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Interaction and fluorescence quenching study of levofloxacin with divalent toxic metal ions

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Abstract

The binding interaction of levofloxacin (LF) with toxic metal ions Cd^{+2}, Hg^{+2} and Pb^{+2} was investigated in aqueous acidic medium by absorption and fluorescence spectrophotometry. The experimental results showed that the metal ions quench the fluorescence intensity of LF by forming LF_2 -metal complex. It was found that static quenching was the main reason of fluorescence quenching. Quenching of LF by toxic metal follows the order Hg>Cd>Pb. The stoichiometry and logK of formed chelate was determined by absorption and fluorescence spectrophotometry. The quenching constant Ksv and the binding sites "n" were determined together with their thermodynamic parameters at 25 ºC and 35 ºC. The positive entropy change indicated the gain in configurational entropy as a result of chelation. The process of interaction was spontaneous and mainly ΔS-driven.

Keywords:

Levofloxacin; Fluorescence Quenching; binding constant

1. Introduction

Levofloxacin (LF),(-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate, [Fig.1] is one of the commonly used fluoroquinolone antimicrobials, is the active S-isomer isolated from the racemic ofloxacin. Its antibacterial action is twice as active as the racemate ofloxacin *in vitro*. Levofloxacin possesses a broad spectrum of activity against various bacteria, including gram-positive and gram-negative microorganisms [1]. It is also active against causes of atypical respiratory infection such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* [2]. Because of its excellent antibacterial activity and low frequency of adverse effects on oral administration, levofloxacin has been widely used for the treatment of infectious diseases, such as community-acquired pneumonia and acute exacerbation of chronic bronchitis [3]. Levofloxacin inhibits bacteria type II and IV DNA gyrase. Levofloxacin like other fluoroquinolone, inhibits the A subunits of DNA gyrase; two subunit encoded by gyrA gene. This result in strand breakage on a bacterial chromosome, supercoiling and resealing, DNA replication and transcription is inhibited [4]

The antibacterial action of the quinolones is not linearly proportional to their concentration. As the concentration of the drug in the human body falls the bacterial population increases. However, such an optimum concentration must be maintained before the surviving bacteria start regrowing [5]. In the case of FQs, the number of the surviving bacteria is lower, but the unusual dependence is retained. Mainly limiting concentration of

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FQs has been determined. The pharmacopoeia method is based on a microbiological test involving the diffusion of the antibiotic into agar (nutrient medium) and the growth inhibiting effect of the known concentrations of the drug on the microorganism with reference to samples [6]. The intrinsic fluorescence of FQs is used for their determination in biological samples after their preliminary extraction with organic solvents [7]. A method was proposed for determining these antibiotics in biological fluids using a mixed-ligand complex formed by terbium and triphenylphosphine oxide, the detection limit was 1.2 pico mole [8].

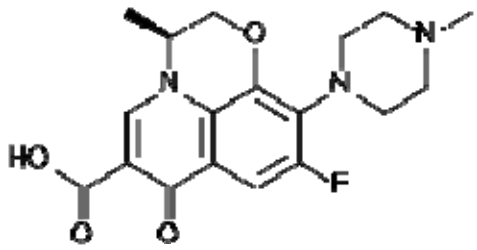


Fig.1. Chemical structure of Levoflaxacin (LF)

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 [9]. Since the metal ions cause fluorescence quenching of the drug spectrofluorimetric method for quantitative determination of the quinolone type drugs has been developed [10] besides titrimetric [11] spectrophotometeric [12], electrochemical [13], electrophoretic [14] and chromatographic [15] techniques.

Since the study of that stability of the complexes formed between LF and toxic metal ions has not been undertaken thus far, we have determined the binding constant (logK) and binding site (n) of the complexes by absorption and fluorescence emission spectrophotometry. In addition the thermodynamic parameters of the process were also proposed in this work.

2. Material and Methods

2.1. Instruments

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.

2.2. Reagents

Levofloxacin was purchased from Morpen Laboratory Ltd. (India). All solvents and chemicals were of analytical grade. Double distilled water was used throughout. NaOH, $CdCl_2.H_2O$, $HgCl_2$, and $Pb(NO_3)_2$ (Merck Ltd., Mumbai, India) and HCl (Ranbaxy fine chem. Ltd., India) were used as received.

2.3. Preparation of solutions

The stock solution of levofloxacin (3×10^{-2} mol L^{-1}) prepared in 1×10^{-2} mol L^{-1} HCl was stored at 4°C and those of the metal salts (1×10^{-2} mol L^{-1}) were prepared in water respectively. All working solutions were prepared by dilution with water.

2.4. Spectrophotometeric methods

Solutions of equimolar concentration (5×10^{-5} mol L^{-1}) of levofloxacin and metal ions were prepared. The ratio of metal to levofloxacin was determined by Job's method. The linearity of levofloxacin was found in the range 2×10^{-5}-5×10^{-4} mg mL^{-1} and the correlation factor (R^2) of 0.9780.

2.5. Fluorescence spectrophotometeric methods

Solution of the levofloxacin (6×10^{-6} mol L^{-1}) and those of metal ions (2×10^{-6} mol L^{-1} to 12×10^{-6} mol L^{-1}) 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 water. Spectra were recorded immediately after sample preparation by scanning wavelength range from 360 to 600 nm at optimum excitation wavelength of 330nm. For calibration curve an aliquot of stock solution (4.5×10^{-8}-0.5×10^{-5} mg mL^{-1}) was prepared which showed linearity with correlation factor (R^2) of 0.986.

3. Results and Discussion

3.1. Absorption studies

The absorption spectrum of levofloxacin run at room temperature and at constant pH 6.25 displayed a strong peak at 278 nm and, a weak absorption at 325 nm (Fig. 2).

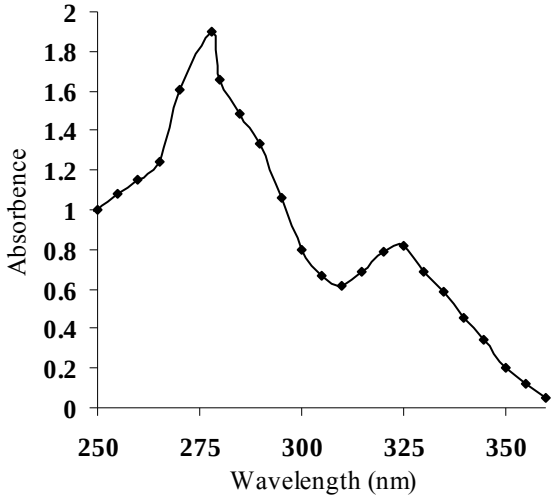


Fig.2. Absorption spectrum of LF (5×10^{-5} M) at pH 6.25 at 25 ºC.

The binding constant of the complexes were calculated by the continuous variation method at 25 ºC and 35 ºC using the following equation [16]

$$K = \frac{A / A_{ex} C_x}{(C_M - A / A_{ex} C_x)(C_L - nA / A_{ex} C_x)^n} \quad (1)$$

Where, K is the binding 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.3. The ratio of LF **:**metal, is 2**:**1, which is quite obvious. The fairly large value of logK of the two complexes suggests that they are pretty stable in acidic medium only. The drug under this condition must be acting as ionophore. The calculated binding constant (logK) and thermodynamic parameters are shown in Table 1 and the thermodynamic parameters are discussed in section 3.3.

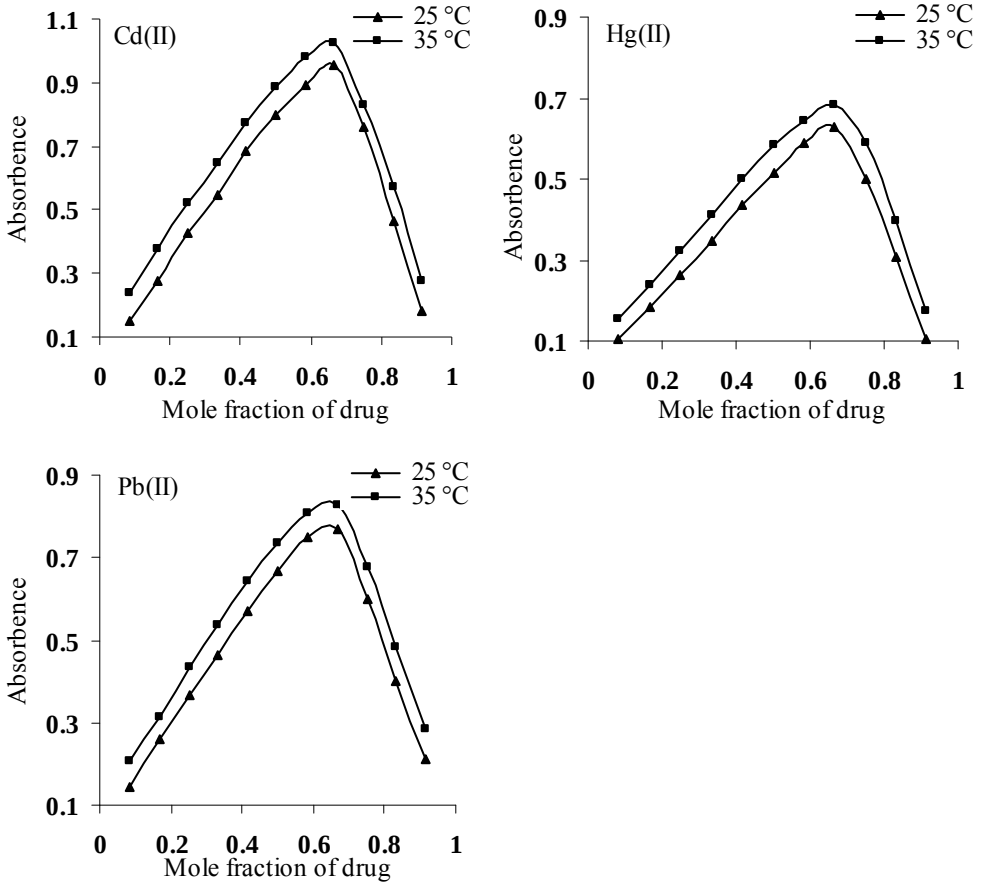


Fig.3. Continuous variation plots of complexes of LF with Cd(II), Pb(II), and Hg(II).

Table 1 Stability constant and thermodynamic parameters of LF complexes (Job's method)

Metal	logK		-ΔG (kJ.mol^{-1})		ΔH (J.mol^{-1})	ΔS (J.mol^{-1} K^{-1})	
	25 °C	35 °C	25 °C	35 °C		25 °C	35 °C
Cd(II)	9.399	9.579	53.629	56.490	301.58	180.97	184.38
Hg(II)	9.499	9.684	54.199	57.109	226.18	182.63	186.15
Pb(II)	9.459	9.522	53.971	56.154	206.00	181.80	182.98

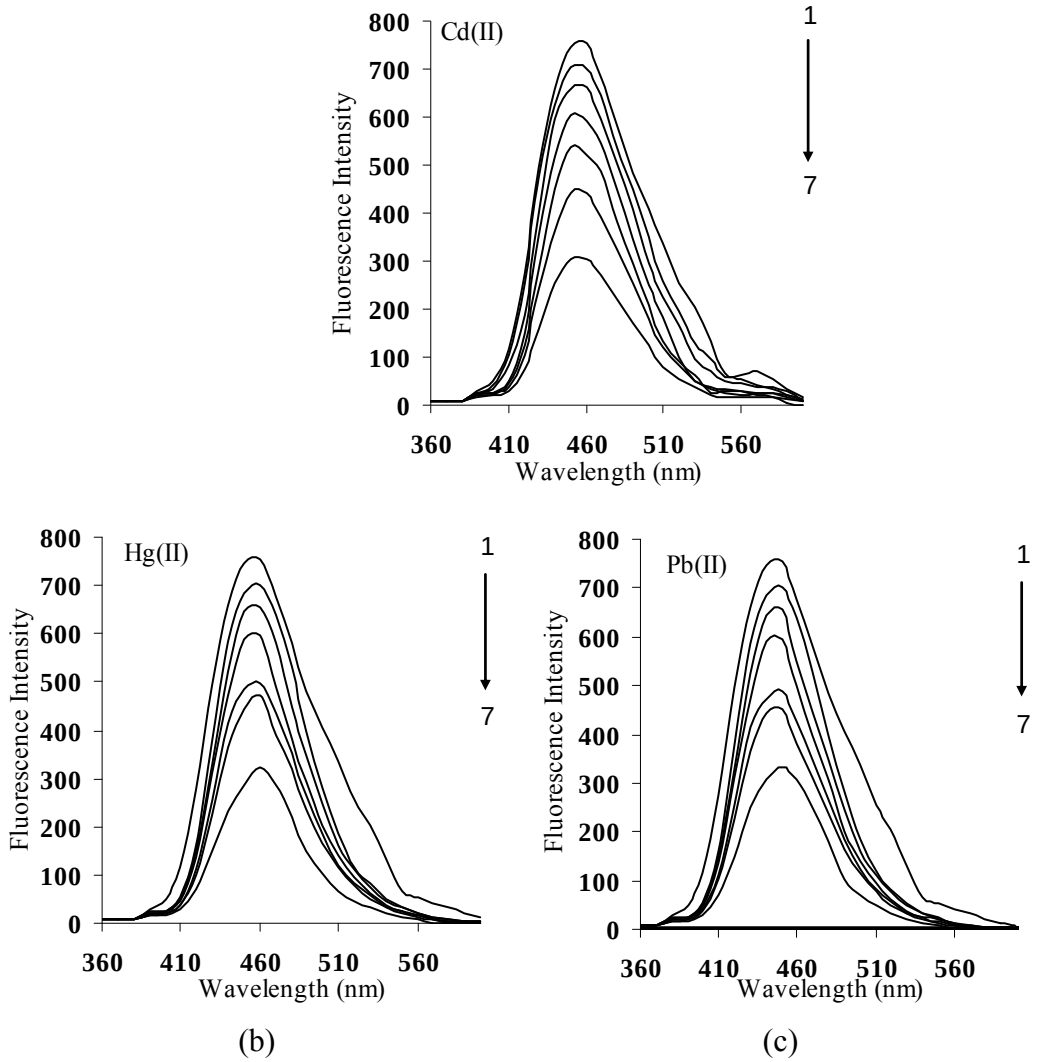
3.2. Fluorescence study

The emission spectra of levofloxacin in presence of various concentrations of metal ions showed in Fig. 4. It was observed that the fluorescence intensity of levofloxacin decreased regularly with the increasing concentration of metal ions without any change in emission maxima and shape of peak. As there was no significant λ_{em} shift with the addition of metal ions ,it indicated that metal ion can quench inner fluorescence of levofloxacin and that

the interaction between levofloxacin and metal ion indeed existed without inducing any conformational change in it under the condition studied here. Quenching can occur by a variety of molecular interactions, viz. excited-state reactions, molecular rearrangement, energy transfer, ground state complex formation (static quenching) If the K_{sv} decreased with increased temperature it could be concluded that the quenching process is static rather than dynamic [17,18]. Static quenching implies either the existence of a sphere of effective quenching or the formation of a ground state non-fluorescent complex, whereas collisional or dynamic quenching involves the collision and subsequent formation of a transient complex between an excited state fluorophore and a ground state quencher. The excited state complex dissociates upon radiative and non-radiative deactivation. In order to confirm the quenching mechanism the procedure of fluorescence quenching was first assumed to be dynamic. For dynamic quenching the mechanism can be described by the Stern-Volmer equation [19].

$$F_0 / F = 1 + K_{sv}[Q] \qquad (2)$$

Here Fo and F are the fluorescence intensities in the absence and presence of the quencher respectively. Ksv is the dynamic quenching constant [Q] is the concentration of quencher Fig.5 displays the Stern-Volmer plots of quenching of LF by metal ions at different temperature.



(b) (c)

Fig.4. Fluorescence quenching (at λ_{ex} 330 nm) of LF with Cd(II), Hg(II) and (c) Pb(II) at 25 ºC, (1) 6.0 µM LF, from (2) to 7: 2, 5, 6, 7, 19, 12, µM of metal ions

Based on the experimental data in Fig.5, the dynamic quenching constants at different temperature are shown in Table 2 It is observed that Ksv decreased with increasing temperature for all metal ions. It can be therefore, concluded that quenching is not initiated by dynamic, but probably by static process. Cu^{+2} is well known as a strong quencher because of its electronic structure (d^9).Quenching by this type of substance most likely involve the donation of an electron from the fluorophore to the quencher, the ion dipole interaction between Cu^{+2} and the molecule will also be strong due to the large nuclear charge and the relatively small size compared with other metals. Cu^{+2} usually introduces easily accessible low energy levels, which can give rise to energy and electron transfer processes and is capable of quenching the fluorescent excited state of the molecule [20].

Table 2. Stern-Volmer constant (Ksv, binding constant (logK), binding site and regression coefficient at 25 ^oC and 35 ^oC.

Metal	Ksv/mol^{-1}		logK		n		R^2	
	25 °C	35 °C	25 °C	35 °C	25 °C	35 °C	25 °C	35 °C
Cd(II)	4.22×10^4	3.57×10^4	9.466	9.602	1.895	2.009	0.9817	0.9652
Hg(II)	4.88×10^4	3.72×10^4	9.598	9.717	1.921	1.991	0.9809	0.9904
Pb(II)	4.71×10^4	3.65×10^4	9.585	9.680	1.918	1.994	0.9810	0.9573

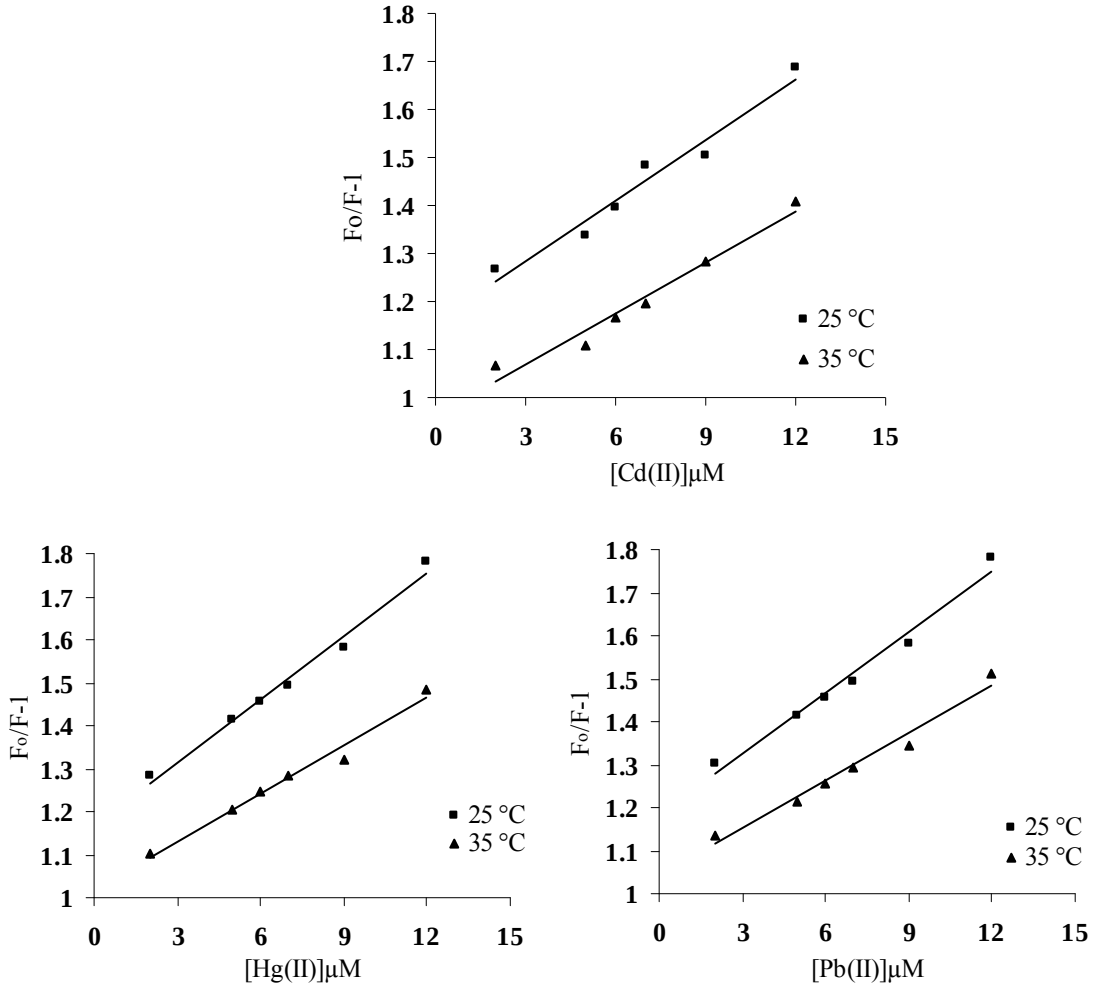


Fig.5. Stern-Volmer plot of LF with Cd(II), Hg(II) and Pb(II) at 25 ºC and 35 ºC.

3.2.1. 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]

$$log(F_0 - F)/F = log\,K + n\,log[Q] \quad (3)$$

where K is the binding constant (Table.2.), n is the number of binding sites per LF. After the fluorescence quenching intensities on LF at 330 nm were measured, the double-logarithm algorithm was assessed by equation (3) Fig. (6) shows double-logarithm curve 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 LF agrees well with the site- binding model underlying equation (3). The results illustrate that there is a strong binding force between LF 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 LF and metal ions form 2:1 complex.

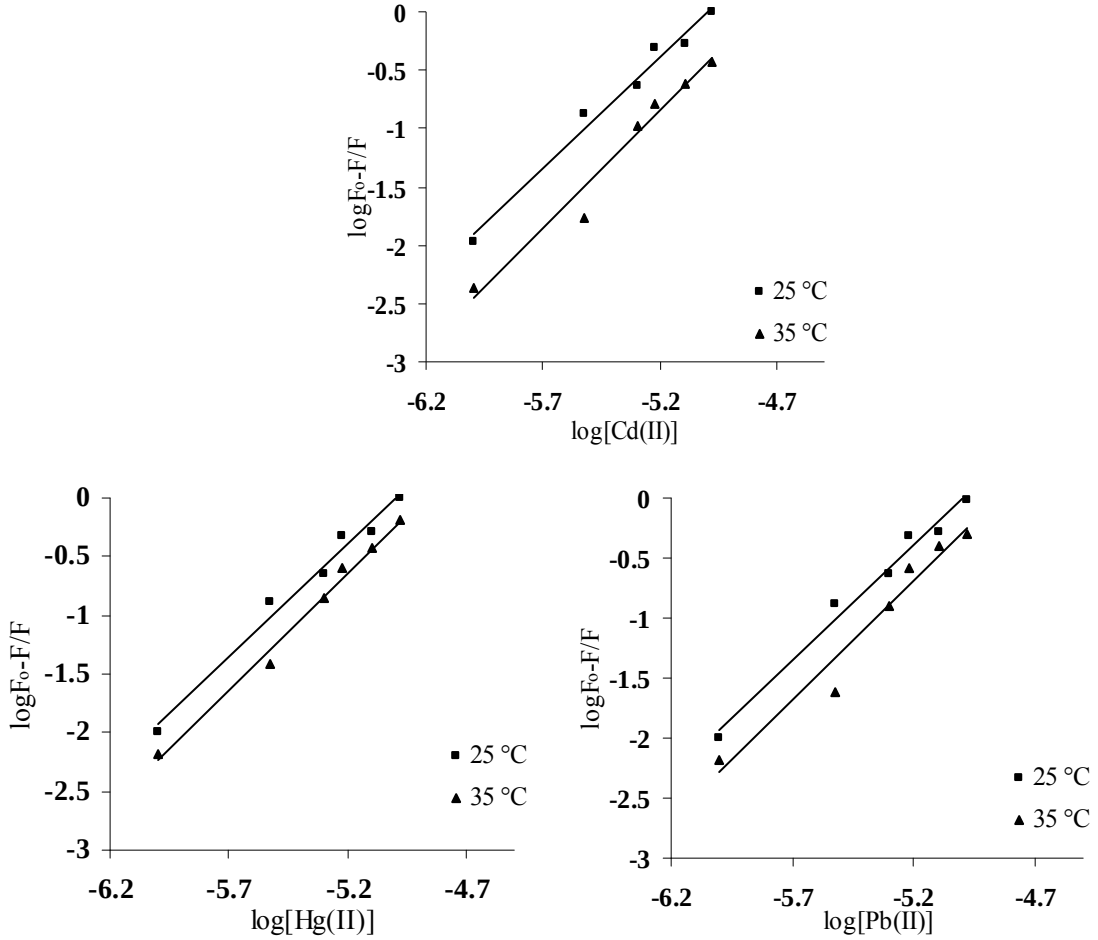


Fig.6. Double reciprocal plots of LF with Cd(II), Hg(II) and Pb(II) at 25 ºC and 35 ºC.

3.3. 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.

$$\Delta G = -2.303RT \log K \quad (4)$$

where R is the gas constant T is the experimental temperature, 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:

$$\log K_2 / K_1 = [1/T_1 - 1/T_2]\Delta H / 2.303R \quad (5)$$

The entropy changes can be calculated by using equation:

$$\Delta G = \Delta H - T\Delta S \quad (6)$$

The Thermodynamics parameters for the interaction of metal ions and LF 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 LF 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 LF is mainly ΔS-driven, with little contribution from the enthalpy factor.

Table 3. Thermodynamics parameters at 25 ^{o}C and 35^{o}C.

Metal	-ΔG /kJ mol^{-1}		ΔH/ J.mol^{-1}	ΔS /J.mol^{-1}K^{-1}	
	25 °C	35 °C		25 °C	35 °C
Cd(II)	54.011	56.625	227.86	182.01	184.58
Pb(II)	54.764	57.262	187.64	184.40	186.52
Hg(II)	54.690	57.085	159.16	184.05	185.87

4. Conclusion

In this paper the nature and magnitude of the interaction of LF with toxic metal ion was investigated by fluorescence spectra and UV spectra. The experimental result indicated the formation of 2:1 complex of LF with metal ions in acidic medium. The thermodynamic parameters showed that the interaction between LF and toxic metal ion was spontaneous, and that the hydrophobic force was a major factor in the interaction.

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