Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology A: Chemistry 162 (2004) 129–137
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Emission of thioflavin T and its control in the presence of DNA

M. Ilanchelian, R. Ramaraj*

School of Chemistry, Madurai Kamaraj University, Madurai 625 021, India

Received 31 August 2002; received in revised form 27 June 2003; accepted 5 July 2003

Abstract

The interaction of thioflavin T, 3,6-dimethyl-2-(4-dimethylaminophenyl)-benzothiazolium cation (TFT) with DNA is studied using absorption and emission spectral methods. The observed hypochromism in the absorption spectra suggests that the TFT binds with DNA. The emission spectral changes show two different emission bands at 450 and 485 nm for TFT in the presence of DNA. The emission intensity observed at 450 nm decreases upon increasing the concentration of DNA. This further confirms the binding of TFT with DNA. The new emission band observed at 485 nm for TFT increases with increasing the concentration of DNA. It is proposed that the binding of TFT with DNA facilitates the formation of emittive TFT dimer. An isoemissive *point* was observed at 464 nm when the DNA concentration was higher than 1.35×10^{-5} mol dm^{-3}. This observation indicates the conversion of monomer TFT to emittive TFT dimer. Both the absorption and emission spectral studies show that two types of bindings such as groove binding and electrostatic interaction are present in the TFT–DNA system. The addition of NaCl to TFT–DNA showed the removal of the electrostatic binding between TFT and DNA. The intensity of 485 nm emission band is decreased upon the addition of NaCl to TFT–DNA system. The interaction of Na^+ ions with the phosphate groups of the DNA blocked the electrostatic interaction of TFT cations with DNA. The emission band at 485 nm shows only one type of interaction viz. the electrostatic interaction. The three-dimensional emission spectral studies shows only one contour due to monomer TFT emission in the absence of DNA. However, in the presence of DNA, TFT shows two contours due to monomer TFT and emittive TFT dimer.

Keywords: Thioflavin T; DNA; Thioflavin T dimer; Groove binding; Electrostatic binding

1. Introduction

As a consequence of the biological significance the spectroscopic properties of molecules containing amino and imino groups, particularly their fluorescence behaviors, have stimulated many spectroscopic studies [1–4]. The interactions of these molecules with DNA and dynamics have been extensively investigated by both theoretical and experimental methods [1–7]. Small molecules bind to DNA double helix by three modes of binding: electrostatic, groove and intercalative binding [1–10]. Very recently, the electron-transfer reactions between molecules and DNA have been reported [11–18]. Recently, Sugiyama et al. [7] clearly demonstrated that the dimer drug molecules could effectively interact with the DNA minor groove. They also reported that the guanine–cytosine (GC) sequences prefer to bind dimer molecules (a homodimer or a heterodimer) in the minor groove. Thioflavin T, 3,6-dimethyl-2-(4-dimethylaminophenyl)-benzothiazolium cation (TFT) (Fig. 1), is a benzothiazolium dye and it has been used for staining amyloid and paraamyloid tissues [19]. The photophysical properties at low temperatures [20] and the interactions of TFT with DNA have been reported [21,22]. Studies on the photophysical and photochemical properties of molecules in constrained media have received much attention in the past few years [23,24]. The formation of dimers, either in the ground state or in the excited state, has been observed for many molecules [25]. Dimer formation in solution is a diffusion-controlled process and is governed by the solubility of the compound and other diffusion controlled factors. In contrast, the formation of the dimer in the solid state is primarily affected by the topochemical alignment of the molecules [25]. Recently, we have reported the emission properties of TFT and its off–on control in cyclodextrin [26] and polymer membrane [27]. In this paper, we present the details on the absorption and emission properties of the TFT dye molecule in the presence of DNA.

It is interesting to know how spectroscopic characteristics of TFT vary as a function of DNA binding, i.e. whether different modes of DNA binding might be distinguished by

* Corresponding author. Tel.: +91-452-2459084; fax: +91-452-2459139.
E-mail address: ramarjar@yahoo.com (R. Ramaraj).

1010-6030/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/S1010-6030(03)00320-4

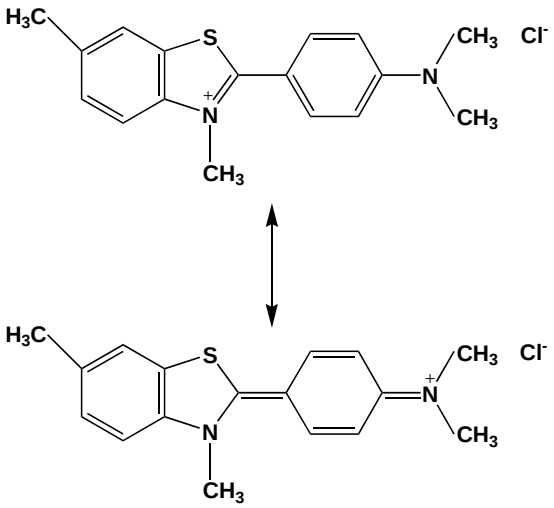


Fig. 1. Structure of thioflavin T (TFT).

studying the photophysical properties so as to optimize the sensitivity and utility of the spectroscopic probes for DNA. In the presence of DNA, the TFT exhibits emission for the monomer and dimer dye molecules. The emission observed for the dimer TFT molecule is eliminated upon the addition of NaCl.

2. Experimental methods

TFT (Aldrich) was thrice recrystallized from methanol and characterized by its absorption spectrum [20]. *Escherichia coli* (50% GC) DNA purified by phenol extraction using literature procedure [28] was used in this study. The purity of DNA was checked by monitoring the absorption and the ratio of the absorbances at 260 and 280 nm was calculated as (≈1.9). The ratio indicated that the DNA was sufficiently free of protein [29]. The DNA and TFT were dissolved in TE buffer pH 8 (10 mM Tris (Tris(hydroxymethyl)aminomethane–HCl) and 1 mM EDTA mixture). The DNA concentration per nucleotide (c(P)) was determined by absorption spectroscopy using the molar extinction coefficient value of 6600 $dm^3\,mol^{-1}\,cm^{-1}$ at 260 nm [30]. Other reagents were of analytical grade and used as received.

Absorption spectral studies were carried out using a JASCO 7800 spectrophotometer. Emission studies were performed on a HITACHI F4500 fluorescence spectrophotometer. The TFT dye was excited at 330 and 450 nm, respectively, and the corresponding emission was monitored. The excitation spectra of TFT were recorded for their corresponding emission wavelengths 450 and 485 nm, respectively. The emission and excitation slit widths used throughout the experiments were both 5 nm. The molecular dimensions of TFT were measured by Biosym-Insight II molecular modeling software on a silicon graphics computer system. All the measurements were carried out at room temperature (25 °C). Water used in this investigation was doubly distilled over alkaline potassium permanganate using an all-glass apparatus. TFT solution (≈10^{-5} mol dm^{-3}) was always freshly prepared before use. Excess concentration of TFT was used when compared to DNA in all experiments.

3. Results and discussion

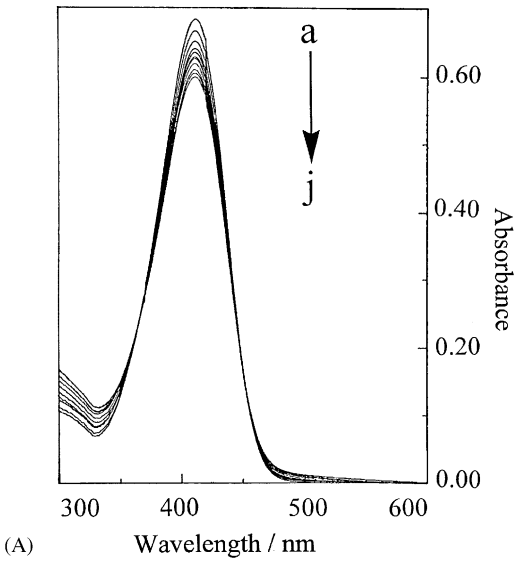
3.1. Absorption spectral studies

TFT in buffer (pH 8) solution shows an absorption band at 412 nm [20] and an intense emission band at 450 nm [20,26]. Shirra observed the formation of dimer TFT in aqueous solution at low temperatures [20] and it showed an increase in the intensity of the absorption bands at 364 and 460 nm caused by the dimer TFT. In the present investigation, we have not observed such absorption bands for the dimer in our experimental conditions (at room temperature) even at higher concentration of TFT. This could be because of the very low extinction coefficient of the ground state dimer. The absorption spectra of TFT in the presence of different concentrations of DNA are shown in Fig. 2A. The absorption intensity of TFT is decreased (hypochromism) with the increase in the concentration of DNA (Fig. 2A). The observed hypochromism is suggested to be due to the interaction of TFT with DNA base pairs [31]. From the absorption spectral data the binding constant of TFT with DNA was calculated by Eq. (1) [32].

$$\frac{1}{\Delta A} = \frac{1}{(\varepsilon_1 - \varepsilon_0)[C_0]K_{\text{bin}}}\frac{1}{[\text{DNA}]} + \frac{1}{(\varepsilon_1 - \varepsilon_0)[C_0]} \quad (1)$$

where ΔA is the difference in absorption intensities of TFT in the absence and presence of DNA ($\Delta A = (A_0 - A)$, A_0 the absorption intensity of TFT in the absence of DNA, A the absorption intensity of TFT at a given concentration of DNA), ε_1 and ε_0 are molar extinction coefficients of TFT and DNA, respectively. $[C_0]$ is initial concentration of TFT and K_{bin} is the binding constant. The double reciprocal plot of TFT–DNA system is shown in Fig. 2B. The plot is not a single straight line as expected from Eq. (1). Fig. 2B shows two different regions, and indicates the existence of two different modes of binding for TFT with DNA. There may be an intercalative or groove binding and electrostatic interaction. Most of the researchers have reported a single straight line for the binding of organic molecules with DNA [33–37]. It has been suggested that the organic molecules intercalate into DNA and shows only a single binding constant.

To check the electrostatic interaction between TFT and DNA, a strong electrolyte sodium chloride (NaCl) was used. A recent report clearly shows that higher concentrations of NaCl would hinder small cationic molecules from binding with DNA [38]. The absorption spectra of TFT–DNA at different concentrations of NaCl are shown in Fig. 3. As mentioned earlier (Fig. 2A), a decrease in



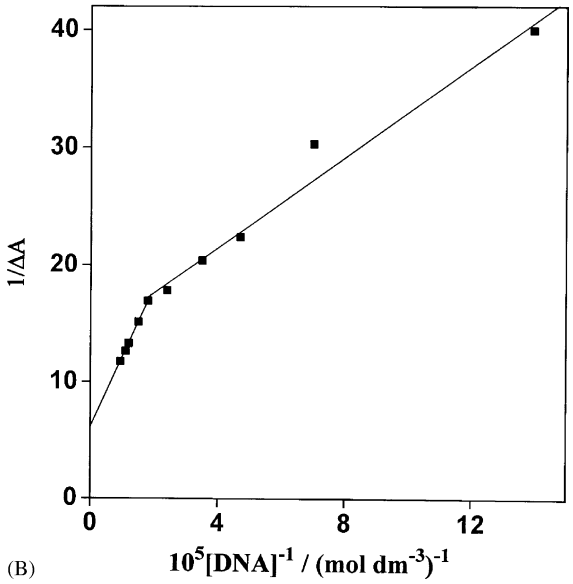


Fig. 2. (A) Absorption spectra of TFT (2.65×10^{-5} mol dm^{-3}) at various DNA concentrations. [DNA]: (a) 0; (b) 6.52×10^{-7}; (c) 1.30×10^{-6}; (d) 2.57×10^{-6}; (e) 3.82×10^{-6}; (f) 5.0×10^{-6}; (g) 6.24×10^{-6}; (h) 7.42×10^{-6}; (i) 8.57×10^{-6} and (j) 9.7×10^{-6} mol dm^{-3}. (B) Double reciprocal plot obtained from the absorbance spectral data for the TFT–DNA system.

the absorption intensity was observed after the addition of DNA to TFT solution. However, the absorption intensity of TFT (Fig. 3b) increased with increasing the concentration of NaCl (Fig. 3c–h) in the presence of DNA. The increase in the absorption intensity of TFT is owing to the release of electrostatically interacted TFT with DNA. A complete release of TFT–DNA binding was not achieved even when the concentration of NaCl was increased to 0.25 mol dm^{-3}. This is because of the intercalative or groove binding of TFT with DNA. These observations show that the TFT binds to DNA by both electrostatic and other type of interactions [39].

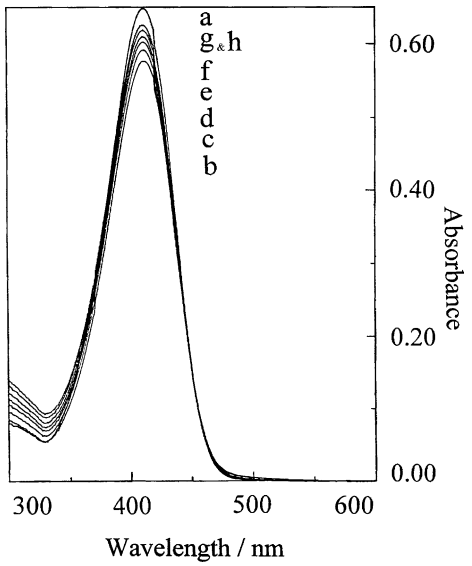


Fig. 3. Absorption spectra of TFT (2.57×10^{-5} mol dm^{-3}). (a) TFT alone and (b–e) TFT in the presence of DNA (6.48×10^{-6} mol dm^{-3}). [NaCl]: (b) 0; (c) 0.025; (d) 0.05; (e) 0.1; (f) 0.15; (g) 0.2 and (h) 0.25 mol dm^{-3}.

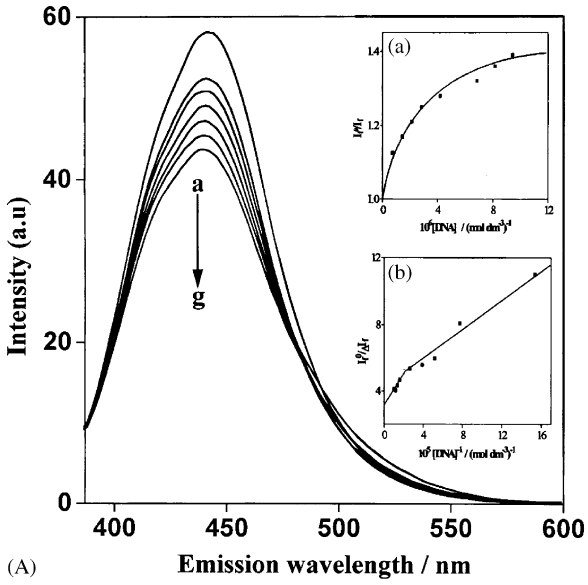
3.2. Emission spectral studies

In aqueous solution, TFT molecule shows an intense emission band at 450 nm [26,27]. The emission intensity increases with increase in concentration of the dye and at higher concentrations a decrease in emission intensity accompanied by a shift in the emission maximum is observed. The decrease in emission intensity observed at higher concentrations could be because of the self-quenching of the excited state TFT. The emission spectral studies of TFT in the presence of DNA showed emission bands at 450 and 485 nm [26,27]. When the emission wavelength was fixed at 485 nm TFT showed two excitation bands, i.e. one at 330 and another band at 450 nm in the presence of DNA. The reason for the observed two excitation bands for TFT in the presence of DNA will be discussed latter.

The emission spectra recorded for TFT with different concentrations of DNA are shown in Fig. 4A. The emission spectrum of TFT in the absence of DNA shows an emission maximum at 450 nm, when TFT was excited at 330 nm (Fig. 4A(a)). After the addition of 8.57×10^{-6} mol dm^{-3} of DNA to TFT (Fig. 4(b–g)), the emission intensity at 450 nm was decreased with no significant shift in the emission maximum. The decrease of emission intensity of TFT at 450 nm upon increasing the concentration of DNA show that the TFT binds with DNA [40,41]. The decrease in the emission intensity of TFT at 450 nm in the presence of DNA was analyzed using the Stern–Volmer Eq. (2) [42].

$$\frac{I_f^0}{I_f} = 1 + K_{SV}[Q] \quad (2)$$

where I_f^0 and I_f are the emission intensities of TFT in the absence and presence of DNA. [Q] is the concentration of



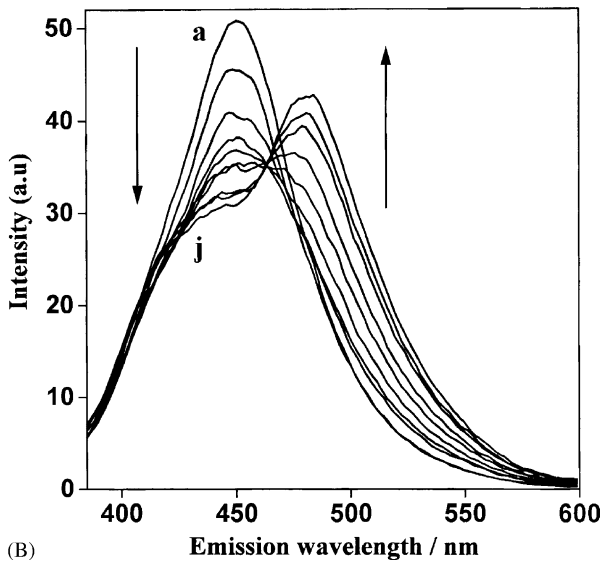


Fig. 4. (A) Emission spectra of TFT (2.65×10^{-5} mol dm^{-3}) at various DNA concentrations. [DNA]: (a) 0; (b) 6.52×10^{-7}; (c) 1.30×10^{-6}; (d) 1.94×10^{-6}; (e) 3.82×10^{-6}; (f) 5.0×10^{-6} and (g) 8.57×10^{-6} mol dm^{-3}. ($\lambda_{ex} = 330$ nm). Inset (a) plot of quenching of TFT (2.65×10^{-5} mol dm^{-3}) by DNA. (b) Double reciprocal plot obtained from the emission spectral data for the TFT–DNA system. (B) Emission spectra of TFT (2.75×10^{-5} mol dm^{-3}) at various DNA concentrations. [DNA]: (a) 0; (b) 1.77×10^{-6}; (c) 1.0×10^{-5}; (d) 1.37×10^{-5}; (e) 2.01×10^{-5}; (f) 2.93×10^{-5}; (g) 3.8×10^{-5}; (h) 5.3×10^{-5}; (i) 5.92×10^{-5} and (j) 6.52×10^{-5} mol dm^{-3}. $\lambda_{ex} = 330$ nm.

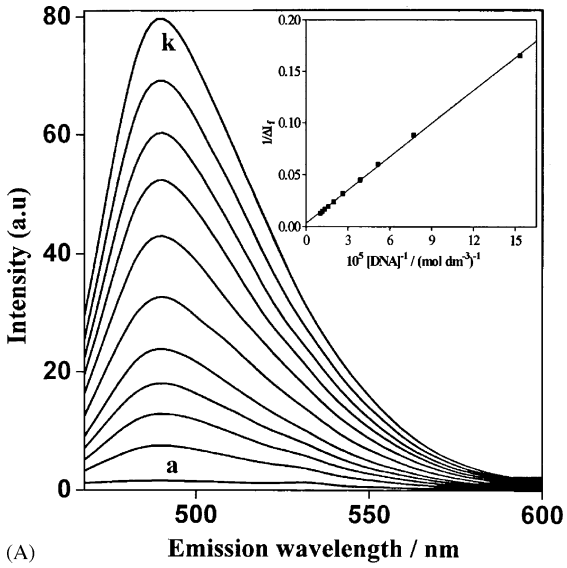
quencher (DNA) and K_{SV} is the Stern–Volmer constant. The Stern–Volmer plot (Fig. 4A (inset a) shows deviation from linearity. The quenching curve tends towards the *x*-axis at higher concentrations of DNA. The changes in the emission spectra of TFT after adding DNA (Fig. 4A (inset a)) indicate that there were strong interactions between the TFT chromophore and the base pairs of DNA. Combined with the changes of absorption spectra, the strong interaction should be considered as the groove binding and electrostatic interactions. Sugiyama et al. [7] reported that the heterodimer drug molecules bound to the minor groove of DNA. In the present case, we expect that the different bindings involved in TFT with DNA are groove binding and electrostatic interaction. The interaction of TFT with DNA was understood from the emission spectral data ($\lambda_{em} = 450$ nm) using Eq. (3) [42].

$$\frac{I_f^0}{\Delta I_f} = \frac{1}{I_f^a K_{bin}[DNA]} + \frac{1}{I_f^a} \quad (3)$$

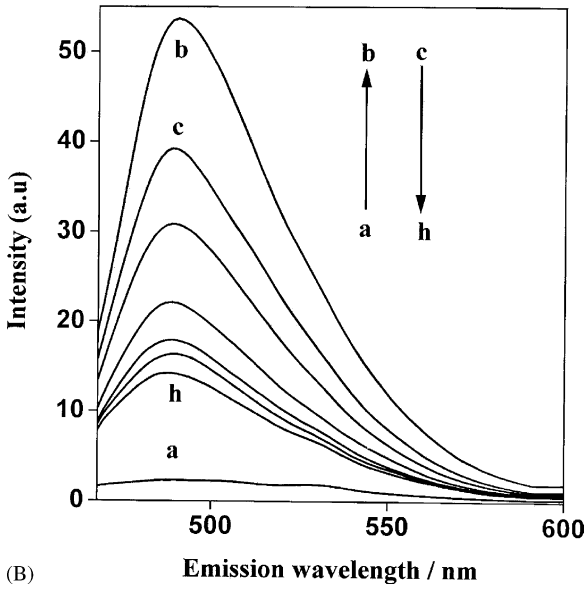
where I_f^0 is the emission intensity of TFT in the absence of DNA, ΔI_f the difference in emission intensities of TFT in the absence and presence of DNA ($\Delta I_f = I_f^0 - I_f$), I_f the emission intensity of TFT at a given concentration of DNA, I_f^a the fraction of the initial fluorescence which is accessible to quencher and K_{bin} is the binding constant. The plot of $I_f^0/\Delta I_f$ versus $[DNA]^{-1}$ (Fig. 4A (inset b) shows a very similar trend as we observed in the absorption spectral studies (Fig. 2B). This clearly indicates the presence of two modes of bindings of TFT with DNA.

Further, we recorded the emission spectra of TFT in the presence of higher concentrations of DNA (>9.7×10^{-6} mol dm^{-3}) (Fig. 4B). The emission intensity at 450 nm decreased with increase in the concentration of DNA (upto 1.35×10^{-5} mol dm^{-3}) but further increase in the concentration of DNA leads to the formation a new emission band. The emission intensity of the new band at 476 nm increased and the band shifted to 485 nm whereas the emission band at 450 nm decreased simultaneously upon increasing the concentration of DNA with an isoemissive point at 464 nm. The emission spectral studies at higher concentrations of DNA indicate the existence of two TFT species in the excited state. Further, the emission band observed at 485 nm was found to be due to the excitation wavelength at 450 nm.

The emission intensity of TFT at 485 nm increased with an increase in the concentrations of DNA (Fig. 5A). Recently, we have reported the emission spectral studies of γ-cyclodextrin (γ-CD) inclusion complex of TFT [26,43] and TFT in polymer membrane [27]. The emission intensity of TFT at 485 nm is increased in the presence of γ-CD due to the formation of emittive dimer. The emittive dimer can be formed between the excited state and ground state species of TFT inside the γ-CD cavity. Increasing hydrophobicity around TFT stabilizes the excited state species and the less polar rigid microenvironment provided by the γ-CD cavity leads to the formation of the emittive dimer. In the present study, we believe that the increased emission intensity ($\lambda_{em} = 485$ nm) of TFT is possibly due to the formation and stabilization of the emittive dimer in the hydrophobic environment provided by DNA due to electrostatically interacting excited state species of TFT with DNA. It is worth mentioning that as the TFT molecule is in a planar configuration, the dimerization can occur in a sandwich form with an anti-conformation because of the positive charge density of the thiazole ring [20]. The new excitation band appearing



(A)



(B)

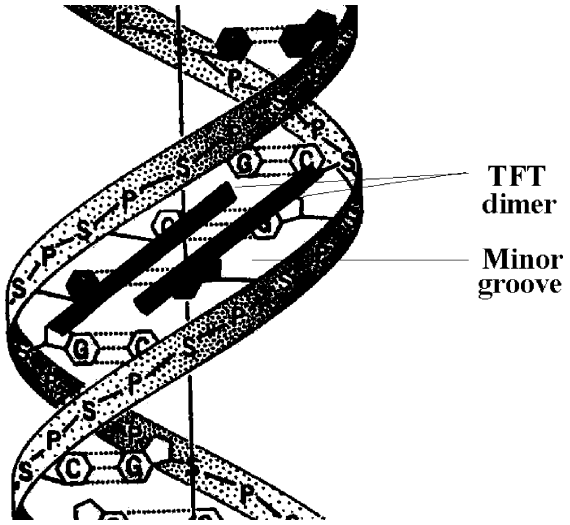
Fig. 5. (A) Emission spectra of TFT (2.65×10^{-5} mol dm^{-3}) at various DNA concentrations. [DNA]: (a) 0; (b) 6.52×10^{-7}; (c) 1.30×10^{-6}; (d) 1.94×10^{-6}; (e) 2.57×10^{-6}; (f) 3.82×10^{-6}; (g) 5.0×10^{-6}; (h) 6.24×10^{-6}; (i) 7.42×10^{-6}; (j) 8.57×10^{-6} and (k) 9.7×10^{-6} mol dm^{-3}. $\lambda_{ex} = 450$ nm. Inset: double reciprocal plot obtained from the emission spectral data for the TFT–DNA system. (B) Emission spectra of TFT (2.57×10^{-5} mol dm^{-3}). (a) TFT alone and (b–h) TFT in the presence of DNA (6.48×10^{-6} mol dm^{-3}). [NaCl]: (b) 0; (c) 0.025; (d) 0.05; (e) 0.1; (f) 0.15; (g) 0.2 and (h) 0.25 mol dm^{-3}.

at 450 nm in the presence of DNA assigned to the dimer TFT. Here it should be mentioned that the absorption spectrum of TFT–DNA does not show any absorption band for the dimer even at higher concentrations of TFT. As the absorption spectrum does not show any band for the dimer, it can be concluded that the ground state TFT dimer has a very low extinction coefficient. However, as it shows very high fluorescent intensity, as evidenced by the intense emission at 485 nm and the excitation band at 450 nm, the ground state dimer could have a high fluorescence quantum yield.

The binding of TFT with DNA was understood by using Benesi–Hildebrand Eq. (4) by monitoring the emission at 485 nm [44].

$$\frac{1}{\Delta I_f} = \frac{1}{K_{bin}(I'_f - I^0_f)[DNA]} + \frac{1}{(I'_f - I^0_f)} \quad (4)$$

where $\Delta I_f = (I_f - I^0_f)$, I^0_f is the emission intensity of the TFT in the absence of DNA, I_f the emission intensity of TFT at a given concentration of DNA and I'_f the emission intensity of the complex. The plot (Fig. 5A (inset)) shows a single straight line. This reveals that the binding of TFT to DNA involves only one type of interaction. The emission spectra shown in Fig. 5A are observed when the TFT–DNA is excited at 450 nm where only ground state TFT dimer can be excited. Thus, the data in Fig. 5A (inset) clearly shows that the dimer TFT binding to DNA involves only one type of interaction, i.e. electrostatic interaction between the cationic TFT and DNA. This was further confirmed by salt effect experiments. It has already been shown that the electrostatic interaction would be weakened by the addition of Na^+ ion [38]. The emission spectra monitored at 485 nm after the addition of NaCl to TFT–DNA system are shown in Fig. 5B. The emission intensity of TFT–DNA was decreased by the addition of NaCl (Fig. 5B(c–h)). However, the complete release of TFT–DNA interaction was not occurred. The TFT molecules interact with DNA by groove binding and electrostatic interaction. In this case the dominant interaction is electrostatic in nature. It has been suggested by Sugiyama et al. [7] that organic dimer molecule effectively interact with the DNA minor groove wall hydrophobically, stabilizing the complex (Scheme 1). Considering the molecular size of the TFT dimer, the intercalative binding of TFT dimer with DNA base pairs is ruled out. The majority of the TFT dimer molecules experienced the electrostatic interaction are



Scheme 1. Schematic representation of groove binding of the dimer TFT with DNA.

released by the Na^+ ions and the small fraction of the TFT dimer interacted by groove binding assisted by hydrophobic force are not released by Na^+ ions.

3.3. Emission spectral studies of TFT with DNA containing NaCl

The emission spectra of TFT in the presence of DNA containing 0.5 and 1.0 mol dm^{-3} concentrations of NaCl were studied. When DNA with 0.5 mol dm^{-3} of NaCl was added to TFT the emission intensity was decreased at 450 nm and reached saturation level at higher DNA concentrations. However, further decrease in the emission intensity of TFT was not observed upon the addition of DNA containing higher concentrations of NaCl (1.0 mol dm^{-3}). The double reciprocal plots ($I_f^0/\Delta I_f$ versus $[DNA]^{-1}$) (Fig. 6A) showed the release of one mode of binding at the increased concentration of NaCl (1.0 mol dm^{-3}) (Fig. 6A). From these plots the lower concentration of NaCl (0.5 mol dm^{-3}) shows two types of binding (Fig. 6A(a)) and at higher concentration of NaCl (1.0 mol dm^{-3}) (Fig. 6A(b)) a single straight line was observed when compared to Fig. 4A (inset b). This indicates that only one mode of interaction (i.e. other than electrostatic interaction) exists between TFT and DNA at higher concentration of NaCl ($\geq$1.0 mol dm^{-3}). Thus there is only one mode of binding, i.e. groove binding is observed for TFT with DNA at higher concentrations of NaCl. From the above experiments, we suggest that both groove binding and electrostatic interactions operate between TFT and DNA in the absence of NaCl whereas purely groove binding exists between a small fraction of TFT and DNA at higher concentration of NaCl. Recently, the influence of Na^+ ions on the binding of negatively charged ligands of metal complexes with DNA has been reported [45]. It has been shown that the presence of Na^+ ions on the surface of DNA favor the interaction of negatively charged ligands with DNA [45]. In the present case, the presence of Na^+ ions on the surface of DNA will repel the positively charged TFT from binding to DNA while it favors the groove binding of TFT with DNA.

The emission spectra (λ_{em} = 485 nm) of TFT with increasing concentrations of DNA containing various concentrations of NaCl (0, 0.5, 1.0 and 1.5 mol dm^{-3}) were recorded at λ_{ex} = 450 nm. The emission spectra of TFT in the presence of DNA containing lower concentration of NaCl (0.5 mol dm^{-3}) showed a very similar spectral change as shown in Fig. 5A but with lower emission intensity. However, the emission intensity of TFT at 485 nm in the presence of DNA with higher concentration of NaCl (1.5 mol dm^{-3}) showed a saturation level with lower emission intensity even after the addition of 9.7×10^{-6} mol dm^{-3} of DNA (Fig. 6B (inset)) when compared to Fig. 5A. This indicates that the surface binding of TFT with DNA was prevented by Na^+ ions. The binding constants were calculated using the emission spectral data observed at 485 nm. The double reciprocal plots observed for the TFT with DNA containing various concentrations of NaCl (0, 0.5, 1.0 and 1.5 mol dm^{-3}) are

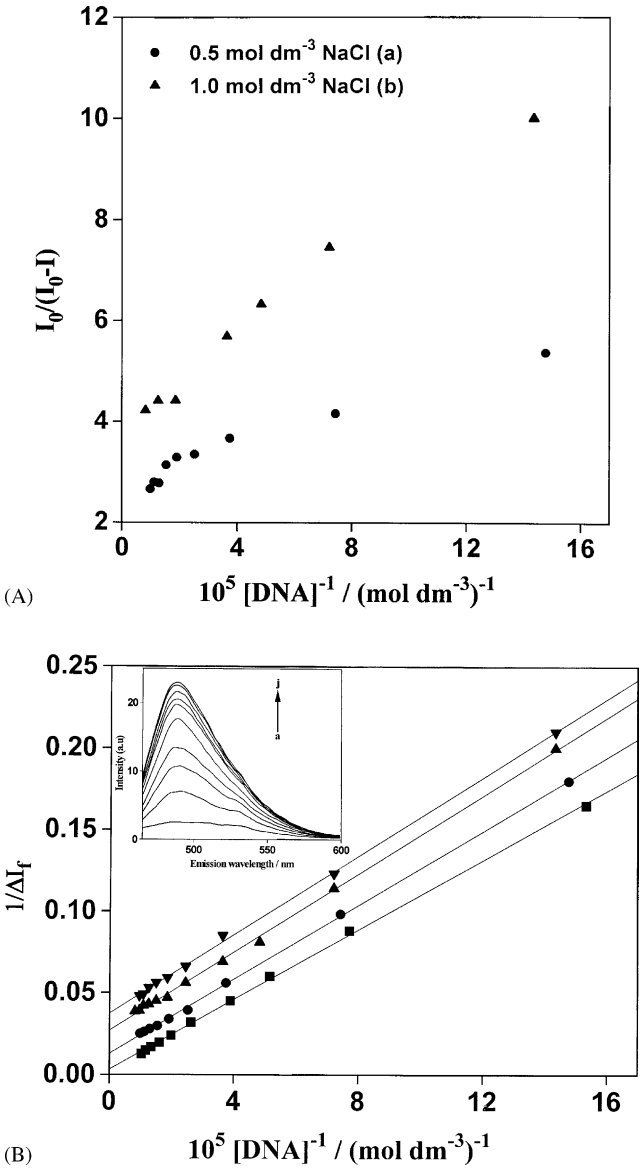


Fig. 6. (A) Double reciprocal plot obtained from the TFT emission spectral data (450 nm band) for the DNA containing 0.5 and 1.0 mol dm^{-3} of NaCl. (B) Double reciprocal plot obtained from the TFT emission spectral data (485 nm band) for the DNA containing 0, 0.5, 1.0 and 1.5 mol dm^{-3} of NaCl. Inset: emission spectra of TFT (2.65×10^{-5} mol dm^{-3}) at various concentrations of DNA containing 1.5 mol dm^{-3} of NaCl. [DNA]: (a) 0; (b) 6.52×10^{-7}; (c) 1.30×10^{-6}; (d) 1.94×10^{-6}; (e) 2.57×10^{-6}; (f) 3.82×10^{-6}; (g) 5.0×10^{-6}; (h) 6.24×10^{-6}; (i) 7.42×10^{-6} and (j) 9.7×10^{-6} mol dm^{-3}. λ_{ex} = 450 nm.

shown in Fig. 6B. The binding constants were calculated from the intercepts and slopes and the values are summarized in Table 1. The increase in the concentration of NaCl increases the TFT–DNA binding constant values (Table 1). The addition of NaCl to TFT–DNA (Fig. 5B) leads to a decrease in the dimer TFT emission intensity. The dimer emission intensity at 485 nm becomes very low at higher concentration of NaCl. However, complete decrease of dimer TFT emission intensity at 485 nm was not observed for TFT–DNA even after the addition of higher

Table 1
Binding constants for the TFT–DNA and TFT–DNA containing various concentrations of NaCl

[NaCl] (mol dm^{-3})	Binding constant, $\times 10^4$ dm^3 mol^{-1} (λ_{em} = 485 nm)
0.0	3.0
0.5	11.3
1.0	22.4
1.5	31.0

concentrations of NaCl (Fig. 5B). This means that a small fraction of dimer TFT is still bound to DNA. Sugiyama et al. [7] have reported that the dimer drug molecules bound to the minor groove of DNA. Sigman [10] Sigman and co-workers [46] have also reported the minor groove binding of copper complexes of substituted 1,10-phenanthroline ligand with DNA. In the present case, we assume that the binding of a small fraction of dimer TFT into the minor groove of the DNA would be possible in the presence of NaCl due to the hydrophobic interaction. The complete elimination of dimer TFT emission was not observed in the presence of DNA containing higher concentration of NaCl (1.5 mol dm^{-3}). We assume that the groove binding of a small fraction of dimer TFT with DNA is stronger than the electrostatic interaction. The hydrophobic interaction [7,10,46] between the small fraction of dimer TFT and DNA might enhance the groove binding. The binding constants are calculated with very low emission intensity (in the presence of 1.0 or 1.5 mol dm^{-3} of NaCl) and the spectral data might reflect the groove binding of a small fraction of TFT dimer.

To further confirm the presence of two different emittive TFT species observed in the TFT–DNA system, the three dimensional emission and excitation spectral studies were carried out. The three-dimensional emission contours recorded for TFT in the absence and presence of DNA are shown in Figs. 7 and 8, respectively, and the corresponding

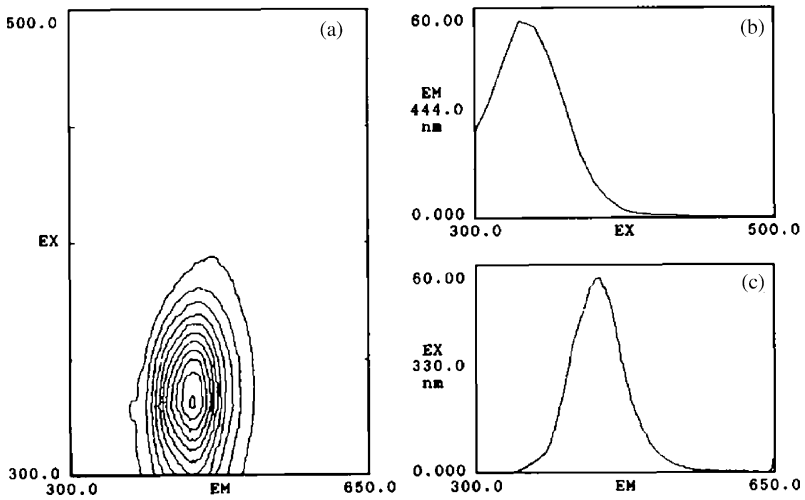


Fig. 7. Three dimensional emission spectra of TFT (2.75×10^{-5} mol dm^{-3}) in the absence of DNA: (a) three dimensional contour plot; (b) corresponding excitation spectra and (c) emission spectra.

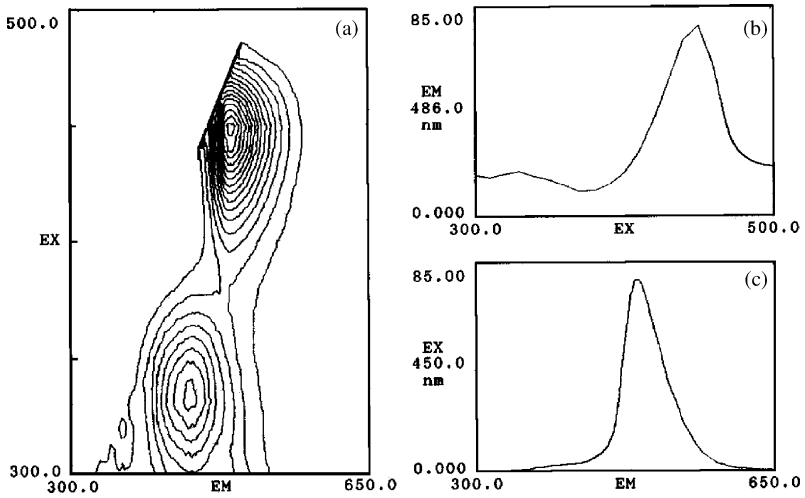


Fig. 8. Three dimensional emission spectra of TFT (2.75×10^{-5} mol dm^{-3}) in the presence of (a) 1.10×10^{-5} mol dm^{-3} of DNA: (a) contour plot; (b) corresponding excitation spectra and (c) emission spectra.

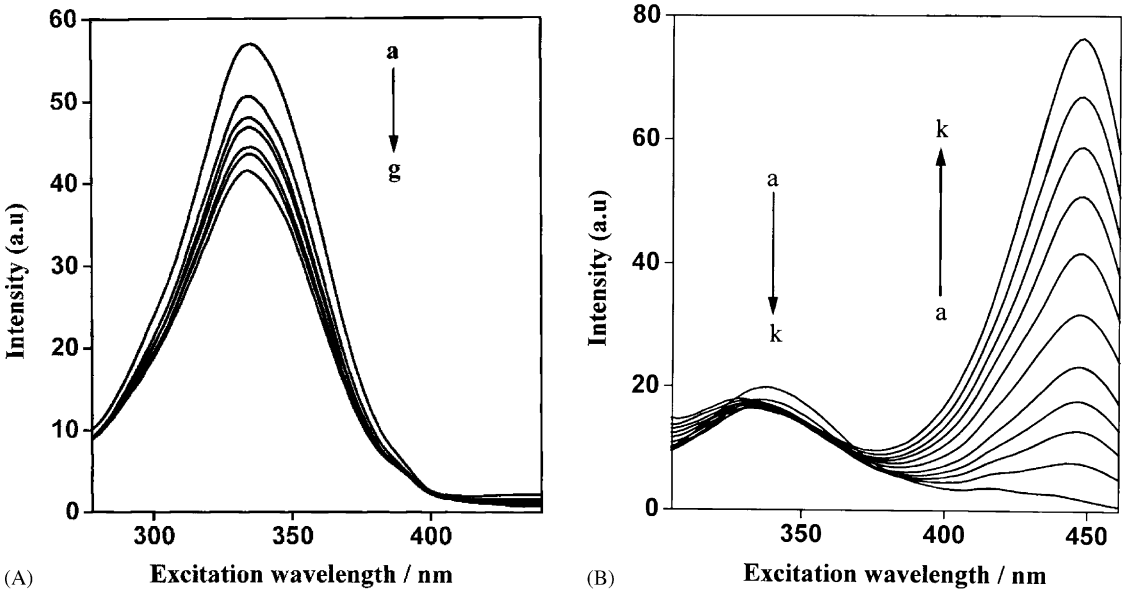


Fig. 9. (A) Excitation spectra of TFT (2.65×10^{-5} mol dm^{-3}) at various DNA concentrations. [DNA]: (a) 0; (b) 6.52×10^{-7}; (c) 1.30×10^{-6}; (d) 1.94×10^{-6}; (e) 3.82×10^{-6}; (f) 5.0×10^{-6} and (g) 8.57×10^{-6} mol dm^{-3}. $\lambda_{em} = 450$ nm. (B) Excitation spectra of TFT (2.5×10^{-5} mol dm^{-3}) at various DNA concentrations. [DNA]: (a) 0; (b) 6.52×10^{-7}; (c) 1.30×10^{-6}; (d) 1.94×10^{-6}; (e) 2.57×10^{-6}; (f) 3.82×10^{-6}; (g) 5.0×10^{-6}; (h) 6.24×10^{-6}; (i) 7.42×10^{-6}; (j) 8.57×10^{-6} and (k) 9.7×10^{-6} mol dm^{-3}. $\lambda_{em} = 485$ nm.

excitation and emission spectra are also shown. In the absence of DNA, TFT shows only one contour and the corresponding excitation and emission spectra show only one band each (Fig. 7). As shown in Fig. 8, two contours were observed for TFT in the presence of DNA (1.10×10^{-5} mol dm^{-3}) and the corresponding excitation and emission spectra show two excitation bands at 330 and 445 nm and two emission bands at 450 and 485 nm due to the presence of two emittive TFT species. No further decrease was observed in the contour intensity of TFT–DNA at lower wavelength band ($\lambda_{em} = 450$ nm) whereas the intensity at higher wavelength band ($\lambda_{em} = 485$ nm) showed an increase in the intensity at higher concentration of DNA (Fig. 8). The new contour observed at higher wavelength in the presence of DNA is assigned to the electrostatically interacted excited state species of TFT dimer. The excitation spectra recorded for TFT with different concentrations of DNA are shown in Fig. 9A. Fig. 9A shows only one excitation band at 330 nm, which corresponds to the emission at 450 nm. The excitation spectra recorded for TFT with DNA

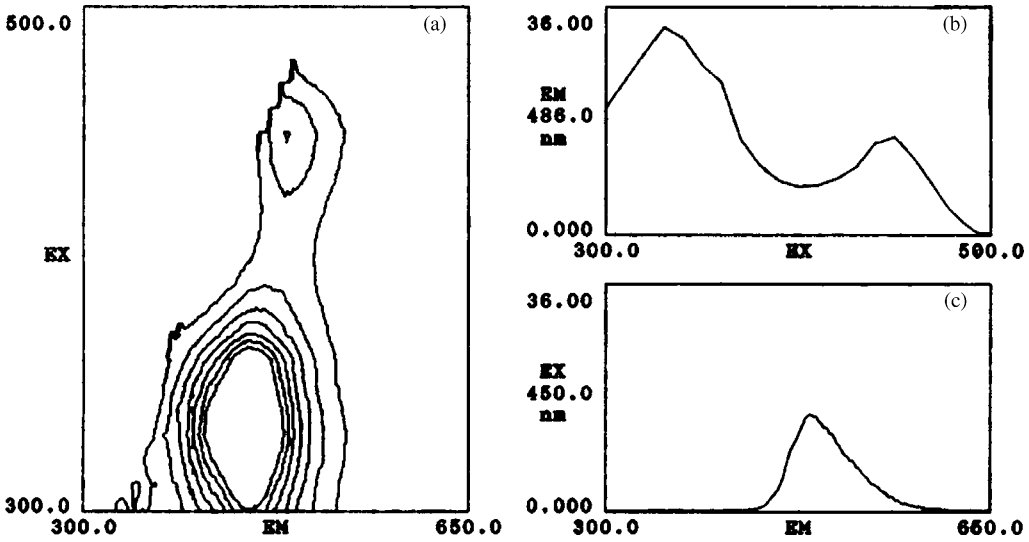


Fig. 10. 3D emission spectra of TFT (2.76×10^{-5} mol dm^{-3}) in the presence of (a) 1.10×10^{-5} mol dm^{-3} of DNA in 1.5 mol dm^{-3} NaCl: (a) contour plot; (b) corresponding excitation spectra and (c) emission spectra.

showed two excitation bands at 330 and 450 nm when the TFT emission is fixed at 450 nm (Fig. 9B). Fig. 9B shows that the intensity of 330 nm band decreased and the band at 450 nm due to the dimer TFT increased with increasing concentrations of DNA. Fig. 10 shows the three dimensional emission spectra recorded for TFT with DNA containing 1.5 mol dm^{-3} of NaCl. In the presence of NaCl the new contour observed for the emittive TFT dimer decreased to a large extent due to the elimination of the electrostatic interaction between the TFT and DNA thereby eliminating the formation of the dimer TFT.

4. Conclusion

The present study shows both groove binding and electrostatic interactions between TFT and DNA. The electrostatic interaction between TFT and DNA facilitates the formation of emittive TFT dimer. The addition of NaCl to TFT–DNA solution eliminates the electrostatic interaction between the TFT and DNA. The excitation and three-dimensional emission spectral studies of TFT–DNA system confirm the formation of emittive TFT dimer. Two bands are observed in the excitation spectrum (330 and 450 nm) for TFT and the new contour observed at $\lambda_{ex} = 450$ nm and $\lambda_{em} = 485$ nm upon the addition of DNA is ascribed to the formation of emittive TFT dimer. In the presence of NaCl, the new contour is almost eliminated in the three dimensional spectra. The presence of NaCl eliminates the electrostatic interaction between the TFT and DNA. The electrostatic interaction between TFT and DNA is responsible for the formation of emittive dimer TFT.

Acknowledgements

R.R. acknowledges the financial support from the Department of Science and Technology (DST). M.I. acknowledges the Council of Scientific and Industrial Research (CSIR) for the student fellowship.

References

[1] A. Felgner, G.M. Ringold, Nature 337 (1989) 387.
[2] N. Ghoneim, J. Photochem. Photobiol. A: Chem. 60 (1991) 175.
[3] S. Mitra, R. Das, S. Mukherjee, J. Photochem. Photobiol A: Chem. 87 (1995) 225.
[4] D.V. Bent, E. Hayon, J. Am. Chem. Soc. 97 (1995) 2612.
[5] M.M. Tirado, J.G. Garcia de la torre, J. Chem. Phys. 71 (1979) 2581.
[6] M.E. Ferrari, V.A. Bloomfield, Macromolecules 25 (1992) 5266.
[7] H. Sugiyama, C. Lian, M. Isomura, I. Saito, A.H.-J. Wang, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 14405.
[8] A. Wolfe, G.H. Slimer Jr., T. Meehan, Biochemistry 26 (1987) 6392.
[9] V.Ya. Shafirovich, A. Dourandin, N.P. Luneva, N.E. Geacintor, J. Phys. Chem. B 101 (1997) 5863.
[10] D.S. Sigman, Acc. Chem. Res. 19 (1986) 180.
[11] G.D. Reid, D.J. Whittaker, M.A. Day, D.A. Turton, V. Kayser, J.M. Kelly, G.S. Beddard, J. Am. Chem. Soc. 124 (2002) 5518.
[12] G.D. Reid, D.J. Whittaker, M.A. Day, C.M. Creely, E.M. Tuite, J.M. Kelly, G.S. Beddard, J. Am. Chem. Soc. 123 (2001) 6953.
[13] S.C. Weatherly, I. Yang, H.H. Thorp, J. Am. Chem. Soc. 123 (2001) 1236.
[14] M.F. Sistare, S.J. Codden, G. Heimlich, H.H. Thorp, J. Am. Chem. Soc. 122 (2000) 4742.
[15] A.I. Kononov, E.B. Moroshkina, N.V. Tkachenko, H. Lemmetyinen, J. Phys. Chem. 105 (2001) 535.
[16] B. Armitage, Chem. Rev. 98 (1998) 1171.
[17] G.B. Schuster, Acc. Chem. Res. 33 (2000) 253.
[18] H. Hidaka, S. Horikoshi, N. Serpone, J. Knowland, J. Photochem. Photobiol. A: Chem. 111 (1997) 205.
[19] S. Waldrop, H. Puchtler, S.N. Meloan, J. Histotechnol. 7 (1984) 123.
[20] R. Shirra, Chem. Phys. Lett. 119 (1985) 463.
[21] A. Parker, T.A. Joyce, Photochem. Photobiol. 18 (1973) 467.
[22] R.B. Cundall, A.K. Davies, P.G. Morris, J. Williams, J. Photochemistry 17 (1981) 369.
[23] J. Kalfter, J.M. Drake, Molecular Dynamics in Restricted Geometries, Wiley, New York, 1989.
[24] V. Ramamurthy, Photochemistry in Organized, Constrained Media, VCH, New York, 1991.
[25] J.B. Birks, Organic Molecular Photophysics, vol. 1, Wiley, New York, 1975.
[26] C. Retna Raj, R. Ramaraj, J. Photochem. Photobiol. A: Chem. 122 (1999) 39.
[27] C. Retna Raj, R. Ramaraj, Photochem. Photobiol. 74 (2001) 752.
[28] T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1982, p. 458.
[29] J. Marmur, J. Mol. Biol. 3 (1961) 208.
[30] M.E. Reichmann, S.A. Rice, C.A. Thomas, P. Doty, J. Am. Chem. Soc. 76 (1954) 3047.
[31] E.C. Long, J.K. Barton, Acc. Chem. Res. 23 (1990) 273.
[32] S. Hamat, Bull. Chem. Soc. Jpn. 55 (1982) 2721.
[33] Y. Baba, C.L. Beatty, A.C. Gebelein Jr. (Eds.), Proceedings of the ACS Symposium Series 186 on Biological Activity of Polymers, American Chemical Society, Washington, DC, 1982, Chapter 14, pp. 177–189.
[34] M.J. Waring, J. Mol. Biol. 13 (1965) 269.
[35] J.-B. Lepecq, C. Paoletti, J. Mol. Biol. 27 (1967) 87.
[36] A.M. Brun, A. Harriman, J. Am. Chem. Soc. 113 (1991) 8153.
[37] A.M. Brun, A. Harriman, J. Am. Chem. Soc. 114 (1992) 3656.
[38] G. Prativel, J. Bermadou, B. Meunier, Angew. Chem. Int. Ed. Engl. 34 (1995) 746.
[39] A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro, J.K. Barton, J. Am. Chem. Soc. 111 (1989) 3051.
[40] R. Tamilarasan, D.R. Mc Millin, Inorg. Chem. 29 (1990) 2798.
[41] M.J. Waring, Ann. Rev. Biochem. 50 (1981) 159.
[42] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum Press, New York, 1983 (Chapter 9).
[43] C. Retna Raj, R. Ramaraj, Chem. Phys. Lett. 273 (1997) 285.
[44] S. Nigam, G. Durocher, J. Phys. Chem. 100 (1996) 7135.
[45] S. Mahadevan, M. Palaniandavar, Inorg. Chem. 37 (1998) 693.
[46] T.B. Thederahn, M.D. Kuwabara, T.A. Larsen, D.S. Sigman, J. Am. Chem. Soc. 111 (1989) 4941.