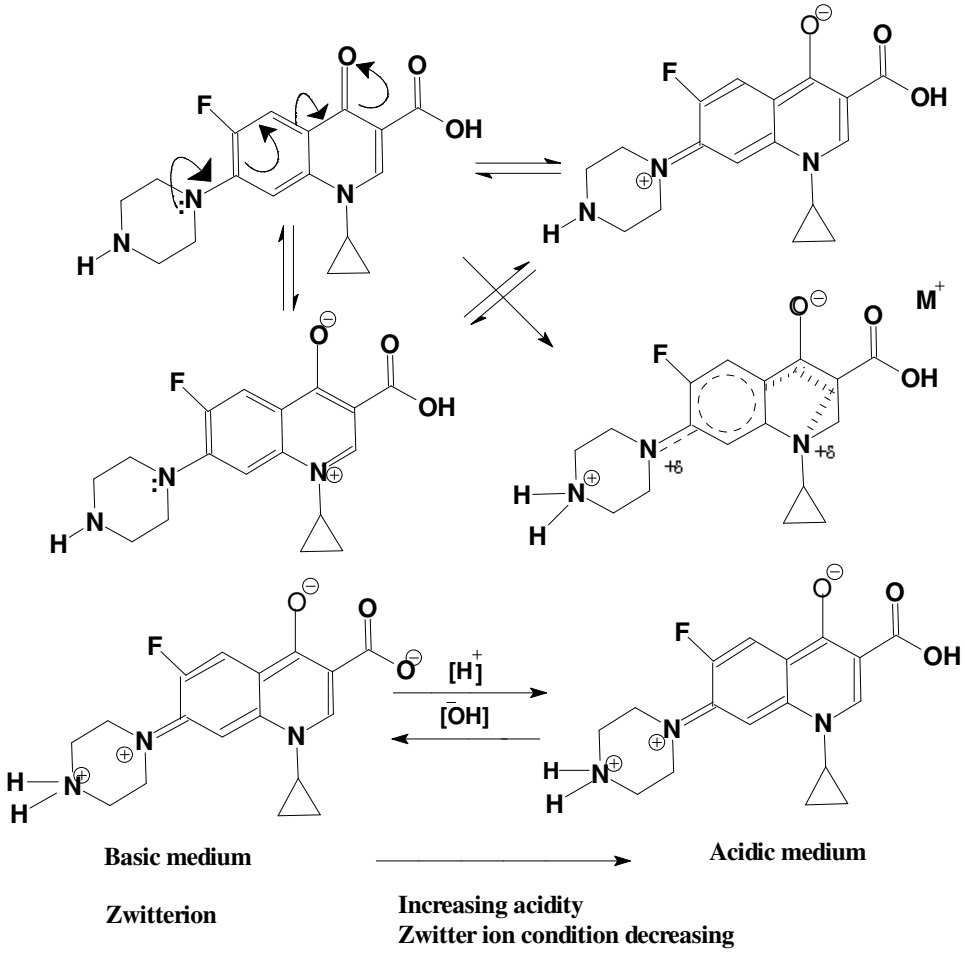

Fig. 5. Zwitterion formation and intramolecular charge transfer of drug in solution phase at varying pH

electron donor while the keto group acts as electron acceptor. Since all investigations were done in acidic medium, ciprofloxacin exists in zwitter ionic form. The peaks in the absorption spectra are due to intramolecular charge transfer, the greater the number of resonating structures the stronger the fluorescence emission.

The fluorescence spectra are very sensitive to the nature of the metal quencher, which is reflected from the emission spectra of the drug in presence of the Fe(III) and Zn(II) quencher. They showed a consistent decrease in the intensity of fluorescence with increasing concentration of the metal ion until it was nearly completely quenched (Fig. 6) as a consequence of the complex formation between the metal ions and ciprofloxacin.

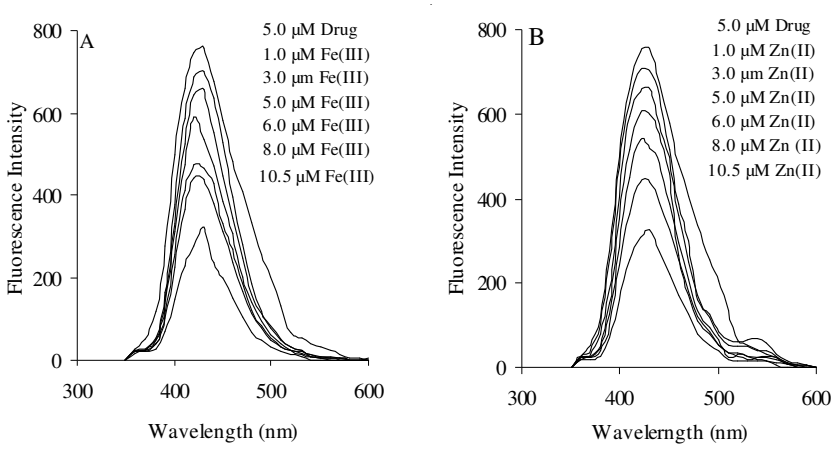

Fig. 6. The fluorescence emission spectra of drug quenched (A) Fe(III) and (B) Zn(II)

Many workers[19,20] have studied the interaction of drug with metal ions at temperature exceeding 37 °C in order to show the Stern-Volmer plot although such experiments above body temperature do not *mimic* the biological system. We, therefore, did all the experiments at room temperature. The fluorescence intensity increases rapidly with decrease in temperature. The lower stability at higher temperature also supports lowering in quenching. The fluorophore is quenched both by collision and complex formation with the metal ions. The complex formation is mainly due to ion dipole interaction.

Fluorescence quenching refers to any process in which the fluorescence intensity of a given fluorophore decreases upon adding a quencher. Assuming that the fluorescence intensity of a fluorophore-quencher complex is negligible as compared to an unquenched fluorophore, the intensity in the absence (F_o) and presence (F) of the quencher is expressed by Stern-Volmer equation

$$F_o / F = 1 + Ksv\,[Q] \quad (3)$$

where [Q] is the concentration of quencher, Ksv is the Stern-Volmer constant which is the equilibrium constant of the complex formed in the static quenching process. If a system obeys the Stern-Volmer equation, a plot of F_o/F-1 *vs.* [Q] will give straight line with a slope of Ksv and y-axis intercept. Fig. 8 represents the quenching of ciprofloxacin by Fe(III) and Zn(II), respectively. The linearity of Stern-Volmer plot (Fig. 7) increases with increasing concentration of quencher. The values of Ksv and correlation coefficient are shown in Table-2.

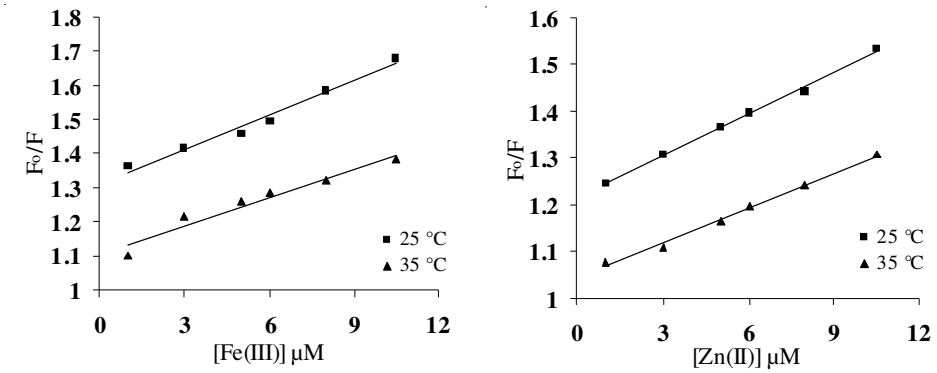

Fig. 7. Stern-Volmer plot for the quenching of drug with Fe(III) and Zn(II)

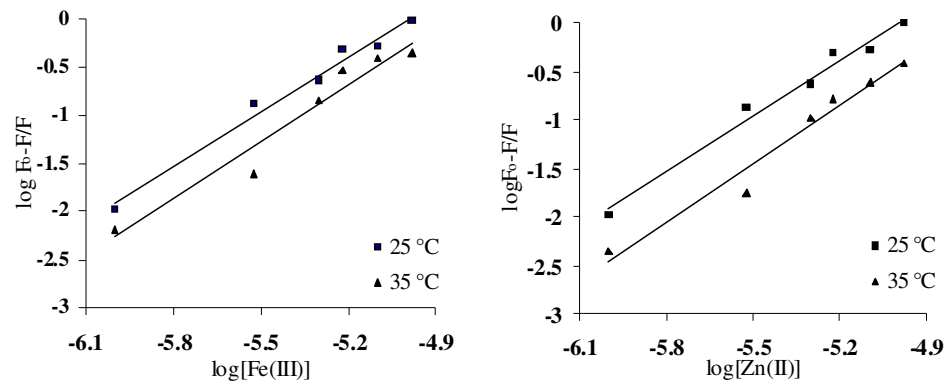

Fig. 8. Double reciprocal plot of drug with Fe(III) and Zn(II)

TABLE-2
STERN-VOLMER CONSTANT (Ksv, BINDING CONSTANT (log k),
BINDING SITE AND REGRESSION COEFFICIENT AT 25 and 35 ºC

Metal	Ksv/mol^{-1}		log K		n		R^2	
	25 ºC	35 ºC	25 ºC	35 ºC	25 ºC	35 ºC	25 ºC	35 ºC
Fe(III)	3.36×10^{-4}	2.77×10^{-4}	9.42	9.58	1.88	1.97	0.982	0.950
Zn(II)	2.97×10^{-4}	2.48×10^{-4}	9.46	9.60	1.89	2.01	0.981	0.965

The metal ions with large nuclear charge and small size will experience greater ion-dipole interaction and hence the emission intensity in the case of Zn(II) should be less than that for Fe(III) although Fe(III) has greater nuclear charge and smaller ionic radius.

The large value of stability constant of the complexes formed in solution reflects strong interaction between ciprofloxacin and the metal ions.

Binding constant and binding sites: For static quenching, the relationship between intensity and the concentration of quencher can be described by the binding constant formula[21,22]:

$$\log(F_o\text{-}F)/F = \log K + n \log [Q] \quad (4)$$

where K is the binding constant (Table-3), n is the number of binding sites per ciprofloxacin. After the fluorescence quenching intensities on ciprofloxacin at 315 nm were measured, the double-logarithm algorithm was assessed by eqn. 4. Fig. 8 shows double-logarithm curve and Table-2 gives the corresponding calculated results. The linear correlation coefficient for all the curves are larger than 0.945, indicating that the interaction between metal ions and ciprofloxacin agrees well with the site-binding model underlying eqn. 4. The results illustrate that there is a strong binding force between ciprofloxacin and metal ions and approximately two binding site would be formed in each case which is consistent with the previous studies that in acidic medium ciprofloxacin and metal ions form 2:1 complex.

TABLE-3
THERMODYNAMICS PARAMETERS AT 25 AND 35 ºC

Metal	ΔG (kJ mol^{-1})		ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	
	25 ºC	35 ºC		25 ºC	35 ºC
Fe(III)	-53.77	-56.54	118.59	180.85	183.96
Zn(II)	-54.01	-56.62	98.94	181.57	184.16

Thermodynamic parameters and nature of binding forces: Considering the dependence of the binding constant on the temperature a thermodynamic process was considered to be responsible for this interaction. Therefore, the thermodynamic parameters dependent on temperature were analyzed in order to further characterize the forces acting between drug and metal ions. The thermodynamic parameters enthalpy changes (ΔH), entropy changes (ΔS) and free energy changes (ΔG) are the main evidences to determine the binding mode. If the temperature does not vary significantly, the enthalpy changes (ΔH) can be regarded as constant. The free energy change (ΔG) can be estimated from the following equation, based on the binding constant at different temperatures.

$$\Delta G = - 2.303 RT \log K \quad (3)$$

where R is the gas constant, T is the experimental temperature and K is the binding constant at the corresponding temperature.

From the value of stability constant at different temperature the enthalpy changes can be calculated by using equation:

$$\log K_2/K_1 = [1/T_1\text{-}1/T_2]\Delta H/2.303R \quad (4)$$

The entropy changes can be calculated by using equation:

$$\Delta G = \Delta H\text{-}T\Delta S \quad (5)$$

Thermodynamics parameters for the interaction of metal ions and ciprofloxacin are shown in Table-3. The negative value of ΔG means that the interaction process is spontaneous. The +ve ΔS value obtained for all investigated complex is characteristic of chelation. It occurs because the water molecules that are normally arranged

in an orderly fashion around the ciprofloxacin and metal ions have acquired a random configuration as a result of chelation. This is referred as gain in configurational entropy[23]. The +ve value of ΔH indicate that the processes are endothermic and binding between metal ions and ciprofloxacin is mainly ΔS-driven, with little contribution from the enthalpy factor.

Conclusion

It is concluded that the drug stays as zwitter ions. Ionization of carboxylic group and protonation of piperazinyl group occurs. The two isosbestic points indicate the presence of three chemical species in solution. The result shows that the complex of ciprofloxacin with Fe(III) and Zn(II) are fairly stable. The thermodynamic parameters showed that the interaction between ciprofloxacin and metal ion was spontaneous and that the hydrophobic force was a major factor in the interaction.

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