

Electron-Transfer Quenching of the Singlet State of Protonated and Unprotonated Basic Dyes

Silmara F. Buchwieser, Fergus Gessner, Luiz C. Vetere and Miguel G. Neumann*

Instituto de Física e Química de São Carlos, Universidade de São Paulo, Caixa Postal 369, 13560 São Carlos, SP, Brasil

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A supressão da fluorescência dos corantes Tionina e Vermelho Neutro, nas suas formas protonadas e não-protonadas, por vários doadores de elétrons, foi estudada por fluorometria de estado estacionário. Os valores das constantes de velocidade dependem da energia livre de transferência do elétron, de acordo com a teoria de Rehm e Weller. A influência da carga do corante sobre a energia de reorganização do solvente, λ , foi menor que o esperado, possivelmente devido à sua deslocalização no sistema aromático. Também foram estimadas diferenças entre os pK_a dos estados singletes e das espécies radicalares semireduzidas correspondentes, que mostraram às mesmas tendências que outros sistemas de corantes excitados.

The fluorescence quenching of the protonated and unprotonated forms of Thionine and Neutral Red, by several electron donors has been studied by static fluorometry. The rate constants show a dependence on the electron transfer free energy as expected from Rehm and Weller theory. The charge on the dye was found to influence the solvent reorganization energy, λ , by less than expected, possibly due to its delocalization over the aromatic system. The differences between the pK_a of the singlet states and those corresponding to the semireduced radicals were estimated and found to follow the same trend as for other excited dye systems.

Key words: *Neutral red; thionine.*

Introduction

A large number of electron transfer reactions involving dyes have been studied^{1,2} since the development of the theory of Rehm and Weller³. The results for many of these reactions have been rationalized in terms of that theory using the classical Marcus⁴ expression for the rate constant of the electron transfer event, k_{23}

$$k_{23} = v \times \exp \left[-\frac{\lambda}{4RT} \left(1 + \frac{\Delta G_{23}}{\lambda} \right)^2 \right] \quad (1)$$

This equation states the influence of the electron transfer free energy ΔG_{23} and the solvent reorganization energy λ on the rate constant. The reorganization energy can be related to the activation free energy for an isoenergetic process $\Delta G^\ddagger(0)$, and involves two terms, one corresponding to solvent reorganization around the new species and the other to internal bond changes, the former being larger in most cases.

From the point of view of the energetics of the process differences in the electron transfer reactivity of protonated and unprotonated species can be related to the pK_a differences between the reactants and the products²

$$\Delta \Delta G^\ddagger = 2.3 RT (pK_a^R - pK_a^S) \quad (2)$$

In this sense, Traber *et al.*⁵ found that the difference in reactivity between the neutral and protonated forms of triplet flavin were shifted by 0.25 eV, which compares quite well with the value of 0.22 eV calculated using experimental pK_a values in Eq. 2.

Values of λ parameters for several systems were determined by adjusting the experimental $\log k$ vs. ΔG curves with the Rehm and Weller expression in Eq. 1. In general, values between 5 and 40 kcal/mol were obtained, depending mainly on the solvent and the charges of the species involved in the electron transfer.¹ As far as we know, no studies have been reported comparing the λ values for reactions involving electron acceptors (or donors) with similar structures, differing only by their charge. Basic dyes seem to be appropriate for this kind of study, as their structures remain practically the same when in their protonated and neutral forms.

Continuing our studies on the reactions of the excited states of basic dyes, (which include the photoreduction of Safranine⁷ and Methylene Blue⁷ by nitrogen compounds, energy transfer between the singlet states of the dyes,⁸ the behaviour of transients in the presence of polyelectrolytes, and, more recently, the comparison between electron-transfer quenching of the singlet and triplet states of Safranine⁹), we present results on the quenching of the excited singlet states of the protonated (single-charged) and unprotonated (neutral) forms of Neutral Red and Thionine.

Experimental

Both dyes, Thionine, ThH⁺, and Neutral Red, NRH⁺, were from Merck and recrystallized from methanol before use. The solid quenchers, p-chloroaniline, p-toluidine, p-anisidine, p-nitroaniline, 1,4-dimethoxynaphthalene (1,4-DMN), 1,4-dimethoxybenzene (1,4-DMB), 2-methoxynaphthalene (2-MN) and 1,3,5-trimethoxybenzene (1,3,5-TMB) were purified by sublimation. All the other quenchers were vacuum distilled before use.

All experiments were performed at 25±0.5°C in methanol at pH 7.0 and 13.5, where the dyes are in their protonated (charged) and unprotonated (uncharged) forms, as confirmed by the uv spectra. The pH were regulated with 0.02 M phthalate buffer solutions. Dye concentrations were kept at 1.2×10⁻⁵ M and the quenchers were changed between 0.0025 and 0.200 M, except for anisole, which was used up to 0.7 M.

Fluorescence quenching was determined on an Aminco-Bowman, mod. J4 spectrofluorimeter with a Hamamatsu R928 photomultiplier. Dyes were excited at 600 nm (ThH⁺), 514 nm (Th), 535 nm (NRH⁺), and 460 nm (NR), and the emission monitored at 610 nm (ThH⁺), 620 nm (Th), 610 nm (NRH⁺), and 590 nm (NR), as shown in Figs. 1 and 2.

Fluorescence lifetimes of the dyes were estimated from the integration of the visible (lowest energy) absorption band and the fluorescence quantum yields,¹⁰ determined from comparison with quinine sulfate¹¹ in 0.1 M H₂SO₄ and fluorescein¹² in 0.1 M NaOH.

The half-wave reduction potentials of the dyes were determined by square wave voltammetry, using a dropping

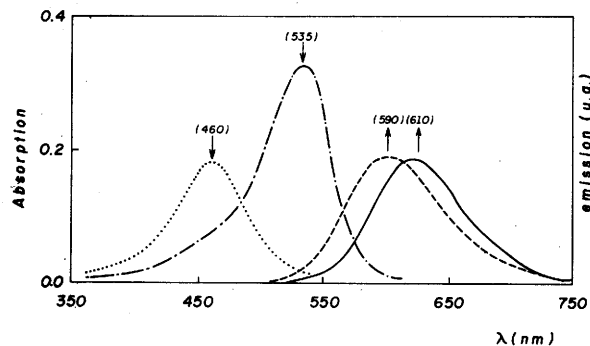


Figure 1. Absorption and emission spectra of Neutral Red in the protonated (pH 7.0) [abs - - - - ; em —] and unprotonated (pH 13.5) [abs ; em —] forms. Arrows indicate the excitation (↓) and emission detection wavelengths (↑).

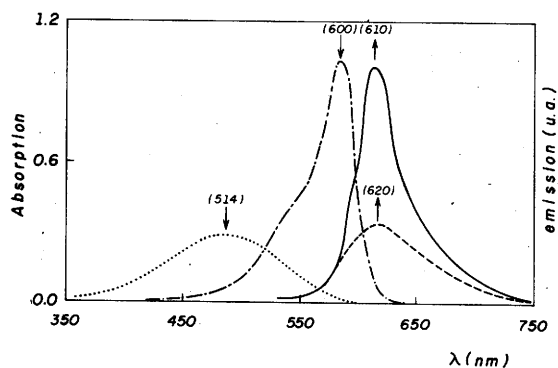


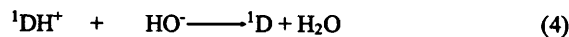
Figure 2. Absorption and emission spectra of Thionine in the protonated (pH 7.0) [abs - - - - ; em —] and unprotonated (pH 13.5) [abs ; em ----] forms. Arrows indicate the excitation (↓) and emission detection wavelengths (↑).

mercury electrode and a Ag⁺/AgCl reference electrode. The oxidation potentials of the aromatic amines were determined by cyclic voltammetry, using a vitreous carbon electrode and a Ag⁺/AgCl reference electrode. Measurements were done using a Parc mod. 384B polarograph and a Parc mod. 273 potentiostat-galvanostat.

Results

The rate constants for the quenching of the fluorescence emission of ThH⁺, Th, NRH⁺ and NR by several electron donating compounds are shown in Table I. For the experiments with the neutral dyes, care was taken to make sure that only the unprotonated species was present. This was attained by adjusting the pH to values where only the spectra corresponding to the neutral species were observed.

The observed quenching reaction rates were fast enough to make degassing of the solutions unnecessary. Quenching of the excited singlet species by protonation or deprotonation



can be ruled out due to the small concentrations of protons (<10⁻⁶M) and hydroxide ions (<10⁻⁴M) present in the working solutions. Even considering those reactions to be diffusion controlled, their rates will not compete with the quenching by the organic substrates,¹³ or even with spontaneous excited state decay (τ⁻¹).

The lifetimes used to calculate the experimental quenching rate constants from the Stern-Volmer constants, shown in Table 2, were determined from their radiative lifetimes (obtained from integration of the absorption band and the corresponding emission quantum yields). These values match quite well with those determined directly¹⁴ and provide reasonable values for the diffusional limit, except for neutral NR, for which the diffusional constant was too high by a factor of four. In this case, the lifetime was assumed to be that which gave a diffusional limit compatible with that obtained for the other cases.

Table 1. Rate constants for the fluorescence quenching of charged and neutral dyes by electron donors

Quencher	$E_{ox}^{O(a)}$ (V)	log k			
		NRH ⁺	NR	ThH ⁺	Th
p-anisidine	0.45*	10.39	10.63	10.39	10.42
p-toluidine	0.64*	10.43	10.61	10.34	10.39
m-anisidine	0.75*	10.36	10.53	10.37	10.32
m-toluidine	0.78*	10.34	10.50	10.28	10.34
p-chloroaniline	0.84*	10.43	10.45	10.48	10.28
Aniline	0.86*	10.44	10.35	10.40	10.37
m-chloroaniline	0.86*	10.32		10.41	10.26
m-nitroaniline	0.89 ^b	10.35	10.55		
1,4-DMA	1.06 ^b	10.47	10.60	10.49	10.20
1,4-DMA	1.30 ^b	10.49	9.83	10.37	10.28
1-MN	1.34 ^b	10.47	8.92	10.47	10.10
2-MN	1.38 ^b	10.53	10.05	10.40	10.28
1,3-DMB	1.45 ^b	9.72	9.51	10.11	9.73
1,3,5-TMB	1.45 ^b		8.44		9.98
Anisole	1.72 ^b		8.12	9.56	9.32
	$E_{red}^{O(a)}$ (V)	-0.394*	-0.570*	-0.042*	-0.310*

* Determined in this work

^a) Referred to Ag⁺/AgCl electrode^b) From references¹⁸

Table 2. Lifetimes and fluorescence quantum yields for charged and neutral Neutral Red and Thionine

	NRH ⁺	NR	ThH ⁺	Th
Φ	0.031		0.038 0.050*	0.022
τ (ns)	0.34	(0.51)*	0.35	0.31

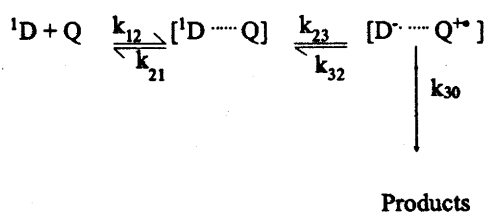
* Extrapolated value. See text.

*Reference 24

The quenching rate constants are plotted as a function of the reaction free energies ΔG_{23} in Figs. 3 and 4. These free energy changes were calculated from

$$\Delta G_{23} = \Delta G^\circ(Q/Q^+) - \Delta G^\circ(D/D^-) + \Delta\omega - E_{00} \quad (5)$$

where $\Delta G^\circ(Q/Q^+)$ and $\Delta G^\circ(D/D^-)$ are the oxidation and reduction free energies of the quenchers and the dyes, respectively, derived from the one-electron potentials (meas-



Scheme I

ured or from the literature, see Table 1); $\Delta\omega$ is the coulombic energy gained in the formation of the encounter complex; and E_{00} is the 0-0 energy of the singlet state of the dye.

The dependence shown in these figures is consistent with a quenching process involving electron transfer, as described below according to Rehm and Weller³.

where D and Q represent the dye and the quencher, respectively, the first species in brackets the encounter complex, and the second one the charge transfer complex; k_{12} and k_{21} are diffusion rate constants, k_{23} and k_{32} are the rate

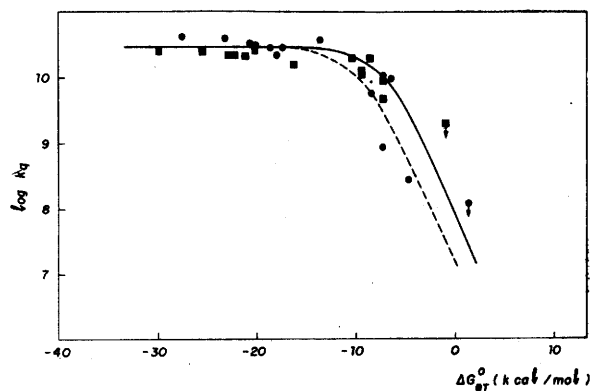


Figure 3. Rehm and Weller plot for the quenching of the fluorescence of the dyes (● NRH; ♦ ThH) in their protonated form by organic electron donors. The theoretical curves were calculated with eq. 1 using $l = 18$ and 24 kcal/mol.

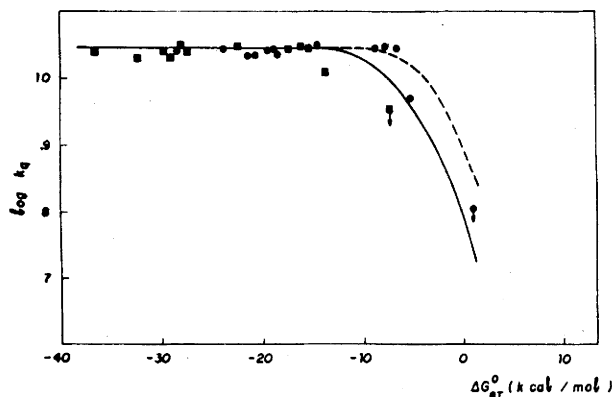


Figure 4. Rehm and Weller plot for the quenching of the fluorescence of the dyes (● NR; ♦ Th) in their unprotonated form by organic electron donors. The theoretical curves were calculated with eq. 1 using $l = 24$ and 28 kcal/mol.

constants for electron transfer and back-transfer, and k_{30} is the rate of formation of products or separation of the ions. For protonated dyes, the corresponding radical species in the charge transfer complex will be neutral.

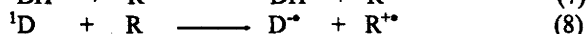
Discussion

The theoretical curves shown in Figs. 3 and 4 were calculated using the expression derived from Scheme I for the observed quenching rate constant k_q

$$\frac{1}{k_q} = \frac{1}{k_{12}} = \frac{1}{K_D \cdot k_{23}} \quad (6)$$

using $K_D = 2.0 \text{ M}^{-1}$ and $k_{12} = 3 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, and eq. 1 for k_{23} with $\nu_{23} = 10^{12} \text{ s}^{-1}$; values which are within what can be expected for these kinds of systems¹.

The effect of the charge of the dye molecule. When comparing the reactions of the protonated and unprotonated dyes, it should be noted that in the former case the electron transfer process will lead to a neutral dye radical and the cation radical of the reductant, whereas in the latter an anion radical and a cation radical will be formed:



Thus, a small solvent reorganization energy would be expected for reaction (7), where both the reactants and the products consist of a neutral and a cationic species. On the other hand, in reaction (8) two ion radicals are formed from two neutral species, implying a larger solvent reorganization energy.

According to theory, the λ parameters determined from Figs. 3 and 4 include two factors,

$$\lambda = \lambda_o + \lambda_i \quad (9)$$

namely, the solvent reorganization energy λ_o and an internal reorganization energy λ_i , related to the changes in structure associated with the electron transfer process. For the systems studied here, the molecular mainframe is quite

rigid, so that this contribution can be assumed to be negligible.

As can be seen from Figs. 3 and 4, there seems to be a small difference between the λ parameters which best adjust the experimental results for the protonated and unprotonated forms to the theoretical Rehm and Weller - Marcus equation. The best adjustment for the neutral dye species is obtained for λ values between 24 and 28 kcal/mol, whereas for the singly charged protonated dyes, it is somewhere between 18 and 24 kcal/mol. Due to the experimental errors involved in the determinations, it is quite difficult to ascertain exactly these parameters. In principle, from what has been discussed above, one might expect a larger difference between the λ parameters for these two cases. Nevertheless, two alternative, or perhaps concurrent, explanations can be given for the small sensitivity of these parameters to the presence of a charge on the excited acceptor. First, since the charges on the cationic dye or the radical anion of the reduced dye will be reasonable delocalized, i.e., it may be only weakly solvated, so that there will be no great difference in the reorganization energies for these species when passing to the neutral reduced radical or the neutral dye, respectively. Thus, all the influence on the λ parameter would then be due to the pair Red/Red⁺.

According to Marcus theory, the reorganization energy for the whole process can be assumed to be

$$\lambda = (\lambda_A + \lambda_D)/2 \quad (10)$$

where λ_A and λ_D correspond to the solvent reorganization energy for the electron self exchange processes of the acceptor and donor species, respectively. For the systems studied in this work, the values of λ_A can be assumed to be around 8 kcal/mol, similar to those determined for the 9-phenylacridinyl radical¹⁵ in methanol (8.0 kcal/mol) and phenothiazine¹⁶ and 10-methylphenothiazine¹⁶ in acetonitrile (5.4 and 8.8 kcal/mol), for which the extent of delo-

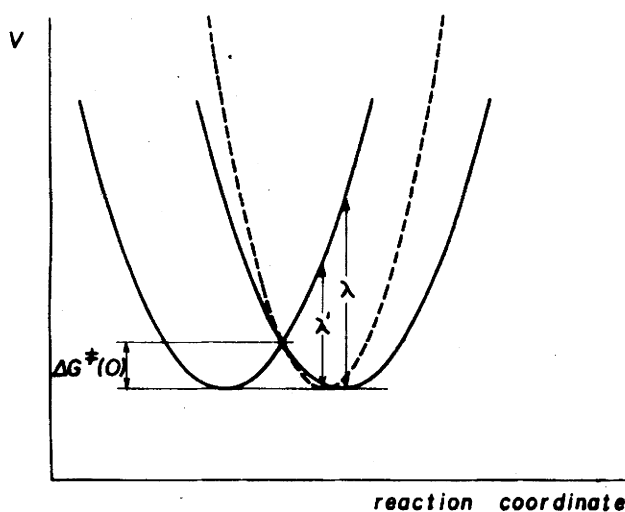
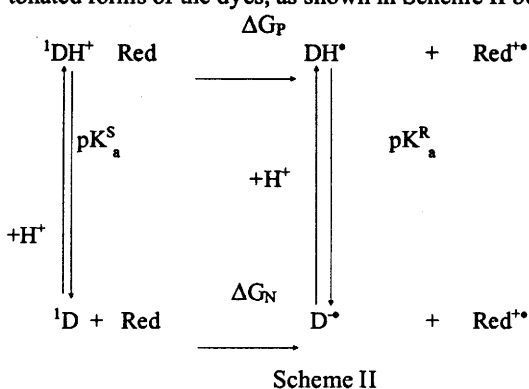


Figure 5. Relationship between the activation free energy $\Delta G^\ddagger(0)$ and the solvent reorganization energy λ for isoenergetic electron transfer reactions. Solid lines represent systems with equal force constants, and the dashed line one with higher solvent binding energy.

calization of the charge is comparable to that of the excited dye molecules. On the other hand, for the quenchers, which are mainly substituted anilines and methoxy-substituted aromatic hydrocarbons the λ_D values should be similar to those for alkyl-substituted aromatic hydrocarbons¹⁷ in acetonitrile (20 - 30 kcal/mol). Thus, the estimated value for the λ parameters, irrespective of whether the dye is in the protonated or neutral form, will be around 20 kcal/mol, in good agreement with the values found experimentally.

Secondly, for the unprotonated dyes, the radical ion products, due to their charges, will interact more strongly with the solvent molecules than the neutral reactants, resulting in a larger solvation energy change. At the same time, the charges on the radical ions will attract each other, shortening the internuclear distance. As can be seen from Fig.5, both these effects will decrease the relationship between the reorganization energy λ and the activation free energy $\Delta G \neq (0)$ below the value of 4 as deduced for electron self-transfer reactions. The net effect is, again, a decreased sensitivity of the quenching reaction rate to the variation in charge on the excited acceptor species.

The pK values of the excited singlet and semireduced forms of the dye. The pK_a 's for the species involved in the electron transfer process can be calculated from the difference between the reactivities of the protonated and unprotonated forms of the dyes, as shown in Scheme II below



where ${}^1\text{D}$, ${}^1\text{DH}^+$, D^* , and DH^+ are, respectively, the neutral

(basic) and protonated dye in their excited singlet states and the corresponding semireduced (radical) forms. Using a thermodynamic cycle, similar to the Foerster one, the relationship between the pK_a 's for both states of the dye is

$$\Delta G_P - \Delta G_N = 2.3 RT(pK_a^R - pK_a^S) = 2.3 RT \Delta \Delta pK_a \quad (11)$$

The difference in free energies between the two forms of the dyes can be obtained from the curves of $\log k_q$ vs. quencher oxidation potential, as shown in Fig.6. The values obtained for $\Delta \Delta pK_a$ in the systems studied in this work are 2.4 for the Neutral Red pair and 1.4 for the Thionine pair. These differences show the same trend as found for the triplet states of other basic dyes, which are stronger acids in their excited states than in the corresponding semireduced (radical) forms, e.g. Lumiflavin¹⁹ ($\Delta \Delta pK_a = 5.0$); Thionine²⁰ ($\Delta \Delta pK_a = 3.4$); Acridine Orange²¹ ($\Delta \Delta pK_a = 6.0$); Safranin²² ($\Delta \Delta pK_a = 2.0$); Flavin⁵ ($\Delta \Delta pK_a = 4.8$); and Neutral Red²³ ($\Delta \Delta pK_a = 3.0$).

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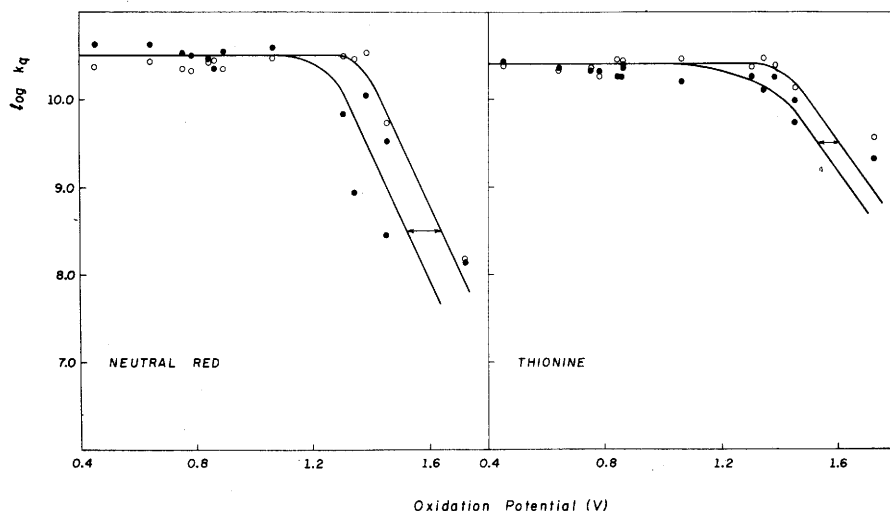


Figure 6. Rehm and Weller plots for the quenching of the fluorescence of a) Neutral Red (. NR; , NRH) and b) Thionine . Th; , ThH), as a function of the oxidation potential of the quenchers.

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