J Fluoresc (2011) 21:1789–1796
DOI 10.1007/s10895-011-0873-8

ORIGINAL PAPER

Fluorimetric Study on the Interaction between Norfloxacin and Proflavine Hemisulphate

Vishalkumar R. More · Prashant V. Anbhule · Sang H. Lee · Shivajirao R. Patil · Govind B. Kolekar

Received: 4 December 2010 / Accepted: 23 February 2011 / Published online: 30 March 2011

Abstract The interaction between Norfloxacin (NF) and Proflavine hemisulphate (PF) was investigated by spectroscopic tools like UV–VIS absorption and Fluorescence spectroscopy. It was proved that fluorescence quenching of NF by PF is due to the formation of NF-PF complex which was supported by UV–VIS absorption study. The study of thermodynamic parameters suggested that the key interacting forces are hydrogen bond and van der Waal's interactions and the binding interaction was spontaneous. The distance r between NF and PF was obtained according to the Förster's theory of non-radiative energy transfer. The fluorescence quenching mechanism was applied to estimate PF directly from pharmaceutical samples.

Keywords Norfloxacin · Proflavine hemisulphate · FRET · Fluorescence quenching · Thermodynamic parameter

Introduction

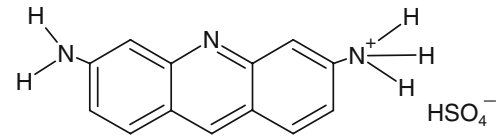
Proflavine hemisulphate (PF), which is generally called as proflavine, is a brightly colored acridine derivative used as a topical and urinary antiseptic in the form of the hemisulphate salt. It has molecular formula $C_{13}H_{11}N_3 . H_2SO_4$ (Fig. 1(a)) and molecular weight 209.25 g mol^{-1}. It was first developed as dye and afterwards it has wide clinical uses as surface antiseptics in both world wars I and II in the treatment of wounds. It is also effective against animal tumor cells under certain experimental conditions [1, 2]. It is disinfectant bacteriostatic against many Gram-positive bacteria. It can be used as antiseptic for first aid treatment for superficial cuts, grazes, wounds, insect bites and burns to prevent infections. It also known to be have a mutagenic effect on DNA by intercalating between nucleic acid base pairs and prevents unwinding prior to DNA synthesis [3]. PF is regarded as one of the wonder drugs. It works systematically to eradicate bacterial infections internally as well as externally which is the next best thing to antibiotic treatments and works very effectively on all strains of pathogenic bacteria .

Norfloxacin (NF) is a synthetic chemotherapeutic agent occasionally used to treat common as well as complicated urinary tract infections [4, 5]. NF is 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid (Fig. 1(b)). It is a white to pale yellow crystalline powder with molecular weight 319.331 g mol^{-1} and empirical formula $C_{16}H_{18}FN_3O_3$. NF is broad spectrum antibacterial agent for oral administration that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase IV enzymes necessary to separate bacterial DNA, thereby inhibiting cell division [6]. The activity of NF against broad range of bacteria is contributed to the F atom at the 6 position which provides increased potency against Gram-negative organisms and the piperazine moiety at the 7 position performs antipseudomonal activity.

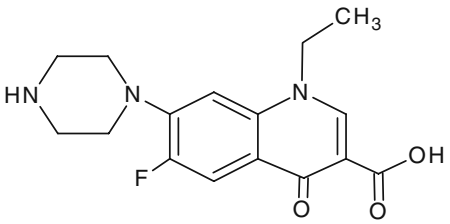
The spectrofluorimetric study of NF with various molecules like Riboflavin [7], DNA [8, 9], p-Amino benzoic acid [10] and divalent transition metal ions [11] have been reported. Similarly, such study of PF with nucleic acids [12], enzymes [13], nitrite ions [14], DNA

V. R. More · P. V. Anbhule · S. R. Patil · G. B. Kolekar (✉)
Department of Chemistry, Fluorescence Spectroscopy Research Laboratory, Shivaji University,
Kolhapur 416 004 Maharashtra, India
e-mail: gbkolekar@yhaoo.co.in

S. H. Lee
Department of Chemistry, Kyungpook National University,
Daegu, South Korea 133–791



(a) Proflavine Hemisulphate



(b) Norfloxacin

Fig. 1 Molecular structure of (**a**) Proflavine hemisulphate (**b**) Norfloxacin

[15, 16], BSA [17] have been carried out. The interaction between NF and PF is reported for first time by using spectrofluorimetric technique in this paper. PF is analyzed by stripping voltammetry [18], electrochemical [19], colorimetric [20], NMR [21] techniques but the spectrofluorimetric technique is superior to these methods used to investigate the molecular interaction between PF and NF.

Therefore it is proposed to carry out study between NF and PF by UV–VIS and fluorescence spectroscopy. The different phenomenon like fluorescence quenching, binding mechanism and energy transfer between donor and acceptor were studied from which the values of Stern-Volmer quenching constant (K_{SV}), binding constant (K), number of binding sites (n), distance between donor and acceptor (r) and thermodynamic parameters like enthalpy change (ΔH), entropy change (ΔS), free energy change (ΔG) are evaluated. The interference of coexisting ions in quenching study has been examined to determine tolerance limit. Finally, method of pharmaceutical analysis to determine PF from commercial sample has been developed by using fluorescence quenching mechanism and Fluorescence (Förster) Resonance Energy Transfer (FRET) study.

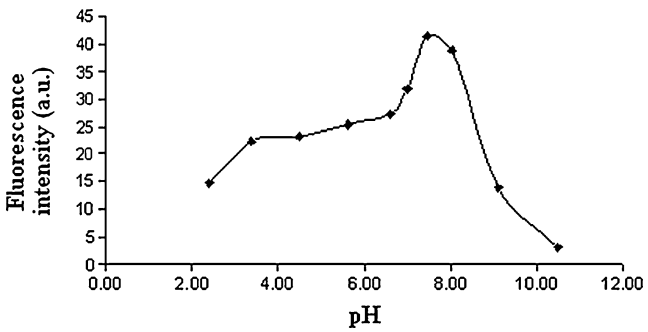


Fig. 2 The effect of pH on fluorescence intensity

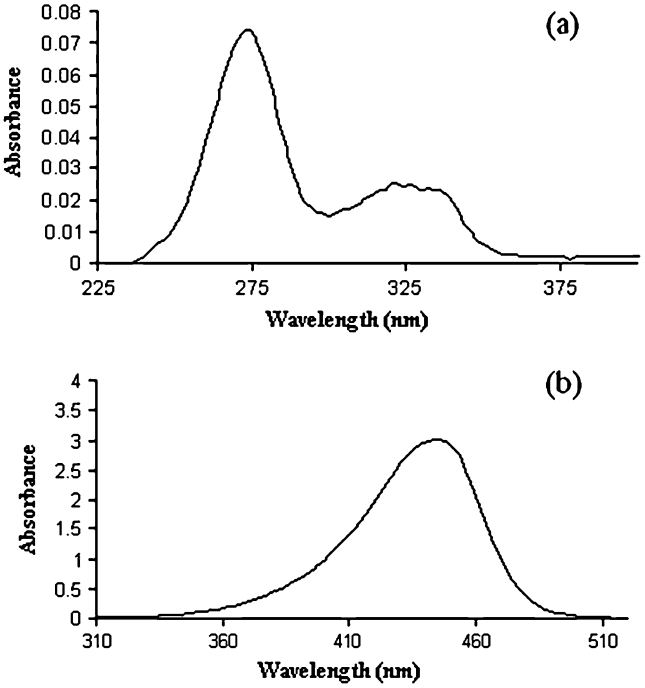


Fig. 3 Absorption spectrum of (**a**) Norfloxacin : [NF]=1×10^{-6} mol dm^{-3} (**b**) Proflavine : [PF]=5×10^{-5} mol dm^{-3}

Experimental

Materials

Norfloxacin (NF) was received as a gift sample from Cipla Limited, Mumbai (Kurkumbh unit). Proflavine hemisulphate (PF) was purchased from Hi-Media Laboratories Ltd., Mumbai. Citric acid, disodium hydrogen phosphate and Tris buffer were used for pH effect study. Tris buffer was purchased from Spectrochem Ltd., Mumbai. NaCl solution (0.5 mol dm^{-3}) was used to keep the ionic strength constant and 0.05 mol dm^{-3} Tris buffer with 0.1 mol dm^{-3} HCl solution was used to maintain the pH of the solution

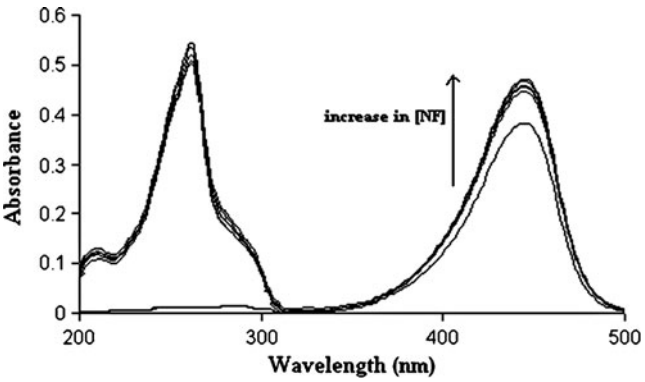


Fig. 4 Absorption spectra showing the formation of the PF-NF ground state complex.[PF]=1×10^{-5} mol dm^{-3}; [NF] ranges from 0.0 to 8×10^{-7} mol dm^{-3}

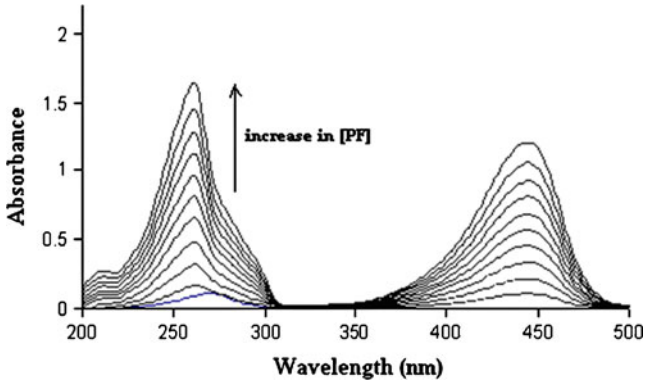


Fig. 5 Absorption spectra showing the formation of the PF-NF ground state complex. [NF]=6×10^{-7} mol dm^{-3}; [PF] ranges from 0.0 to 20×10^{-6} mol dm^{-3}

at 7.40±0.01. The working solution of NF (1×10^{-6} mol dm^{-3}) and PF (5×10^{-5} mol dm^{-3}) were prepared by dissolving the reagents directly in double distilled water. All the reagents were of analytical reagent grade and used without further purification.

Apparatus

UV–vis absorption spectra were recorded on a Shimadzu UV-3600 spectrophotometer with 1 cm quartz cuvette. A JASCO FP-750, Japan PC based spectrofluoriphotometer equipped with 150 W Xenon lamp and 1 cm quartz cell was used for fluorescence measurements. The pH measurements were carried out on ELICO LI-120 pH meter with combined glass electrode. To study the temperature effect, thermostat is used at the time of measurements.

General Procedure

An appropriate quantity of NF (1×10^{-6} mol dm^{-3}) was kept constant while PF was varied from 0.0 to 4.0 ml and diluted

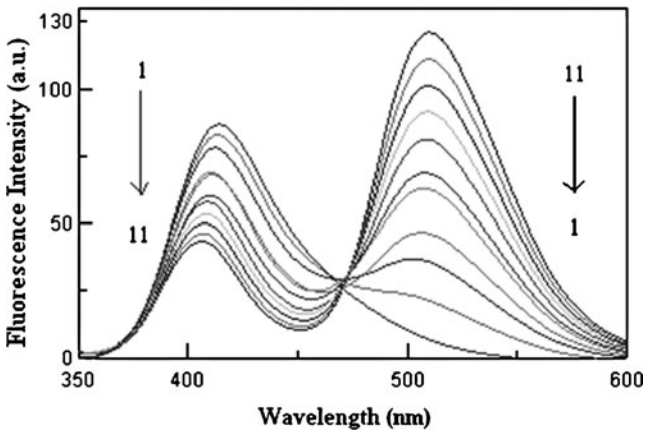


Fig. 6 The fluorescence quenching spectra of NF-PF system. From 1 to 11 : [NF]=6×10^{-7} mol dm^{-3}; [PF]=0.0, 0.2, 0.4, 0.6, 0.8,1.0,1.2,1.4,1.6,1.8,2.0×10^{-5} mol dm^{-3}

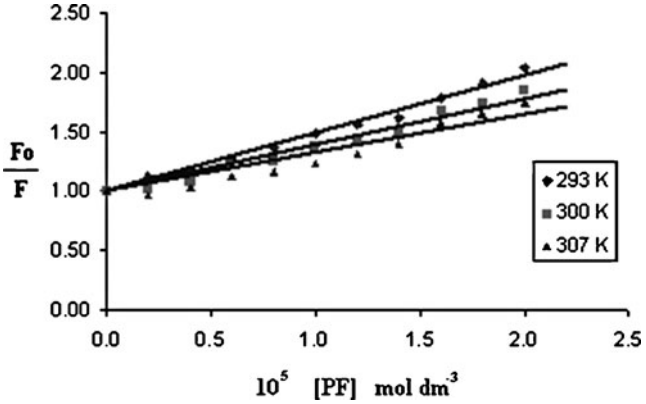


Fig. 7 The Stern-Volmer plots for quenching of NF by PF at three different temperatures

to 10 ml with distilled water for each fluorescence quenching experiment. The solutions were buffered properly prior to the analysis to maintain the pH of solutions to 7.4. The fluorescence emission spectral measurements of NF in absence and presence of varying amounts of PF were carried out in the range 350 nm to 600 nm under the excitation at 278 nm. Both the excitation and emission slit widths were kept at 10 nm. The UV spectra were obtained by scanning the solution on the spectrophotometer in the wavelength range 200 nm to 500 nm.

Results and Discussion

Optimization of Experimental Conditions

Preferentially the various optimal conditions were fixed to get accurate results. The various parameters such as solubility of reagents, medium of solutions, pH of solutions, addition order of solutions, temperature, variation of intensity with concentrations of solutions, excitation and emission wavelengths were studied to obtain optimal experimental conditions.

Effect of pH on Fluorescence Intensity

The Fig. 2 shows the variation of fluorescence intensity with change in pH of system studied in the pH range 2 to

Table 1 Stern-Volmer quenching constants (K_{SV}) for the interaction of PF with NF at different temperatures

Sr.No.	T (K)	10^{-4} K_{SV} (dm^3 mol^{-1})	Correlation coefficient R
1	293	4.53	0.9930
2	300	3.61	0.9985
3	307	3.24	0.9917

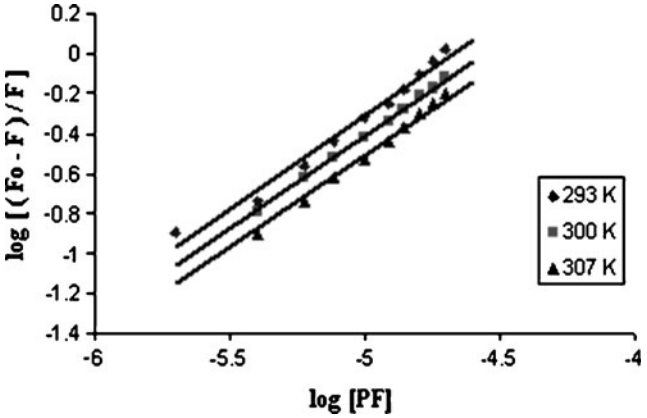


Fig. 8 The plots of log [(F_0-F)/F] against log[PF] at different temperatures

11. From the figure it was observed that the fluorescence intensity reached maximum in the pH range 7 to 8 while in more acidic and alkaline media observed fluorescence intensity gets lowered. Hence in order to get full complexation the pH 7.4 was maintained as optimum pH by using Tris–HCl buffer for every measurement. The buffer prepared from 0.1 mol dm^{-3} citric acid and 0.2 mol dm^{-3} disodium hydrogen phosphate was used for 2 to 6 pH range while the pH 7 to 10 was maintained by using 0.05 mol dm^{-3} Tris–HCl buffer solution.

Effect of Reaction Time

The effect of reaction time upon molecular interaction was studied by recording the fluorescence spectra of NF-PF solution at various time intervals. It was observed that the fluorescence intensity of NF-PF complex has constant value up to 24 h. Therefore it was concluded that the NF-PF complex is stable around 24 h regarding the fluorescence intensity.

Absorption Spectroscopy

The absorption spectra of NF and PF are shown in Fig. 3 (a) and (b) respectively. PF has shown an absorption peak at λ_{max}=444 nm while for NF two peaks are observed at λ_{max}=274 nm and 322 nm.

The absorption spectroscopy is an important tool to prove the formation of complex [17]. To explore this fact, two methods were employed as in first method, the amount of PF was kept constant and then the absorption spectra were recorded by gradually increasing the concentration of NF shown in Fig. 4. It was found that the peak developed at λ_{max}=444 nm whose intensity increased with increase in concentration of NF while in second method, the amount of NF was constant and absorption spectra were recorded with increasing concentration of PF as displayed in Fig. 5. From the figure it is clear that the peak at λ_{max}=262 nm was developed and its intensity increased with increase in concentration of PF.

By using these two methods, the separate contributions of absorbance of NF and PF could be eliminated and it helps to determine the formation of ground state PF-NF complex having its characteristic peaks in the region of absorption spectra characteristic to PF and NF. Thus the absorption spectroscopic studies proved that in the interaction of PF and NF, a ground state complex was formed.

Fluorescence Quenching

The reduction of fluorescence intensity by a competing deactivating process resulting from the specific interaction between fluorophore and acceptor substance is known as fluorescence quenching. The molecular interactions like energy transfer, molecular rearrangements, excited state reactions, collisional quenching and ground state complex formation can cause the fluorescence quenching. These different interactions are mainly classified into two classes viz. dynamic and static quenching involving diffusion and complex formation respectively. It can be distinguished by their different dependence on temperature. As dynamic quenching depends upon diffusion and at higher temperatures, the rate of diffusion increases hence the quenching constants are expected to increase with increasing temperature. On the contrary, increase in temperature is likely to decrease the stability of complex and thereby the static quenching constants are decreased [22].

In the present study, NF was donor while PF acted as acceptor. The Fig. 6 shows the fluorescence spectra of NF quenched by PF at λ_{ex}=278 nm. NF indicates a sharp emission band with λ_{max}=414 nm. It has been observed that with successive addition of PF, the fluorescence intensity of NF decreased regularly with slight blue shift from 414 nm to 406 nm and consequent-

Table 2 The binding parameters for the NF-PF interaction

Sr. No.	T (K)	10^{-4} Binding constant K (dm^3mol^{-1})	Binding sites n	Correlation coefficient R
1	293	2.45	0.94	0.9914
2	300	1.41	0.91	0.9985
3	307	1.26	0.90	0.9937

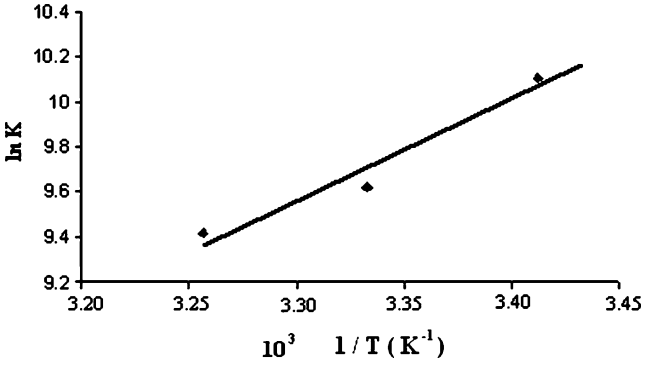


Fig. 9 van't Hoff plot for the binding interaction of PF with NF

ly new emission peak of PF at 511 nm was observed. An isoemissive point has been observed at 470 nm which indicates the existence of equilibrium between bound and free PF.

The fluorescence intensity data was analyzed according to Stern-Volmer equation [23, 24],

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities of NF in the absence and presence of PF respectively, $[Q]$ is the concentration of the quencher i.e. of PF, k_q is the quenching rate constant of donor,τ_0 is the average lifetime of donor without the quencher and K_{SV} is the Stern-Volmer quenching constant which was determined by linear regression of Stern-Volmer equation.

The fluorescence quenching study was carried out at three different temperatures 293, 300 and 307 K to understand the quenching mechanism. The Stern-Volmer plots at different temperatures are presented in Fig. 7. The plots are linear and Stern-Volmer quenching constants obtained from the slopes at various temperatures which were listed in Table 1. From the table, it is clear that with increase in temperature, the K_{SV} values are decreased i.e. K_{SV} is inversely correlated with temperature. This relation implies that the binding process between NF and PF is induced by complex formation rather than dynamic collision. As a consequence, the quenching mechanism was suggested as a static quenching and not dynamic quenching which implies that the quenching was initiated by ground state complex formation and not by collision or diffusion.

Binding Mechanism

The relationship between fluorescence quenching intensity and concentration of quencher can be described by the binding constant formula [22, 25].

$$\log \frac{F_0 - F}{F} = \log K + n \log[Q] \quad (2)$$

where K is the binding constant and n is the number of binding sites. Figure 8 displays the plot of $\log \frac{F_0 - F}{F}$ against $\log[Q]$ which gave the values of K and n from intercept and slope respectively. The values of K and n obtained at various temperatures are reported in Table 2. The data showed that the binding site number (n) is closed to each other at different temperatures which indicates the effect of temperature is not intense and one binding site is present between NF and PF. The considerable value of K indicates that PF has bound to NF effectively though there is only one binding site. The value of K is inversely related to temperature which indicates that NF-PF complex has lowered stability at higher temperatures.

Thermodynamic Parameters and Nature of the Binding Forces

It is known that there are four main types of forces acting in bimolecular interaction which are hydrophobic forces, electrostatic interactions, van der Waals' interactions and hydrogen bonding. The signs and magnitudes of thermodynamic parameters like enthalpy change (ΔH), entropy change (ΔS), free energy change (ΔG) suggests the nature of forces involving in the molecular interaction [26]. In order to elucidate the binding force between NF and PF, it was necessary to obtain the thermodynamic parameters and so the binding studies were carried out at 293, 300 and 307 K. The values of binding constant K are combined with thermodynamic parameters by using following equations.

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

Equations (3) and (4) are van't Hoff equation and Gibb's equation respectively. Here K is the binding constant, R is the gas constant, T is absolute temperature. The plot of ln K

Table 3 Thermodynamic parameters for NF-PF interaction at different temperatures

Sr. No.	T (K)	ΔH (kJ mol^{-1})	ΔS (J mol^{-1}K^{-1})	ΔG (kJ mol^{-1})
1	293			−24.73
2	300	−38.37	−46.56	−24.40
3	307			−24.08

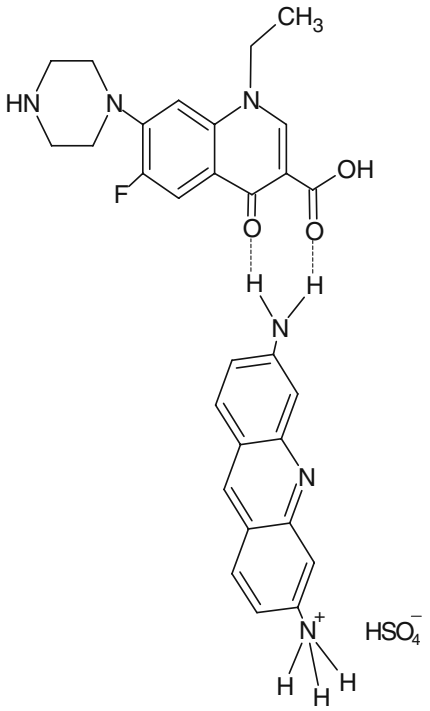


Fig. 10 The interaction between NF and PF involving hydrogen bonding

versus $\frac{1}{T}$ is as shown in Fig. 9 which obtained the values of ΔH *and* ΔS from slope and intercept respectively. By substituting these values in Eq. (4), the values of ΔG at different temperatures were calculated. The results obtained are summarized in Table 3 which shows that $\Delta H<0$, $\Delta S<0$ and $\Delta G<0$ i.e. all these parameters are negative. As both ΔH and ΔS are negative, the major binding forces in this process are hydrogen bonding and van der Waals' interactions and the reaction was mainly enthalpy driven [17]. Also negative ΔG indicates the spontaneity of the binding interaction at all the studied temperatures. The formation of cyclic hydrogen bonding between PF and NF is depicted in Fig. 10. The two hydrogen atoms of amino group of PF forms the hydrogen bonds with carbonyl function of NF.

Energy Transfer and Binding Distance between NF and PF

Fluorescence resonance energy transfer (FRET), a non radiative energy transfer, which is based on distance dependent transfer of energy from a donor molecule to an acceptor molecule. The rate of energy transfer depends upon the extent of spectral overlap of the emission spectrum of donor with the absorption spectrum of the acceptor, quantum yield of donor, relative orientation of the donor—acceptor transition dipoles and the distance between the donor—acceptor molecules. As FRET is sensitive to distance, it has been used to investigate molecular interactions [22, 27].

In present work, the absorption spectrum of PF (acceptor) substantially overlaps with the fluorescence spectrum of NF (donor) as shown in Fig. 11. According to Förster non-radiative energy transfer theory, the energy transfer efficiency (E) is related not only to the distance between the acceptor and donor but also to the critical energy transfer distance (R_0) as given by Eq. (5),

$$E = \frac{R_0^6}{R_0^6 + r^6} \tag{5}$$

where R_0 is the critical distance when 50% of the energy is transferred, r is the distance between donor and acceptor. E is efficiency of energy transfer calculated by Eq. (6),

$$E = 1 - \frac{F}{F_0} \tag{6}$$

where F and F_0 are the fluorescence intensities of NF with and without PF respectively.

R_0 is given by the relation

$$R_0^6 = 8.8 \times 10^{-25}.K^2.n^{-4}.\Phi.J \tag{7}$$

where K^2 is a factor depending upon the orientation of dipoles of the donor and acceptor in space, n is the refractive index of the medium, Φ is the fluorescence quantum yield of the donor in the absence of acceptor and J is the spectral overlap integral describing the degree of spectral overlap between donor emission and acceptor absorption spectra which is given by

$$J = \frac{\Sigma F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\Sigma F(\lambda)\Delta\lambda} \tag{8}$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at wavelength λ, $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength λ .

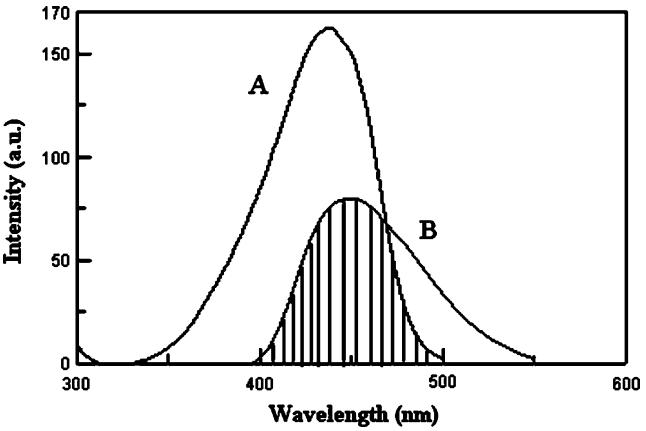


Fig. 11 Spectral overlay of (**a**) excitation of Proflavine hemisulphate and (**b**) emission of Norfloxacin [PF]=5×10^{-5} mol dm^{-3}; [NF]=1×10^{-6} mol dm^{-3}

Table 4 Effect of Foreign ions on fluorescence intensity

Sr. No.	Foreign ion species	% change in intensity	Tolerance limit (μg/ml)
1	Cu^{2+}	−36.82	7.22
2	Zn^{2+}	+68.61	3.87
3	Al^{3+}	+107.68	2.47
4	Fe^{3+}	−86.20	3.53
5	Ca^{2+}	+12.35	21.60
6	Ba^{2+}	−20.30	12.88
7	SO_4^{2-}	−8.48	26.63
8	NO_3^-	+10.72	23.48
9	Br^-	+19.36	13.51
10	I^-	−24.70	15.27

The value of J is evaluated by integrating the spectrum in Fig. 11 which is 17866.01 cm^3 dm^3 mol^{-1}. The critical distance R_0 (3.69 nm) was calculated from Eq. (7), by using $K^2=2/3$ [22], $n_D^{25} = 1.333$ and $\Phi=0.73$. The efficiency of energy transfer, $E=0.26$ and the distance between NF and PF, $r=4.38$ nm were calculated from Eqs. (6) and (5) respectively. The average distance between NF and PF is 4.38 nm, obviously it is much lower than 8 nm and also 0.5 $R_0<r<1.5$ R_0 indicating that the static quenching between NF-PF as well as the energy transfer from NF to PF occurs with high probability [28].

Effect of Interfering Ions

The presence of commonly existing ions may cause interference in spectral analysis of proposed system. Hence the study of such interference was carried out at room temperature by recording the fluorescence intensity of NF-PF complex in presence of each ion separately in the range of 350 nm to 600 nm. The tolerance limit was set up as a concentration of interfering species resulting in ±5% variation in the average fluorescence intensity. The various foreign ions with their tolerance limits and % change in intensity are presented in Table 4. Most of these ions have negligible interference in the present study which shows the method is more sensitive and correct for the analysis of PF.

Analysis of Pharmaceutical Sample

Proflavine hemisulphate which acts as antiseptic is commercially available in the form of ointments and spray. The proposed method of fluorescence quenching mechanism was applied for analysis of commercial pharmaceutical sample such as Lorexane, a veterinary cream, in the form of ointment was used for determination of PF content in it. The appropriate amount of the ointment was weighed and dissolved in distilled water which then diluted to required volume. Afterwards the solution was centrifuged and centrifugate was used for quenching method along with series of standard solutions while undissolved contents of ointment remained in residue. The results were reported in Table 5. The calculated value of PF content is in good agreement with the reported value and the relative standard deviation is also negligible. Thus the method is simple, reproducible and reliable for analysis of such pharmaceutical samples.

Conclusions

The binding interaction between NF and PF has been investigated by using fluorescence and UV–vis spectroscopy. The study of the interaction showed that a complex is formed

Table 5 Determination of PF in pharmaceutical sample

Sample	Composition	Amount of PF (% w/w)		R.S.D.[b] (%)
		Reported	Present method[a]	
Lorexane Galentic Pharma (I) Pvt.Ltd., Rubale, Navi Mumbai. (India).	Gama Benzene Hexachloride IP Proflavine hemisulphate Cetrimide IP	0.10	0.088	2.87

[a] Average of three determinations

[b] Calculated for three repeated measurements

between NF and PF through static quenching mechanism. The UV–vis absorption study supports the formation of ground state complex between NF and PF in this interaction. The binding process was studied at three different temperatures viz. 293, 300 and 307 K to examine the temperature dependence of Stern-Volmer quenching constant K_{SV}. The Stern-Volmer quenching constant K_{SV} is inversely related to temperature which indicates that binding process involves static quenching mechanism. The negative value of ΔG suggested the spontaneity of interaction; similarly ΔH and ΔS are both negative indicated van der Waals' interaction along with hydrogen bond had a major role in binding process. The binding distance $r = 4.38$ nm between NF-PF is obtained according to FRET which indicated high probability of energy transfer. The interference of foreign ions was studied which gave useful information regarding the tolerance limits in present study. The analytical method is applied successfully to estimate PF directly from pharmaceutical sample by using quenching mechanism.

Acknowledgment One of the authors (VRM) express his gratitude to the Department of Chemistry, The New College, Kolhapur for their kind encouragement.

References

1. Mitchel GAG, Buttle GAH (1943) Proflavine in closed wounds. Lancet 242:749
2. Nash SC, Ketcham AS, Smith RR (1962) Effect of local irrigation with Proflavine Hemisulfate on wounds seeded with tumor cells an experimental study. Ann Surg 155(3):465–471
3. Hunt PB, Ford JL (1983) Release of Proflavine hemisulphate from proflavine preparations currently in use within the mersey regional health authority. J Clin Hosp Pharm 8(4):345–348
4. Nelson JM, Chiller TM, Powers JH, Angulo FJ (2007) Food safety: fluoroquinolone-resistant campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. Clin Infect Dis 44(7):977–980
5. Rafalsky VV, Andreeva IV, Rjabkova EL (2006) Quinolones for uncomplicated acute cystitis in women. Cochrane Database Syst Rev 3:CD003597
6. Drlica K, Zhao X (1997) DNA gyrase, topoisomerase IV and the 4-quinolones. Microbiol Mol Biol Rev 61(3):377–392
7. More VR, Mote US, Patil SR, Kolekar GB (2010) Fluorescence quenching studies on the interaction between Riboflavin and Norfloxacin and Analytical application on determination of vitamin B_2. J Solution Chem 39(1):97–106
8. Son GS, Yeo JA, Kim MS, Kim SK, Holmén A, Akerman B, Norden B (1998) Binding mode of Norfloxacin to calf thymus DNA. J Am Chem Soc 120(26):6451–6457
9. Song G, He Y, Cai Z (2004) A fluorescence spectroscopic study of the interaction between Norfloxacin and DNA. Can J Anal Sci Spectrosc 49(4):203–209
10. More VR, Mote US, Patil SR, Kolekar GB (2009) Spectroscopic studies on the interaction between Norfloxacin and p-Amino benzoic acid: analytical application on determination of Norfloxacin. Spectrochim Acta A 74(3):771–775
11. Park HR, Seo JJ, Shin SC, Lee HS, Bark KM (2007) Fluorescence quenching of Norfloxacin by divalent transition metal cations. Bull Korean Chem Soc 28(9):1573–1578
12. Ackermann NB, Haldorson DK, Tendik FH, Elslager EF (1968) Preparation and screening of aminoacridines for induction of lung tumor fluorescence in rats. J Med Chem 11(2):315–321
13. Faller LD, LaFond RE (1971) Binding properties of oligomeric α-chymotrypsin. Biochemistry 10(6):1033–1041
14. Fernandez-Arguelles MT, Canabate B, Costa-Fernandez JM, Pereiro R, Sanz-Medel A (2004) Flow injection determination of nitrite by fluorescence quenching. Talanta 62(5):991–995
15. Lee WE, Galley WC (1988) Perturbations to the intersystem crossing of proflavin upon binding to DNA and poly d(A-IU) from triplet-delayed emission spectroscopy. J Biophys 54(4):627–635
16. Thomes JC, Weill G, Daune M (1969) Fluorescence of Proflavine–DNA complexes: heterogeneity of binding sites. Biopolymers 8(5):647–659
17. Chakraborty B, Basu S (2009) Interaction of BSA with Proflavin: a spectroscopic approach. J Lumin 129(1):34–39
18. Girousi ST, Alexiadou DK, Ioannou AK (2008) An electroanalytical study of the drug Proflavine. Microchim Acta 160(4):435–439
19. Alexiadou DK, Ioannou AK, Kouidou-Andreou S, Voulgaropoulos AN, Girousi ST (2008) Electrochemical study of the interaction mechanism of Proflavine (PF) with DNA using Carbon Paste (CPE) and Hanging Mercury Drop (HMDE) Electrode. Anal Lett 41(10):1742–1750
20. Shaw WHC, Wilkinson G (1952) The colorimetric estimation of small quantities of Proflavine hemisulphate. Analyst 77(912):127–132
21. Evstigneev MP, Mukhina YV, Davies DB (2006) ^{1}H NMR study of the hetero-association of flavin-mononucleotide with mutagenic dyes: ethidium bromide and proflavine. Mol Phys 104(4):647–654
22. Lakowicz JR (2006) Principles of fluorescence spectroscopy, 3rd edn. Plenum Press, New York
23. Mote US, Bhattar SL, Patil SR, Kolekar GB (2010) Interaction between felodipine and bovine serum albumin: fluorescence quenching study. Luminescence 25(1):1–8
24. Bhattar SL, Kolekar GB, Patil SR (2008) Fluorescence resonance energy transfer between perylene and riboflavin in micellar solution and analytical application on determination of vitamin B_2. J Lumin 128(3):306–310
25. Mote US, Bhattar SL, Patil SR, Kolekar GB (2009) Interaction of fluorescein with felodipine: a spectrofluorometric and thermodynamic study. J Solution Chem 38(5):619–628
26. Ross PD, Subramanian S (1981) Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 20(11):3096–3102
27. Bhattar SL, Kolekar GB, Patil SR (2010) Spectroscopic studies on the molecular interaction between salicylic acid and riboflavin (B_2) in micellar solution. J Lumin 130(3):355–359
28. Mote US, Han S-H, Patil SR, Kolekar GB (2010) Effect of temperature and pH on interaction between bovine serum albumin and cetylpyridinium bromide: fluorescence spectroscopic approach. J Lumin 130(11):2059–2064