J. Phys. Chem. A **1998,** *102,* 10579−10587

Transition Metal Ion Induced Fluorescence Enhancement of 4-(*N*,*N*-Dimethylethylenediamino)-7-nitrobenz-2-oxa-1,3-diazole

B. Ramachandram and A. Samanta*

School of Chemistry, University of Hyderabad, Hyderabad 500 046, India

Received: July 21, 1998; In Final Form: September 22, 1998

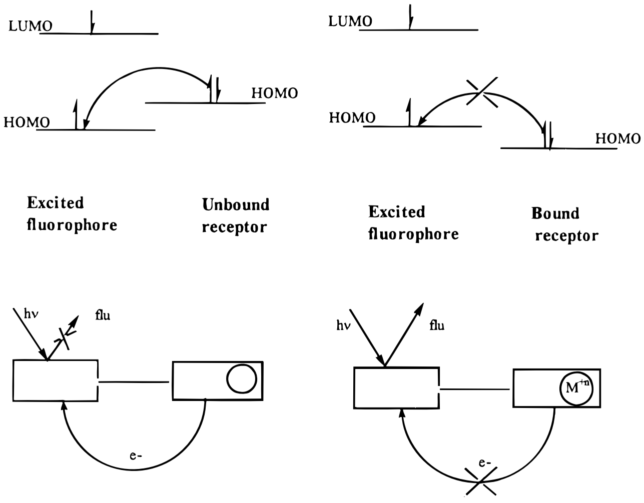
The photophysical properties of a structurally simple *fluorophore−spacer−receptor* supramolecular system involving nitrobenzoxadiazole (NBD) fluorophore, **NEA**, and its fluorescence response toward the *quenching* transition metal ions are reported. It is shown that efficient through-space intramolecular photoinduced electron transfer (PET) between the NBD fluorophore and the amino group, used here as a receptor for metal ions and protons, is responsible for extremely low fluorescence quantum yield and lifetime of **NEA** compared to the system **NAM** which contains the same fluorophore but does not possess the receptor moiety. The interaction between the NBD moiety and the metal ions in the ground and excited state is evident from the metal ion induced changes in the absorption spectra and fluorescence quenching of **NAM**. It is shown that despite a strong interaction between the **NBD** fluorophore and the quenching metal ions, **NEA** exhibits considerable *enhancement* of fluorescence rather than *quenching* in the presence of the transition metal ions. The results show that for an efficiently PET-quenched supramolecular system, the quenching influence of the transition metal ions is not important at moderate concentrations of the ions. This implies that efficient and structurally simple fluorosensors for the quenching metal ions can simply be developed by maximizing PET in the supramolecular systems without paying much attention to the quenching influence of the ions.

1. Introduction

Because of possible application as molecular switches or devices in information processing and computing devices, there is a great deal of current interest in molecular systems capable of performing light-induced logic operations.[1,2] A variety of molecular photonic devices which have been developed in recent years are supramolecular assemblies whose components perform specific operations.[2] The most commonly employed strategy in the design of photonic devices is to develop systems consisting of a photon interaction site (fluorophore) and a guest binding site (receptor).[2] These two components are usually linked by a spacer unit. In the absence of any guest, the interaction (photoinduced electron transfer (PET) is the most commonly exploited mechanism) between the receptor and the fluorophore results in quenching of the fluorescence. In the presence of the guests, the fluorophore−receptor communication gets turned off as a result of the binding of the guests at the receptor site and this leads to recovery of the original fluorescence. Thus, the presence of a guest is indicated by “switching on” of the fluorescence or fluorescence enhancement (FE). The design principle of the fluorosensors is illustrated in Scheme 1 with the help of a frontier orbital diagram.

The fluorosensors for the metal ions based on the PET mechanism commonly employ an amine as the receptor. While a variety of metal ions can be sensed by *fluorophore−spacer−receptor* systems employing simple amines as receptors, it is reported, however, that with such systems no significant FE is possible in the presence of the *transition metal ions* because of their quenching nature.[3] One needs to specially design a receptor for these quenching metal ions such that the metal ions in the bound condition are not accessible to the fluorophore for quenching. One such success has recently been reported where a cryptand receptor has been employed to trap the metal ions in the cavity so as to suppress the quenching influence of the metal ions.[4] Obviously, this approach demands considerable synthetic skill, and the systems developed by this approach are relatively complex. While investigating the fluorescence response of **APEA** and **ANPEA** (Chart 1) toward the transition metal ions[5] we realized that since the fluorophore−metal ion interaction is largely redox in nature, it is possible to reduce the quenching interaction by making the fluorophore electron deficient. With an electron-deficient fluorophore, the redox interaction between the fluorophore and the metal ion is not only minimized but also, such fluorophores, being better acceptors of electrons, enhance PET in the system which helps improve the performance of the system as a sensor. Using this

SCHEME 1

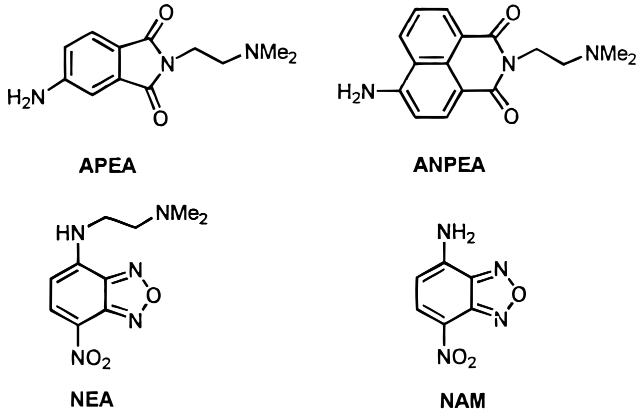


---

* Author to whom correspondence should be addressed. Fax: +91-40-3010120. E-mail: assc@uohyd.ernet.in.

10.1021/jp983106d CCC: $15.00

CHART 1



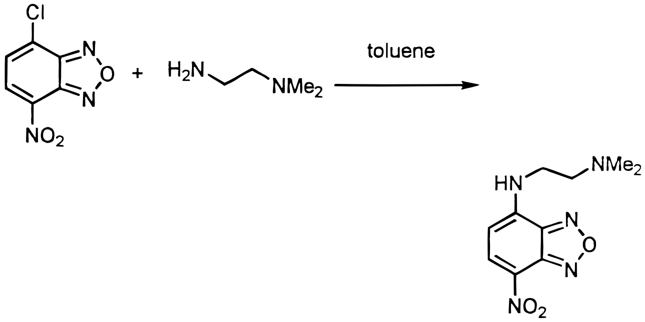
fluorophore selection criterion, we could develop structurally much simpler system such as **APEA** which exhibits considerable enhancement of fluorescence in the presence of the transition metal ions.[5] In this paper, we examine in detail the photophysical properties of a PET system involving NBD fluorophore, **NEA** (Chart 1), and examine its fluorescence response toward several quenching d-block metal ions. NBD derivatives find extensive use in biological applications.[6] NBD chloride[7] has often been used for labeling proteins with a fluorescent group and in studies of protein structure and conformational changes.[8,9] A large number of phospholipids and cholesterol analogues with an NBD group as fluorophore find application in the study of biological and model membranes.[10−12] The fluorescence quenching of the NBD moiety by transition metal ions has also been used in determining the location of the fluorophore in complex media.[13] Excited-state charge transfer and conformational change in some NBD derivatives have been recently investigated.[14] The fluorescence response of a few NBD derivatives toward different guests has been studied.[15−18] However, apart from one of our works, in which the preliminary results of the effect of Cr^{3+} on **NEA** was reported,[19] no other studies have come to our attention where the potential of the NBD derivatives in sensing the *quenching transition metal ions* has been examined. From a detailed study of the influence of the various d-block metal ions it is shown in the present paper that even though the NBD fluorophore interacts strongly with the quenching transition metal ions, **NEA** exhibits considerable FE in the presence of these metal ions. This observation clearly suggests that the quenching influence of the transition metal ions need not be given any special consideration while designing efficient PET fluorosensors.

2. Experimental Section

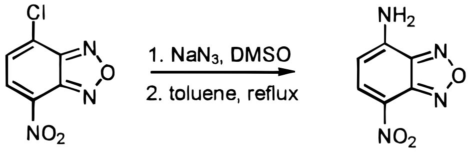
2.1 Materials. **NEA** was prepared according to the reaction scheme of Scheme 2. A 3 mM solution (in toluene) of *N*,*N*-dimethylethylenediamine was added dropwise to a toluene solution of 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (2.5 mM) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then for another 2 h at room temperature. The solid product obtained was purified by repeated crystallization from methanol. The structure of the compound was established by the following analytical data: ^{1}H NMR ($CDCl_3$)/δ 2.3 (s, 6H), 2.7 (t, 2H), 3.5 (t, 2H), 3.7 (s, 1H), 6.15 (d, 1H), and 8.5(d, 1H) IR (KBr) 3447, 3030, 2962, 1587, and 1332 cm^{-1}.

NAM was prepared from 4-chloro-7-nitrobenz-2-oxa-1,3-diazole by following a standard two-step procedure (Scheme 3).[20] The analytical data for the final product was found to be in accordance with its structure.

SCHEME 2



SCHEME 3



The following metal salts were used in the investigation: $Zn(H_2O)_6(ClO_4)_2$, $Cu(H_2O)_3(NO_3)_2$, $Ni(H_2O)_6(ClO_4)_2$, $Co(H_2O)_6(NO_3)_2$, $Fe(H_2O)_6(ClO_4)_3$, $Mn(H_2O)_6(ClO_4)_2$, $Cr(H_2O)_6Cl_3$, and $Co(H_2O)_6Cl_2$. The metal salts used in this study were procured locally and were used without any purification. However, for some specific measurements, a purification procedure was followed (discussed in the text). The anhydrous $CoCl_2$ was prepared from $Co(H_2O)_6Cl_2$ following a standard procedure[21] according to which the hydrated salt was refluxed in dimethoxy propane (Merck, India) for 6 h and subsequently the solvent was removed in a vacuum. The solvents, acetonitrile, tetrahydrofuran, and water, used in this investigation were rigorously purified by standard procedures.[22] The purified solvents were found free from impurities and were transparent in the spectral region of interest. Further, it was confirmed by the measurement of the $E_T(30)$ values of the solvents[23] that acetonitrile and tetrahydrofuran employed in this study for spectroscopic measurements were perfectly dry.

The Tris buffer solution was prepared by addition of an appropriate amount of 0.1 N HCl solution to an aqueous solution of Tris amine [tris(hydroxymethyl)aminomethane] so as to obtain a pH of 7.5.

2.2 Methods. The fluorescence quantum yields of the NBD derivatives were measured using coumarin 153 as the reference compound ($\phi_f = 0.89$ in acetonitrile).[24] A solution of the NBD derivative in tetrahydrofuran/acetonitrile was prepared with an absorbance (OD $= \sim 0.2$) the same as that of the reference compound at the exciting wavelength ($\lambda = 440$ nm). The fluorescence spectra were measured under the same operating conditions and settings, and the quantum yields were determined by comparing the areas underneath the fluorescence spectra by a “cut and weigh” method.

The fluorescence measurements were carried out by using $\sim 10^{-5}$ M solutions of the compounds. The effect of the metal ions on the fluorescence intensity was examined by adding a few microliters of the stock solution of the metal ions to a known volume of the solution of the NBD compound (3 mL). The addition was limited to 100 μL such that the volume change was not significant.

The voltammetric measurements were performed with a Cypres System computer-controlled electroanalytical setup, Model CS-1090. The experiments were carried out in a standard three-component cell which was equipped with a glassy carbon working electrode, a Pt wire as an auxiliary electrode, and a Ag/AgCl electrode as reference electrode. The cyclic voltammograms were recorded in N_2-bubbled acetonitrile containing ~0.1 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. The scan speed was 100 mV/s.

The absorption and the fluorescence spectra were recorded on Jasco UV−vis spectrophotometer (Model 7800) and Hitachi spectrofluorimeter (Model 4010), respectively. The fluorescence decay curves were recorded using an IBH single-photon counting spectrofluorimeter (Model 5000U). The instrument was operated using a thyratron-gated flash lamp filled with hydrogen at a pressure of 0.5 atm. The lamp was operated at a frequency of 40 kHz, and the pulse-width of the lamp under the operating condition was ~1.2 ns. The lifetimes were estimated from the measured fluorescence decay curves and the lamp profiles using a nonlinear least-squares iterative fitting procedure. The goodness of the fit was evaluated by the χ^2 values and the plot of the residuals. A fit was considered acceptable when the plot of the standard deviation was random about zero and the χ^2 value was not more than 1.2.

3. Results and Discussion

3.1. Spectral Properties. The absorption and fluorescence spectra of **NEA** in acetonitrile are shown in Figure 1. The lowest energy absorption band (λ_{max} at 459 nm, $\epsilon_{459} \approx 1.9 \times 10^4$ M^{-1} cm^{-1}) can be assigned to an intramolecular charge-transfer transition between the amino group at the 4-position and the nitro group at the 7-position on the basis of the solvatochromic response of the absorption band (λ_{max} at 455 nm in relatively nonpolar tetrahydrofuran), broad structureless feature of the band, and the literature data[14] on related NBD derivatives. The fluorescence spectrum of **NEA** is also structureless, typical of a charge-transfer transition. The fluorescence maxima of **NEA** have been observed at 523 nm in tetrahydrofuran and 529 nm in more polar acetonitrile. The measured absorption and fluorescence spectral data of **NEA** and **NAM** are collected in Table 1 along with the literature data of *n*-propylamino-NBD (**NPR**). It can be seen that the lowest energy absorption maxima of **NEA** and **NPR** are Stokes-shifted relative to that of **NAM** in any given solvent presumably due to the inductive influence of the alkylamino or alkyl group. Interestingly, however, the fluorescence maxima of **NEA** are slightly blue-shifted with respect to the corresponding maxima of the other two compounds in both tetrahydrofuran and acetonitrile. This small hypsochromic shift of the fluorescence band maximum of **NEA** relative to that of **NAM** or **NPR** is suggestive of a decrease in the charge separation in the fluorophore in the excited state. This can be due to a configurational change of the nitrogen atom attached to the ring in the excited state. A change in the pyramidalization of this nitrogen or a small twisting of the alkylamino group relative to the ring system that may be necessary for through-space overlap of the distal nitrogen lone pair orbital with the π-orbitals of the ring leading to intramolecular electron transfer in **NEA** (vide later) can lead to a decreased separation of charge in the NBD moiety and be responsible for the observed blue shift.

3.2. Fluorescence Yield and Decay Behavior. NBD derivatives are usually highly fluorescent in non-hydrogen bonding solvents. The fluorescence quantum yields (ϕ_f) of **NPR** are reported to be 0.69 and 0.53 in tetrahydrofuran and acetonitrile, respectively.[14] While the measured ϕ_f values (± 10%) of **NAM** are 0.9 and 0.7 in tetrahydrofuran and acetonitrile, respectively, those of **NEA** are lower by a factor of nearly 100. This unusually low fluorescence yield of **NEA** compared to **NAM** is clearly due to the receptor (amino group)-induced PET quenching of fluorescence.

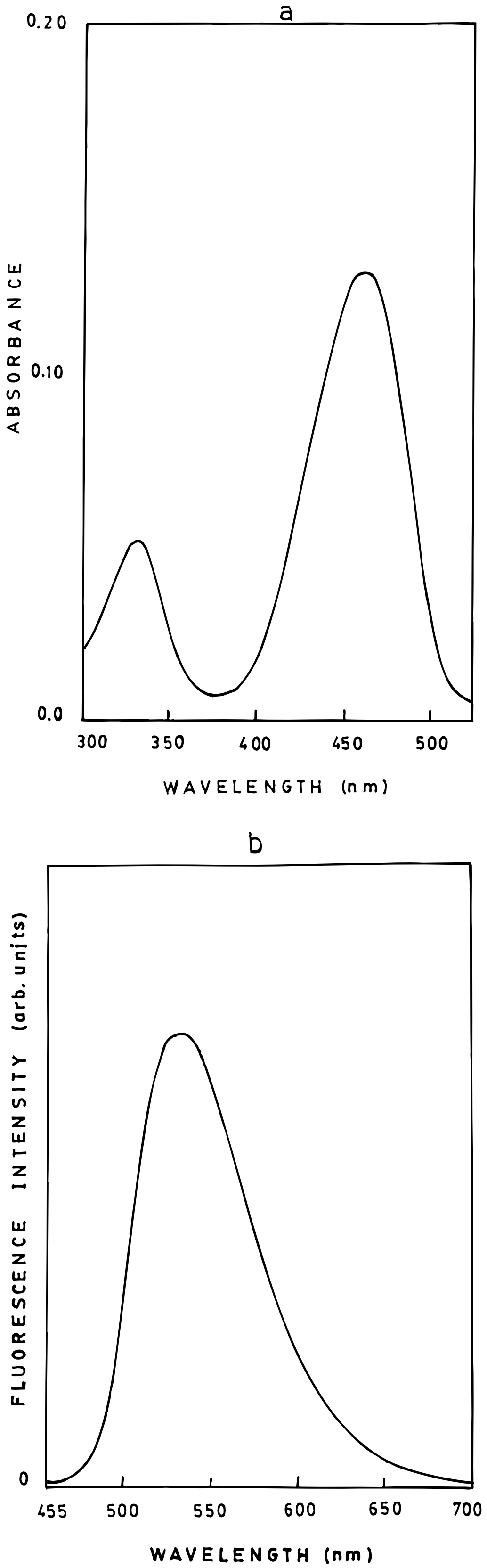


Figure 1. The absorption (a) and fluorescence (b) spectra of **NEA** in acetonitrile. The excitation wavelength for the measurement of the fluorescence spectrum was 440 nm.

TABLE 1: Absorption and Fluorescence Spectral Data of the NBD Derivatives in Acetonitrile and Tetrahydrofuran

	acetonitrile		tetrahydrofuran	
compound	$\lambda_{\max}^{\text{abs}}$	$\lambda_{\max}^{\text{flu}}$	$\lambda_{\max}^{\text{abs}}$	$\lambda_{\max}^{\text{flu}}$
NAM	447	532	445	525
NEA	459	529	455	522
NPR[a]	462	534	457	525

[a] The spectral data for this compound have been collected from ref 14.

That the photoinduced electron transfer between the dimethylamino group and the NBD fluorophore is thermodynamically feasible is evident from the spectral and electrochemical data. The reduction potential of the fluorophore, E_{red} (fluorophore) is measured to be −0.91 V (reversible) while the oxidation of the fluorophore is observed at around 1.59 V (irreversible). The singlet-state energy of the fluorophore ($E_{0,0}$), estimated from the mean position of the absorption and fluorescence maxima, is 58 kcal/mol. Therefore, the free energy change for the PET process in the fluorescent state (ΔG^*) is −26.2 kcal/mol in acetonitrile according to $\Delta G^* = 23.06[E_{\text{ox}}$ (amine) $- E_{\text{red}}$ (fluorophore)] $- E_{0,0}$. In this calculation, the literature value of the oxidation potential of triethylamine (0.49 V, literature SCE value[25] corrected for Ag−AgCl electrode by subtracting 0.27 V) has been used as E_{ox} (amine).

The fluorescence decay behavior of **NEA** further confirms PET-induced fluorescence quenching. While the fluorescence decay of **NAM** is single-exponential with a lifetime of 11.7 and 11.4 ns in tetrahydrofuran and acetonitrile, respectively, the decay behavior of **NEA** is clearly biexponential in both solvents (Figure 2). The fluorescence decay curves of **NEA** consisted of a short-lived major component (∼0.2 ns, 93−98%) and a minor long-lived component with lifetime ranging between 8.1 and 10.4 ns. The predominant short-lived component clearly represents the lifetime of the PET-quenched fluorophore. The long-lived minor component can in principle arise from the products of the electron-transfer reactions such as an exciplex. However, since no second component could be visible in the fluorescence spectrum of **NEA** (which is very similar to that of **NAM**) and the fact that the long-lived component in the fluorescence decay of **NEA** is also present in the polar media such as in acetonitrile (where exciplexes are quite unstable), it is quite clear that this component cannot be due to an exciplex, formed as a result of PET. The biexponential nature of the fluorescence decay of **NEA** can be explained only if a through-space electron-transfer mechanism involving the overlap of the lone pair orbital of the distal nitrogen and the π-orbitals of the ring is considered. The long-lived component arises from species in which the donor and acceptor groups are well-separated, while the short-lived component represents the quenched fluorophore in close proximity with the donor amine. A through-space electron transfer leading to a biexponential decay behavior has also been observed with other flexible donor−acceptor systems such as in **APEA**.[5]

3.3 Effect of the Metal Ions. *3.3.1 Absorption Spectra.* It is generally believed that even though the metal ion binding with the receptor cuts off the PET communication between the fluorophore and the amine receptor, the FE could not be observed in the presence of transition metal ions because of the quenching interaction between the fluorophore and the metal ions.[3] To find out whether the fluorophore interacts with the metal ions in the ground state, we have studied the changes in the absorption spectrum of **NEA** as a function of the metal ion concentrations. A parallel investigation has also been carried out on **NAM**, which does not contain the receptor unit.

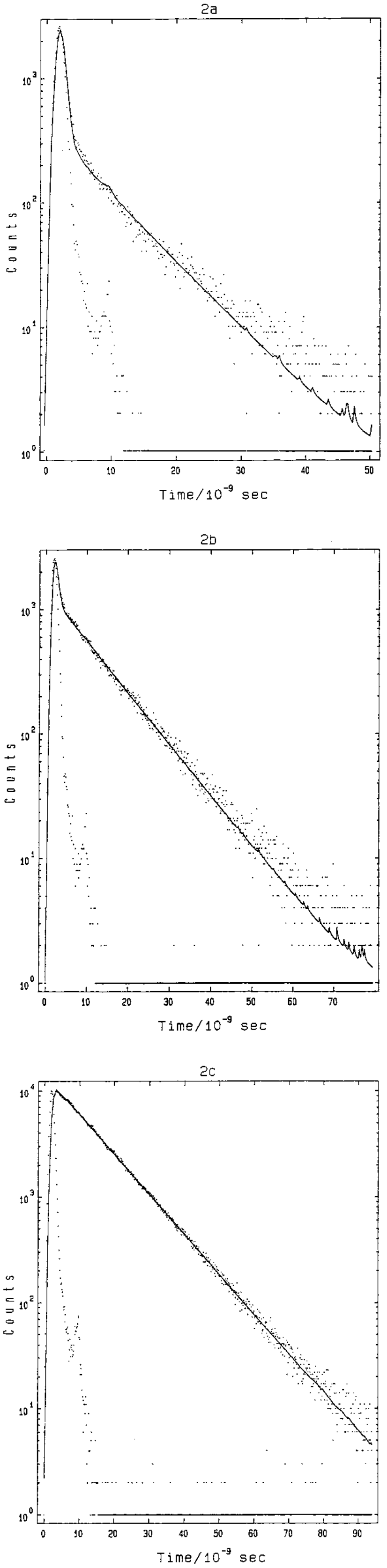


Figure 2. The fluorescence decay curves of **NEA** in THF (a) and acetonitrile (b) and that of **NAM** in acetonitrile (c). The excitation wavelength was 440 nm. Solid lines indicate the fit to the measured decay curves. The decay curves for **NEA** were fitted to a biexponential decay function while that for **NAM** was analyzed using a single-exponential decay function. The exciting lamp profiles are also shown in the figure. The fluorescence was monitored at 530 nm.

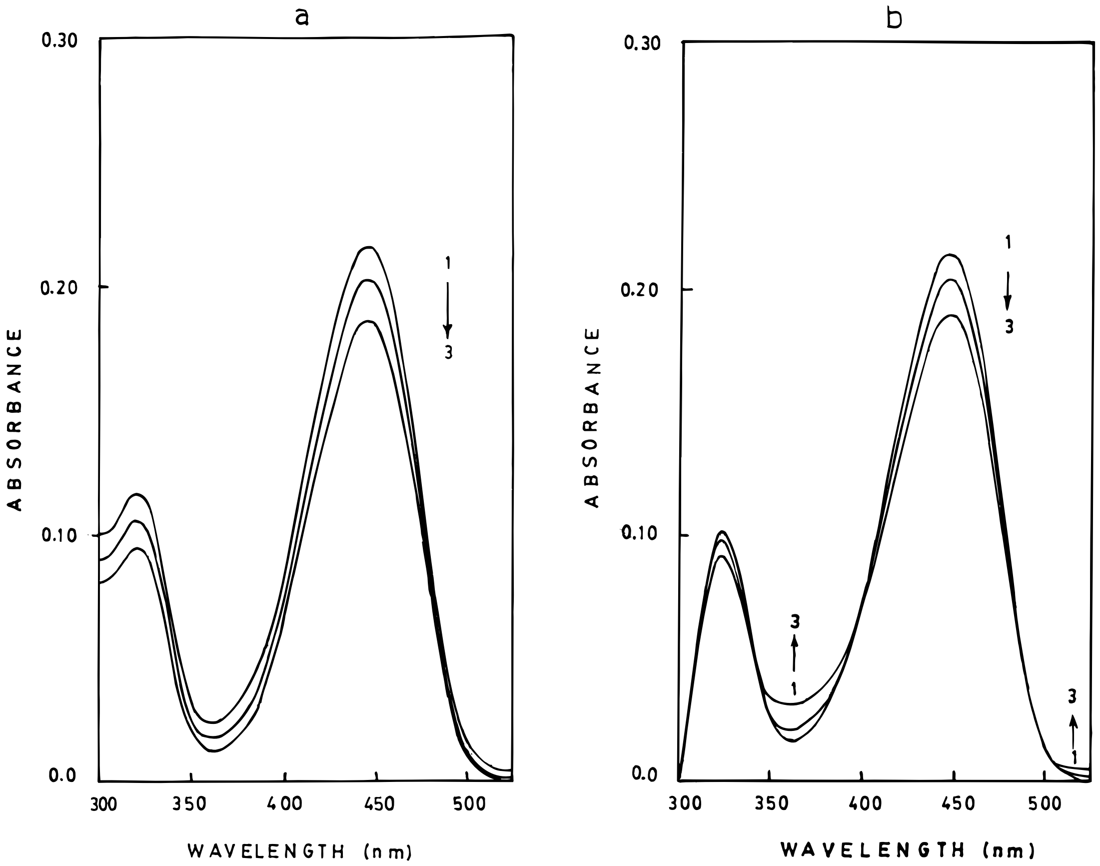


Figure 3. The changes in the absorption spectrum of **NAM** on addition of different amounts of $Zn(H_2O)_6(ClO_4)_2$ (a) and $Ni(H_2O)_6(ClO_4)_2$ (b) in acetonitrile. The concentrations of the Zn^{2+} are (1) 0, (2) 1.6×10^{-3}, and (3) 4.0×10^{-3} M. The concentrations of Ni^{2+} were (1) 0, (2) 1.15×10^{-3}, and (3) 2.87×10^{-3} M.

Figure 3 illustrates the effect of the addition of some of the metal ions on the absorption spectrum of **NAM**. It may be noted that an isosbestic point was observed in the case of Cr^{3+}.[19] Even though a similar observation has been made in the presence of Ni^{2+}, in most other cases no isosbestic point could be observed (Figure 3). The absence of any common pattern of the spectral changes is a reflection of varied coordinating abilities of the transition metal ions and the stabilities of their complexes.[26] Since the coordination of the metal ions by a monodentate or bidentate ligand is a stepwise process with complexes of different stoichiometries expected to be present in the solution, one should not expect the isosbestic point over a large concentration range of the metal ions. However, an isosbestic point can be observed when only one of the various possible equilibria is dominant. It is important to note here that since the fluorophore concentration used in the measurements is rather low ($\sim 10^{-5}$ M), the concentration of the higher complexes would be too low and it may not always be possible to detect them spectrophotometrically under the present experimental condition.

Figure 4 depicts the effect of the metal ions on the absorption spectrum of **NEA**. Unlike in the previous case, the addition of the metal salts to **NEA** leads to a significant blue shift of the absorption maximum. The spectral shift for the various metal ions ranges between 9 and 16 nm in acetonitrile. The charge transfer bands are generally sensitive to small changes in the polarity of the medium. Since the salts used in the experiments contain water of crystallization, one might expect an increase in the local polarity around the fluorophore (on the addition of the salts) is responsible for the shift. However, for the NBD derivatives, this would have resulted in a red shift of the maximum. Further, since no shift could be observed on addition of these metal salts to **NAM**, which contains the same fluorophore, the spectral changes must be ascribed to the binding of the metal ions with the fluorophore. If the distal dimethylamino receptor moiety alone gets bound to the metal ions (assuming **NEA** to act as a monodentate ligand), the energy states of the fluorophore are not expected to be affected as the receptor is connected to the fluorophore by a nonconjugating spacer and there is no evidence of through-bond transfer of charge. However, when the amino nitrogen atom (4-N) is involved in the coordination with the metal ions along with the distal receptor nitrogen, giving rise to a stable 5-membered chelate (Chart 2), the electron density available at 4-N for charge separation within the fluorophore decreases considerably leading to a blue shift of the spectral maxima. This interpretation of the blue shift of the spectra is in accordance with the general principles outlined by Valeur [2g,27−29] and Lapouyade and Rettig.[30−32]

3.3.2 Fluorescence Spectra and Intensity. The addition of the transition metal ions to **NAM** leads to expected fluorescence quenching with no noticeable change in the shape of the spectrum (Figure 5). The quenching constants have been evaluated from a quantitative analysis of the fluorescence intensity data according to the Stern−Volmer equation, $I_0/I = 1 + k_q\tau^0[Q]$, where I_0 and I are the fluorescence intensities in the absence and in the presence of the metal ions, respectively, and [Q] is the quencher concentration. τ^0 is the fluorescence lifetime of **NAM** in the absence of the quencher. A representative Stern−Volmer plot is shown in Figure 6, and the quenching constants are collected in Table 2. As seen from Table 2 the quenching is pronounced in the case of Cr^{3+}, Co^{2+}, and Cu^{2+},

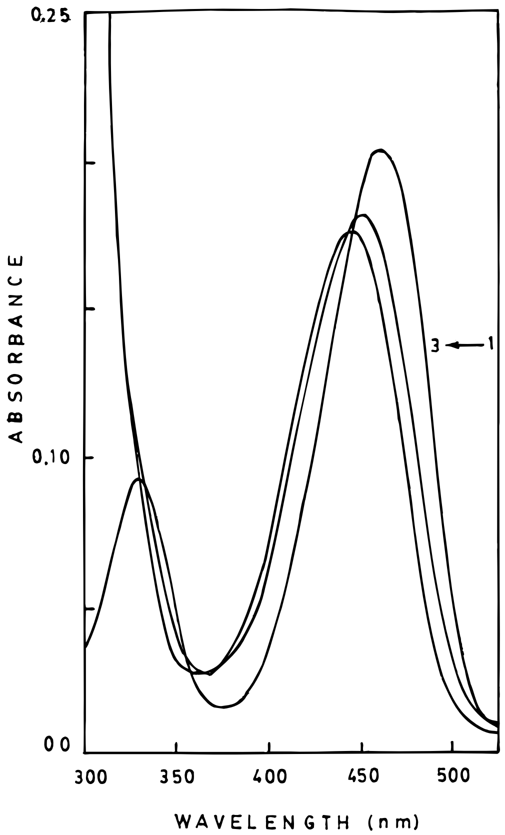
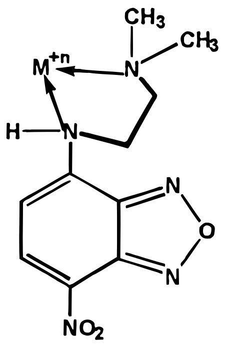


Figure 4. The effect of the addition of $Mn(H_2O)_6(ClO_4)_2$ on the absorption spectrum of **NEA** in acetonitrile. The various concentrations of the metal ions are (1) 0, (2) 3.6×10^{-5}, and (3) 1.1×10^{-4} M.

CHART 2



and the minimum quenching is observed with Zn^{2+}. The observed quenching efficiencies of the metal ions are found to be quite similar to those reported with other fluorophores,[33,34] Fe^{3+} is the only exception. Since the observed quenching is the result of the ground-state interaction (evident from the absorption spectral changes) and dynamic interaction in the excited state, we have studied the variation of the fluorescence lifetime of **NAM** as a function of the metal ion concentration to extract the quenching constant that is solely a measure of the excited-state interaction between the metal ions and the fluorophore. The contribution of the inner filter effect arising from the absorption of exciting light by the colored metal salts appears to be negligible as the extinction coefficients of the metal salts (less than 100 M^{-1} cm^{-1}) at the exciting wavelength (440 nm) were measured to be much lower than that of the fluorophore (~10400 M^{-1} cm^{-1}).

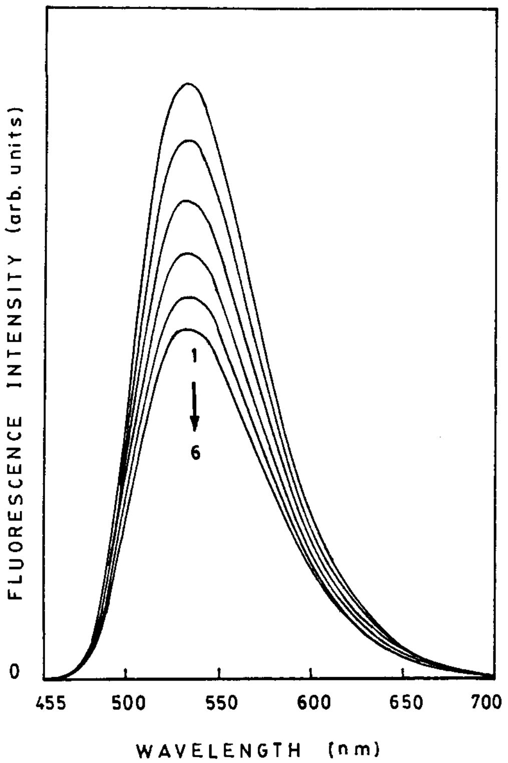


Figure 5. The fluorescence spectra of **NAM** in acetonitrile in the presence of $Co(NO_3)_2(H_2O)_6$. The concentrations of the metal ions are (1) 0, (2) 4.97×10^{-4}, (3) 9.95×10^{-4}, (4) 1.5×10^{-3}, (5) 2.0×10^{-3}, and (6) 2.5×10^{-3} M. The solution was excited at 440 nm.

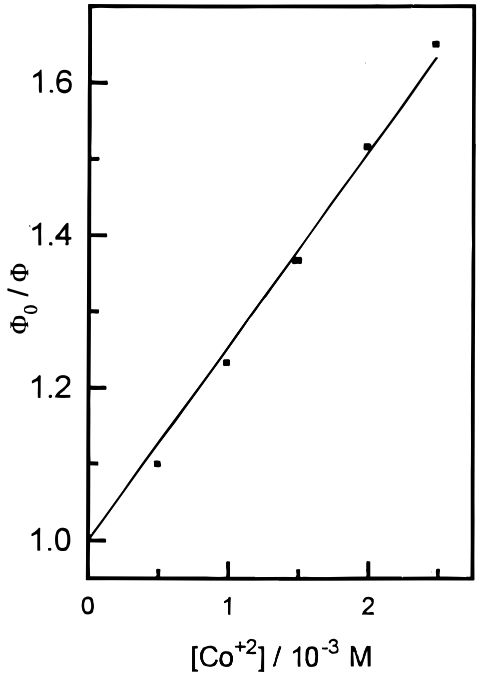


Figure 6. Stern−Volmer plot for the quenching of the fluorescence intensity of **NAM** by $Co(NO_3)_2(H_2O)_6$ in acetonitrile.

A typical plot based on the equation, $\tau_0/\tau = 1 + k_q\tau^0[Q]$ is shown in Figure 7 and the quenching constants obtained from the lifetime data are collected in Table 2. As can be seen, the dynamic quenching constants are significantly lower than the overall quenching constants; however, the quenching trend remains almost the same.

That the NBD moiety interacts with the transition metal ions both in the ground and excited state with quenching constants in many cases close to the diffusion-limited values is clearly evident from the above data. Therefore, it might appear that the choice of the NBD moiety as the fluorophore component

TABLE 2: Quenching Constants Obtained from the Steady-State and Time-Resolved Measurements on NAM

	acetonitrile				tetrahydrofuran			
metal ion	K_{SV}/M^{-1} (overall)	K_{SV}/M^{-1} (dynamic)	$k_q{}^a/10^9$ M^{-1} s^{-1} (overall)	$k_q{}^a/10^9$ M^{-1} s^{-1} (dynamic)	K_{SV}/M^{-1} (overall)	K_{SV}/M^{-1} (dynamic)	$k_q{}^b/10^9$ M^{-1} s^{-1} (overall)	$k_q{}^b/10^9$ M^{-1} s^{-1} (dynamic)
Zn^{2+}	31	10.6	2.74	0.9	70	3.3	6.0	0.3
Cu^{2+}	220	107	19.3	9.4	201	56.3	17.2	4.9
Ni^{2+}	130	76	11.4	6.6	122	23.2	10.5	2.0
Co^{2+}	255	142	22.4	12.4	268	115	23.0	10.0
Fe^{3+}	129	21.4	11.2	1.9	399	82	34.2	7.0
Mn^{2+}	60	10.8	5.25	0.9	59	5.9	5.1	0.5
Cr^{3+}	244	112	21.4	9.8	253	76.2	21.7	6.6

[a] $\tau_0 = 11.4$ ns. [b] $\tau_0 = 11.6$ ns. The excitation wavelength was 440 nm.

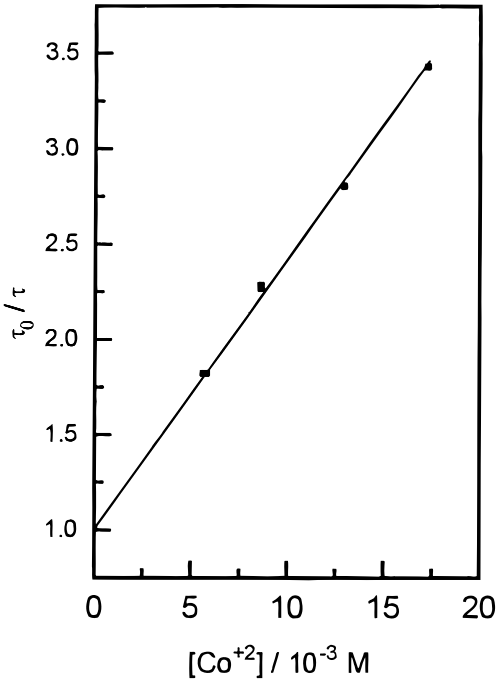


Figure 7. A plot of τ_0/τ of **NAM** versus the concentration of the $Co(NO_3)_2(H_2O)_6$ in acetonitrile.

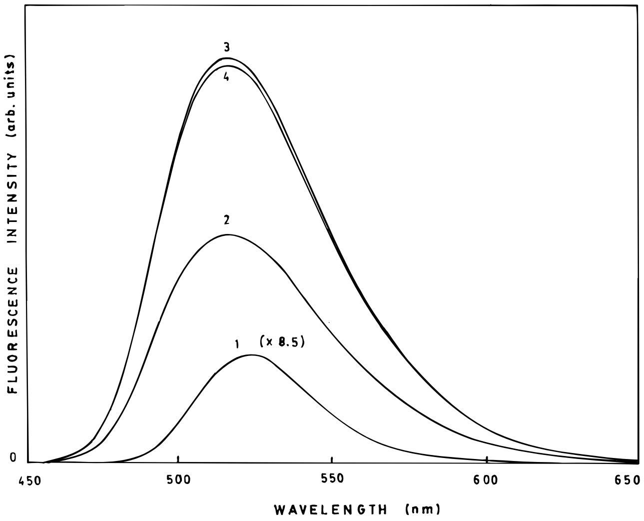


Figure 8. The fluorescence spectra of **NEA** in acetonitrile in the presence of $Co(NO_3)_2(H_2O)_6$. The various concentrations of the metal ions are (1) 0, (2) 1.3×10^{-4}, (3) 1.2×10^{-3}, and (4) 1.67×10^{-3} M. The excitation wavelength was 440 nm.

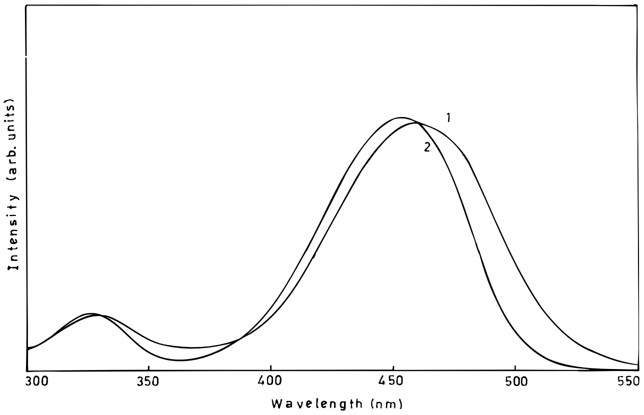


Figure 9. The fluorescence excitation spectra of **NEA** in acetonitrile in the absence (1) and in the presence (2) of 1.67×10^{-3} M of $Co(NO_3)_2(H_2O)_6$. The fluorescence was monitored at 575 nm.

of the supramolecular system, **NEA**, is not appropriate for the quenching metal ion sensing applications. Interestingly, however, **NEA** exhibits excellent fluorescence enhancement in the presence of all of the transition metal ions studied. Figure 8 illustrates the effect of the addition of a metal ion on the fluorescence spectra of **NEA**. The enhancement is associated with a blue shift (ranging from 3 to 10 nm) of the spectra. The fluorescence spectral shift is found to be less pronounced than that observed for the absorption spectrum for any given metal ion. This could be rationalized by taking into consideration the fact that the excited-state dipole moment of the NBD fluorophore is higher than the ground-state dipole moment. Since on electronic excitation, the charge density at 4-N is decreased, a weakening of the metal ion binding with the fluorophore takes place in the excited state which is responsible for a smaller shift of the fluorescence maximum. In this context, it is to be noted that using picosecond pump−probe technique, Valeur and coworkers have demonstrated earlier that a metal ion bound to a donor−acceptor fluoroionophore can be ejected on electronic excitation because of weakening of the binding resulting from a decreased electron density available at the coordinating site of the fluorophore in the excited state.[27] The fluorescence excitation spectra obtained in the presence of the metal ions also display a blue shift of a magnitude similar to that observed for the absorption spectra (Figure 9). In addition to the shift of the spectral maxima that is observed, metal ion addition is associated with a gradual increase in the fluorescence intensity until it reaches a limiting value. It can be seen that the maximum FE values observed with the metal ions, collected in Table 3, are quite high. While poor quenchers such as Zn^{2+} and Mn^{2+} show highest enhancement, as expected, it is interesting to note that some of the efficient quenchers also show excellent enhancement.

The changes in the fluorescence decay behavior of **NEA** on the addition of the metal ions are illustrated in Figure 10. It can be seen that with the addition of the salts the short-lived PET-quenched component is found to disappear gradually and at a certain concentration of the metal ion, the decay becomes

TABLE 3: The Fluorescence Enhancement (FE) Values of NEA in the Presence of Different Metal Ions in Acetonitrile and Tetrahydrofuran

	acetonitrile		tetrahydrofuran	
metal ion	concentration[b]	FE	concentration[b]	FE
Zn^{2+}	6.8×10^{-5}	113	1.09×10^{-3}	295
Cu^{2+}	8.0×10^{-5}	43	3.47×10^{-3}	113
Ni^{2+}	6.3×10^{-3}	70	6.34×10^{-4}	247
Co^{2+}	1.2×10^{-3}	48	4.39×10^{-3}	81
Fe^{3+}	3.4×10^{-5}	113	2.85×10^{-5}	262
Mn^{2+}	8.6×10^{-4}	123	1.36×10^{-3}	258
Cr^{3+}	9.1×10^{-5}	70[a]	4.5×10^{-4}	289

[a] From ref 19. [b] The concentration of the metal ions represents the concentrations for which the FE values were maximum. A further increase in the concentration led to quenching.

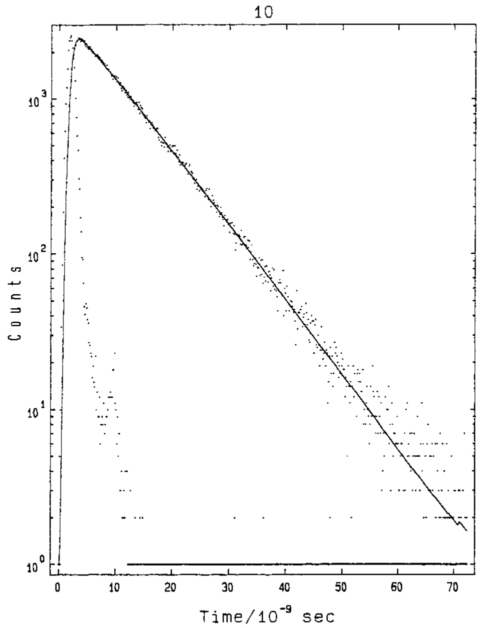


Figure 10. The fluorescence decay curve of **NEA** in acetonitrile in the presence of 2.48×10^{-3} M of $Co(NO_3)_2(H_2O)_6$. Shown also in the figure are the exciting lamp profile and the single-exponential fit to the fluorescence decay. The fluorescence was monitored at 530 nm.

single-exponential with a lifetime very similar to that of **NAM**. This situation corresponds to complete recovery of the fluorescence. A further addition of the metal ions, however, leads to expected quenching of the fluorescence lifetime.

The observation of significantly high FE in the presence of the quenching metal ions may appear quite unusual, particularly when the metal ion-fluorophore quenching interaction is taken into consideration. Since the experiments have been carried out with the hydrated salts of the metals which may be contaminated with protons (resulting from partial hydrolysis of the salts), one can argue that the enhancement may result from the protonation of the dimethylamino group. In fact, we have observed that addition of protons also gives rise to considerable enhancement of the fluorescence signal. However, the possibility of the contaminated protons giving rise to FE has been discounted by some specific experiments. First, in most cases, salts that have been recrystallized several times showed FE not very different from those exhibited by nonrecrystallized salts. Second, one could hardly observe any difference in the FE values with a hydrated salt of cobalt, $[Co(H_2O)_6]Cl_2$, or its anhydrous salt prepared by following a standard procedure described in the Experimental Section. Third, in aqueous buffered solution of pH 7.5 (Tris-buffered solution) when a majority of the receptor sites are protonated we could still observe 1.3-fold FE in the presence of Cu^{2+}. Since the fluorophore itself undergoes deprotonation at pH higher than 8, no experiment could be performed at higher pH required for this experiment.[35]

We now attempt to rationalize whether it is possible to observe FE in the presence of the quenching ions. In the presence of the metal ions, the relative contribution of two opposing factors, the metal ion-receptor binding that leads to enhancement of the fluorescence signal and the fluorophore–metal ion interaction that leads to fluorescence quenching, determines the net effect. It is therefore possible to observe FE in the presence of the quenching ions when the metal ion binding-induced enhancement of fluorescence intensity is greater than the quenching-induced reduction in the fluorescence intensity. We note that even though the metal ions quench **NAM** fluorescence quite efficiently, they are not expected to be as efficient quenchers for **NEA** that contains the same fluorophore. This is because of the difference in the lifetime of the fluorophore in the two systems. In the case of **NAM**, the fluorophore has a long lifetime of 11.4 ns in acetonitrile and 11.65 ns in tetrahydrofuran. On the other hand, the fluorescence lifetime of **NEA** is only ~0.2 ns. Since the lifetimes of the two compounds differ by a factor of ≈55, according to the Stern–Volmer equation, one needs 55-fold higher concentration of the metal ions in the case of **NEA** for identical reduction of the fluorescence intensity of the two compounds due to metal ion induced quenching (assuming the quenching constant to be same in both cases). Obviously, the quenching influence of the metal ions becomes a less important factor for a system whose lifetime is quenched because of PET. That the quenching influence of a given metal ion is not very important in the case of a PET-quenched fluorophore can also be shown in the following manner. According to our quenching data, Co^{2+} is the most efficient quencher of **NAM** with a k_q of $2.2-2.3 \times 10^{10}$ M^{-1} s^{-1}. Therefore, according to the Stern–Volmer equation, 1 mM concentration of Co^{2+} would quench the fluorescence intensity of **NAM** by 20.3–21.1%. On the other hand, the same amount of Co^{2+} would reduce the fluorescence intensity of **NEA** by a factor of only ~0.4%. It is therefore clear that for an already PET-quenched system, an otherwise strongly quenching metal ion acts as a poor quencher. In other words, the transition metal ions at moderate concentration can be treated as nonquenchers for an already PET-quenched system. Obviously, in the absence of quenching interaction, metal ion binding with the receptor will only lead to fluorescence enhancement.

4. Conclusion

We have shown here that it is possible for simple multicomponent systems to exhibit considerable fluorescence enhancement even in the presence of most quenching transition metal ions. What is really interesting is the observation that even when the metal ions interact with the fluorophore both in the ground and excited state, it is possible for the system to exhibit fluorescence enhancement rather than quenching. The results suggest that the simplest way to design an efficient fluorosensor for the quenching metal ion is to select the fluorophore and receptor components such that PET is maximized in the system. For an efficiently PET-quenched system, the transition metal ions can be treated as nonquenching metal ions.

Acknowledgment. This research is supported by the Department of Science and Technology, Government of India and

Council of Scientific and Industrial Research (CSIR). B.R. acknowledges the fellowship received from CSIR.

References and Notes

(1) (a) *Molecular Electronic Devices*; Carter, F. L., Siatkowski R. E., Wohltjen, H., Eds.; Elsevier: Amsterdam, 1988. (b) Lehn, J. M. *Angew. Chem., Int. Ed. Engl*. **1988**, 27, 89. (b) Lehn, J. M. *Angew. Chem., Int. Ed. Engl*. **1990**, 29, 1304. (d) Lehn, J. M. *Supramolecular Chemistry*; VCH: Weinheim, **1995**.

(2) Only leading review articles are cited here. (a) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev*. **1997**, 97, 1515. (b) Bissel, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; McCoy, C. P.; Sandanayake, K. R. A. S. *Top. Curr. Chem*. **1993**, 168, 223. (c) Bissel, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; Sandanayake, K. R. A. S. *Chem. Soc. Rev*. **1992**, 21, 187. (d) Czarnik, A. W. *Acc. Chem. Res*. **1994**, 27, 302. (e) *Fluorescent Chemosensors for Ions and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993. (f) Czarnik, A. W. In *Topics in Fluorescence Spectroscopy*; Lakowicz, J. R., Ed.; Plenum Press: New York, 1994; Vol IV, p 49. (g) Valeur, B. In *Topics in Fluorescence Spectroscopy*; Lakowicz, J. R., Ed.; Plenum Press: New York, 1994; Vol IV, p 21.

(3) One can see for example: ref 2(a), p 1546 and ref 2(b), p 251.

(4) Ghosh, P.; Bharadwaj, P.; Mandal, S.; Ghosh, S. *J. Am. Chem. Soc*. **1996**, 118, 1553.

(5) Ramachandram, B.; Samanta, A. *J. Chem. Soc., Chem. Commun.* **1997**, 1037.

(6) The application of the NBD derivatives has been recently reviewed, Chattopadhyay, A. *Chem. Phys. Lipids* **1990**, 53, 1.

(7) Ghosh, P. B.; Whitehouse, M. W. *Biochem. J.* **1968**, 108, 155.

(8) Fager, R. S.; Kutina, C. B.; Abrahamson, E. W. *Anal. Biochem.* **1973**, 53, 290.

(9) Ferguson, S. J.; Lloyd, W. J.; Radda, G. K. *Biochem. J.* **1976**, 159, 347.

(10) Longmuir, K. J.; Martin, O. C.; Pagano, R. E. *Chem. Phys. Lipids* **1985**, 36, 197.

(11) Schmidt, N.; Gercken, G. *Chem. Phys. Lipids* **1985**, 38, 309.

(12) Silvius, J. R.; Leventis, R.; Brown, P. M.; Zuckermann, M. *Biochemistry* **1987**, *26*, 4279.

(13) Chattopadhyay, A.; London, E. *Biochim. Biophys. Acta* **1988**, 938, 24.

(14) Fery-Forgues, S.; Fayet, J. P.; Lopez, A. *J. Photochem. Photobiol. A: Chem*. **1993**, 70, 229.

(15) Street, K. W., Jr; Krause, S. A. *Anal. Lett*. **1986**, 19, 735.

(16) Lytton, S. D.; Mester, B.; Libman, J.; Shanzer, A.; Cabantchik, Z. I. *Anal. Biochem*. **1992**, 205, 326.

(17) Lytton, S. D.; Cabantchik, Z. I.; Libman, J.; Shanzer, A. *Mol. Pharmacol*. **1991**, 40, 584.

(18) Frost, H.; Pacey, R. E. *Talanta* **1989**, 36, 355.

(19) Ramachandram, B.; Samanta, A. *Chem. Phys. Lett*. **1998**, *290*, 9.

(20) Boulton, A. J.; Ghosh, P. B.; Katritzky, A. R. *J. Chem. Soc. B* **1966**, 1004.

(21) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry: A Comprehensive Text*; Wiley-Interscience: New York, 1988, p 552.

(22) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon Press: New York, 1980.

(23) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*; VCH: Weinheim, **1988**.

(24) Rechthaler, K.; Köhler, G. *Chem. Phys.* **1994**, 189, 99.

(25) Siegerman, H. In *Techniques of Chemistry*; Weinberg, N. L., Ed.; Wiley: New York, 1975; Vol V, part II.

(26) Drago, R. S. *Physical Methods in Chemistry*; Saunders College Publishing: Philadelphia, **1977**.

(27) Martin, M. M.; Plaza, P.; Dai Hung, N.; Meyer, Y. H.; Bourson, J.; Valeur, B. *Chem. Phys. Lett*. **1993**, 202, 425.

(28) Martin, M. M.; Plaza, P.; Meyer, Y. H.; Begin, L.; Bourson, J.; Valeur, B. *J. Fluoresc*. **1994**, 4, 271.

(29) Martin, M. M.; Plaza, P.; Meyer, Y. H.; Badaoui, F.; Bourson, J.; Lefebvre, J. P.; Valeur, B. *J. Phys. Chem*. **1996**, 100, 6879.

(30) Letard, J.-F.; Lapouyade, R.; Rettig. W. *Pure Appl. Chem*. **1993**, 65, 1705.

(31) Dumon, P.; Jonasauskas, G.; Dupuy, F.; Pee, P.; Rulliere, C.; Letard, J.-F.; Lapouyade, R. *J. Phys. Chem*. **1994**, 98, 10391.

(32) Mathevet, R.; Jonasauskas, G.; Rulliere, C.; Letard, J.-F.; Lapouyade, R. *J. Phys. Chem*. **1995**, 99, 15709.

(33) Varnes, A. W.; Dodson, R. B.; Wehry, E. L. *J. Am. Chem. Soc*. **1972**, 94, 946.

(34) Kemlo, J. A.; Shepherd, T. M. *Chem. Phys. Lett.* **1977**, 47, 158.

(35) de Silva, A. P.; Gunaratne, H. Q. N.; Habib-Jiwan, J. L.; McCoy, C. P.; Rice, T. E.; Soumillion, J. P. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 1728.