THE USE OF BASIC DYES AS PHOTOCHEMICAL PROBES

Miguel G. Neumann* and Marcio J. Tiera
Instituto de Física e Química de São Carlos - Universidade de São Paulo - Caixa Postal 369; 13560 - São Carlos - SP - Brazil

The use of Basic Dyes as photochemical probes is described in this review. Their applications in microheterogeneous systems (specially micelles and polyelectrolytes), biological systems and their use to test energy and electron transfer theories are discussed.

Keywords: basic dyes; photochemical probes; micelles; polyelectrolytes.

INTRODUCTION

One of the largest groups of compounds in synthetic and natural chemistry, which has innumerous applications in various fields, is that formed by dyes of various types. Historically, the initial development of this type of compounds can be traced back to their use in the textile industry, although later developments extended their utilization to other important applications such as photography, medicine and biology, food industry, cosmetics, analytical chemistry, and others [1].

In the specific case of the basic dyes, the first applications were related to the dying of cotton, leather and paper. Nevertheless, the research and development of new classes of compounds were enhanced by the introduction of synthetic fibers derived from poly(acrylonitrile), polyesters and modified polyamides [2].

Basic, or cationic, dyes can be classified according to their structures, chromophoric groups and heteroatoms into several classes, like acridines, thiazines, diazines, xanthenes, triarylmethanes, etc., as is illustrated in Figure 1. These dyes have usually a positive charge that is delocalized over the whole Π-system of the molecule, with the largest electron densities placed on the nitrogen atoms.

We will discuss in this paper the use of basic dyes as photochemical and photophysical probes, and their applications in the study of microheterogeneous systems (mainly micelles and polyelectrolytes), as well as in biological systems. Their use in the study of theoretical models for energy and electron transfer will also be presented.

BASIC DYES AS PHOTOCHEMICAL PROBES

Fluorescent molecules can be used as probes for obtaining information about the environment where they are placed, when their spectral and dynamic properties depend on environment. Some of the properties that make basic dyes an interesting choice for their use as probes are:

- Broad absorption bands in the visible and ultraviolet spectra, with quite large extinction coefficients (over 20 000 $M^{-1}cm^{-1}$), as shown in Figure 2. This permits the use of very small concentrations of the dyes, minimizing possible interactions of the system with the probe;
- High fluorescence quantum yields, usually about 20%;
- Very short fluorescent lifetimes (about a couple of nanoseconds), that assure that most properties of the systems will not be change during the measurements;
- Good sensibility to the environment, in the sense that emission and absorption spectra do, in general, depend on the environment where they are placed, changing the emission intensities (or extinction coefficients), as well as the shape of the spectra;
- Low reduction potentials, which allow the use of appropriate reductors to quench their excited states and emissions;
- Possibility of competition between uni- and bimolecular decay mechanisms;
- Being the dyes essentially hydrophobic species, the pres-

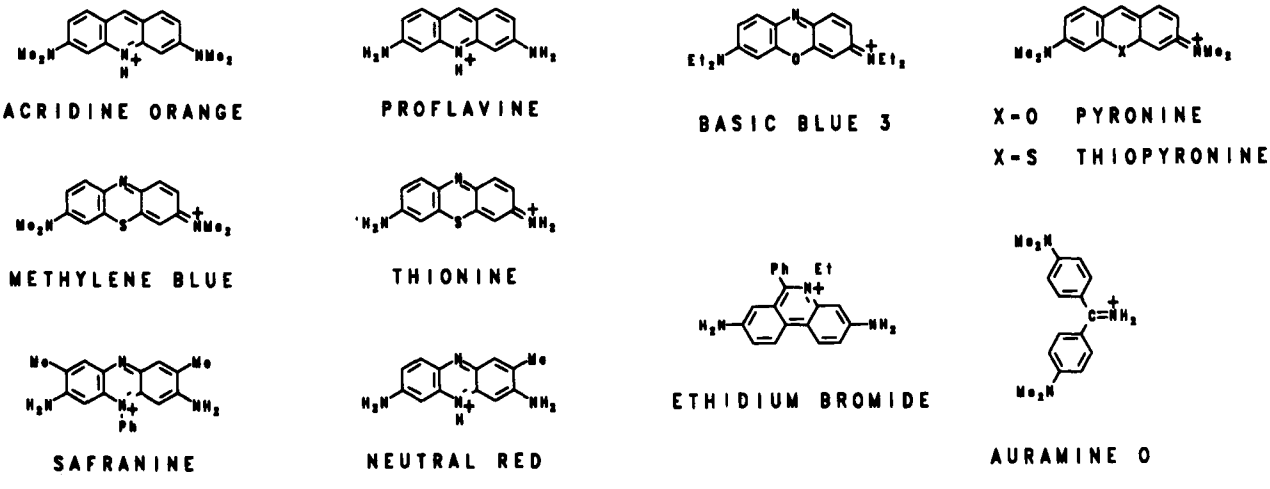


Figure 1. Structures of some Basic Dyes used as probes.

Table 1. Physical constants for some Basic Dyes

Dye	Ground State			Singlet State			Triplet State	
	λ_{max} *nm*	ε_{max} $M^{-1}s^{-1}$	E_{red} *eV*	λ_{max} *nm*	Φ_F	τ_F *ns*	λ_{max} *nm*	τ_P *μs*
Methylene Blue	657	86000	-0.39	665		0.40	420	5.0
Thionine	600	69000	0.04	610	0.05	0.35	390,760	10.0
Neutral Red	535		0.39	610	0.04	0.34	380,580	5.0
Safranine	530	53000	0.29	570	0.13	1.31	820	25.0
Acridine Orange	490	62000	-0.92	525			270,410	100
Acridine Yellow	458		-1.08	525	0.56	3.62	680	20.0
Proflavine	445		-0.73	500	0.28	1.48		

ence of charge allows their localization in hydrophilic regions or interfaces;

- Well known dye aggregation processes in aqueous solutions and induced by microheterogeneous systems;
- Derivatives of the dyes are relatively easy to synthesize and bind to other molecules.

Some of the properties of basic dyes used as probes are shown in Table 1.

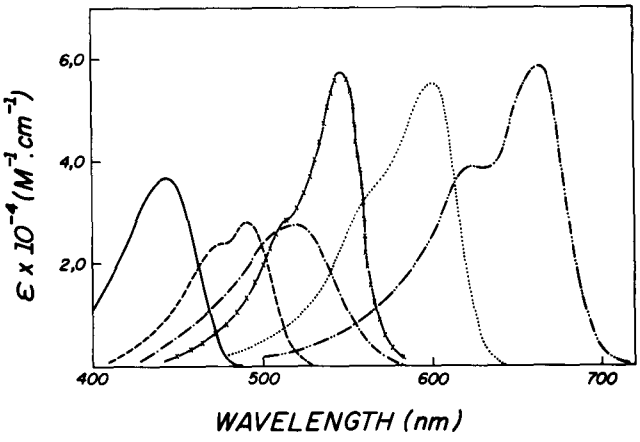


Figure 2. Absorption spectra of basic dyes: (—) Proflavine, (---) Acridine Orange, (-·-·) Safranine, (-x-x-) Pyronine, (···) Thionine, (-··-) Methylene Blue. Concentrations 3.0x10⁻⁵M. [Ref.10d]

BASIC DYES AS PROBES FOR MICELLAR SYSTEMS

Fluorescent dyes are used to determine the critical micelle concentration (*cmc*) of detergents using spectrofluorimetric techniques. The method is based on the observation that the colour of the emission intensity of some dyes changes when adsorbed on micelles [3]. One of the easiest ways to determine the *cmc*'s is by this procedure, using dyes such as Acridine Orange, Proflavine, etc.[4]

The experimental procedure is to dissolve a very small amount (concentration well below the *cmc*) of the dye in aqueous solution. The initial addition of small amounts of detergent will induce the formation of non-fluorescent aggregates [4], decreasing the total emission due to internal conversion, as predicted by the exciton theory [5]. Those non-emitting aggregates have initially equal amounts of detergent and dye molecules, but near the *cmc* the proportion of detergent molecules in the aggregates will increase up to a point where there is only one dye molecule per aggregate (or micelle), which being now precluded from interacting with another molecule of dye, will loose its energy mainly by emission. Thus, the value of the *cmc* can be deduced from the well-known S-shaped plot of the fluorescence intensity as a function of surfactant concentration. (Figure 3). Similarly, spectra in the visible region can also be used for these determinations. The absorbance at the maximum corresponding to the dye monomer will increase as the dye molecules are redistributed between more

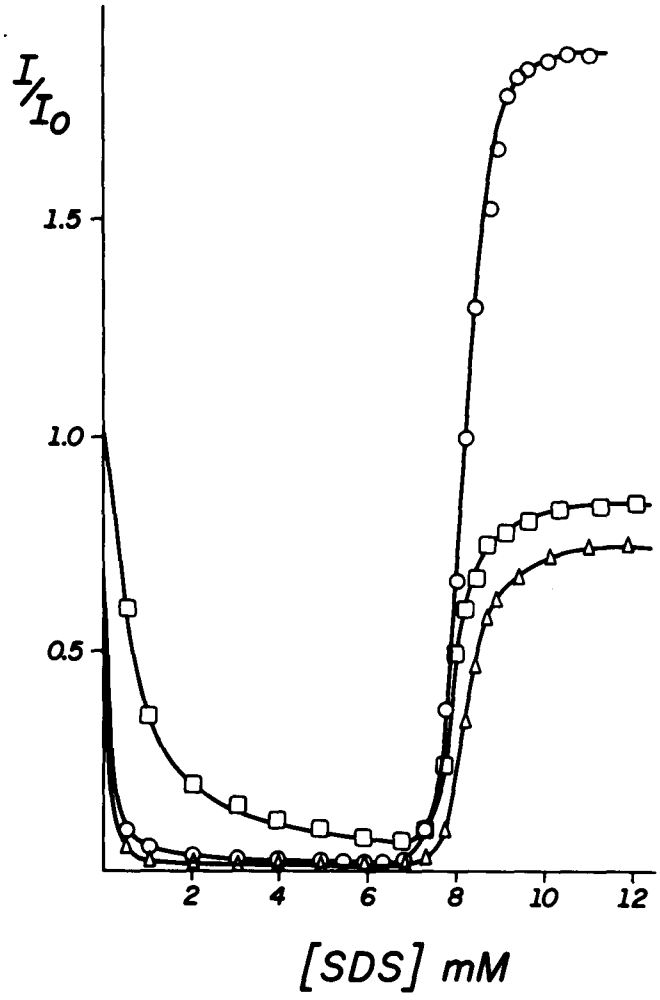


Figure 3. Relative fluorescence intensity of basic dyes in the presence of SDS. (□) Proflavine, (○) Acridine Orange, (Δ) Acridine Yellow. [Ref.4]

surfactant-dye aggregates, or micelles. At the same time, the absorbance corresponding to the dye dimers, or higher aggregates, will decrease [6].

The behaviour of solutions of surfactants below the *cmc* was the aim of several experimental studies [7] and theories [8]. It is now accepted that the surfactant molecules do associate before the formation of true micelles. These associations, called premicelles, may be dimers, trimers, or even higher n-mers, but don't present the properties of micellar solutions [9]. The use of basic dyes can be extended to study the structures of these aggregates and the properties of the surfactant solutions in that region. Assuming a stacked conformation for the dyes in the micellar aggregates [10] it has been possible to calculate theoretically the emission behaviour in the premicellar region [11a]. At concentrations near the *cmc* the proportion between dye and surfactant molecules breaks down, and a new model has to be used to explain the emission behaviour[11b]

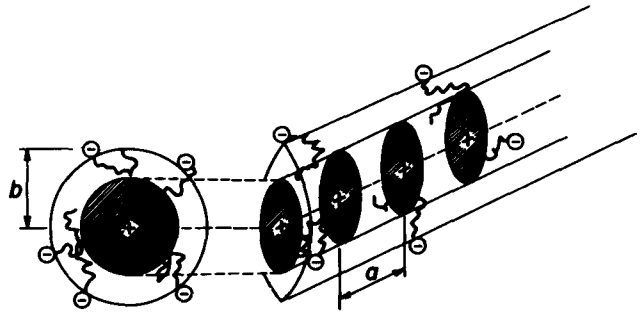


Figure 4. Model for cationic dye aggregates with cylindrical symmetry.[Ref.11a]

Although the models used in these calculations do reproduce quite well the experimental results, some points remain to be looked into more carefully, specially the one related to the eventual induction of surfactant aggregation by the presence of dye molecules.

Another use of basic dyes as probes is its utilization to determine the adsorption, or association, equilibrium constants of substrates to micelles and other microheterogeneous systems. Determinations of this type can be made using static fluorescence emission or dynamic lifetime measurements [12]. When working with anionic or neutral microheterogeneous systems (to which basic dyes will bind due to hydrophobic and electrostatic interactions), it may be possible to determine the local concentrations of different substrates from the quenching of the excited states of the dyes. Acridine Orange and its C12 derivative have been used to determine the association constants of aromatic amines to several types of micelles [12]. The binding constants K_b

$$K_b = \frac{[Q]_{mic}}{[Q]_w \times [Mic]} \qquad (1)$$

(where $[Q]_{mic}$ and $[Q]_w$ are the concentrations of quenchers in the micelles and in water, respectively, and [Mic] is the concentration of micelles), were determined by the method of Lissi and Encinas [13] from where the total quencher concentration $[Q]_T$ can be expressed as a function of the average number of quencher molecules per micelle, $\bar{n}$

$$[Q]_T = \bar{n} \,/\, K_b + \bar{n}.\,[Mic] \qquad (2)$$

In this way, the association constants can be obtained from Stern-Volmer plots, plotting the total concentrations of quenchers necessary to obtain equal quenching efficiencies as a function of micelle concentrations, as shown in Figure 5. In

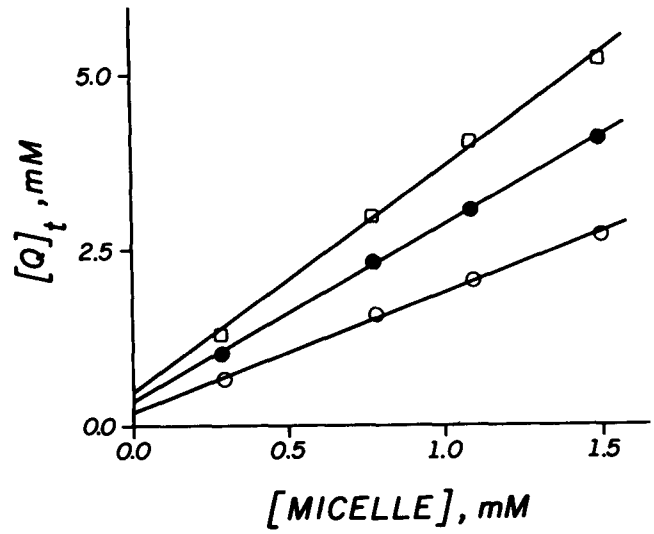


Figure 5. Plots of eqn 2 for the fluorescence quenching of Acridine Orange in the presence of SDS micelles. I_o/I: (○) 1.5; (●) 1.8; (□) 2.1 [Ref.12]

these cases, due to the extremely short lifetimes of the singlet states of the dyes, one can assure that the probe will not change its position during the experiment.

Due to the highly hydrophobic character of the dyes, they can be incorporated to micelles formed by surfactants of different types, allowing a comparison of the binding properties of substrates to, *e.g.*, cationic, anionic and non-ionic micelles, as illustrated in Table 2.

Table 2. Binding constants of aromatic amines to different type of micelles at 298°K. [Ref.12]

Amine	SDS[a]	CTAC[a]	Brij-35[b]
Diphenylamine	0.64×10^4	5.3×10^4	6.4×10^4
2-naphthylamine	0.71×10^4	18.9×10^4	12.0×10^4
N,N-dimethylamine	0.16×10^4	0.53×10^4	0.7×10^4

a) Determined using Acridine Orange
b) Determined using the C12-alkyl derivative of Acridine Orange

The same technique can be used with the triplet state of these dyes, as in general, their formation quantum yields are high, and their reduction potentials low. Thus, knowing the transient spectra of these species, it may be possible to evaluate the local concentration of the quenchers. In the case where more than one ion is present in solution, if at least one of them is a quencher of the excited species, the same method can be used to obtain the ion-exchange constants for the microheterogeneous environments.

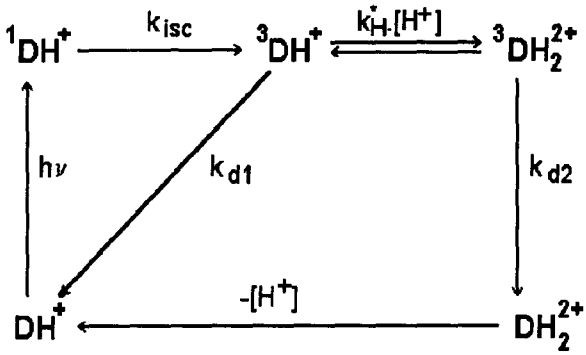
Due to its special properties, pyrene is one of the most used probes for this kind of determinations [14,15]. Nevertheless, in most cases basic dyes can also be used for these purposes. On the other hand, for evaluating proton concentrations in homogeneous solutions, at interfaces, or in microheterogeneous environments, basic dyes seem to be more appropriate.

Basic dyes have, in general, several prototropic forms, with pK values depending on various factors. Their solubilization in aqueous micellar solutions alters the acid-base properties of the dyes [16], which may be influenced by the dielectric constant of the microenvironment, the distribution of counter-ions in the double layer and the nature of the excited state (see Table 3). In the case of the triplet state, the pK's are usually

Table 3. pK values for basic dyes in their ground, singlet and triplet states.

	Ground State		Singlet State		Triplet State	
Dye	D/DH^+	DH^+/DH_2^{2+}	D/DH^+	DH^+/DH_2^{2+}	D/DH^+	DH^+/DH_2^{2+}
Acridine Orange	10.3		13.3		13	
Proflavine	9.5	0.2	12.5	1.5		4.0
Safranine	~12.5	1.5			9.2	7.5
Neutral Red	8.4	0.9	10.0	1.2	9.5	4.8

higher than those of the ground states, so that it is possible to induce pH-jumps, that under appropriate conditions, may allow to monitor the protonation kinetics. From determinations of this type, it may be possible to evaluate the proton concentrations at the probe's site, provided the specific rate constants are known. This process is described in Scheme 1 where DH^+, $^1DH^+$, and $^3DH^+$ correspond to the monoprotonated dye in the ground, and singlet and triplet excited states, respectively, and DH_2^{2+} and $^3DH_2^{2+}$ to the diprotonated dye in the ground and triplet state.



Scheme 1

When the dye is in the monoprotonated triplet state it may decay to the ground state, or if the pH is low enough, take up a proton to form the diprotonated triplet. As the lifetimes of the triplet states of these dyes are usually large and the spectra of the prototropic forms different, it is possible to monitor the protonation rate using flash photolysis techniques.

Several studies on the protonation of the excited states of basic dyes in homogeneous solutions can be found in the literature [17]. In a recent study using Safranine[18], the protonation rate constants of the triplet states in hydroxylic solvents were correlated with their viscosities. The results showed that the protonation process involves a hopping mechanism, as the constants for the more viscous systems were larger than the diffusional rates (Figure 6). The shift of the pK values of basic dyes in micellar systems permits its use to determine local concentrations of various species in the interfaces of microheterogeneous systems. Thus, the pK shift of Acridine Yellow in solutions containing anionic micelles, has been used to evaluate the high concentration of protons in the Stern layer[19].

Interface potentials can also be determined by the study of reactions that involve dyes. The interface potentials at cationic micelles is based on the formation of cations radicals by photoreduction of basic dyes

$$[^3DH^+]_{mic} + [R]_{mic} \longrightarrow [DH^\bullet]_{mic} + R^{\bullet+} \qquad (3)$$

where $[X]_{mic}$ indicates species X at the micelle. Immediately after being formed, these radical cations are expelled from the cationic micellar microenvironment. Later, they may eventually recombine with the semireduced form of the dye that is in the micellar microenvironment.

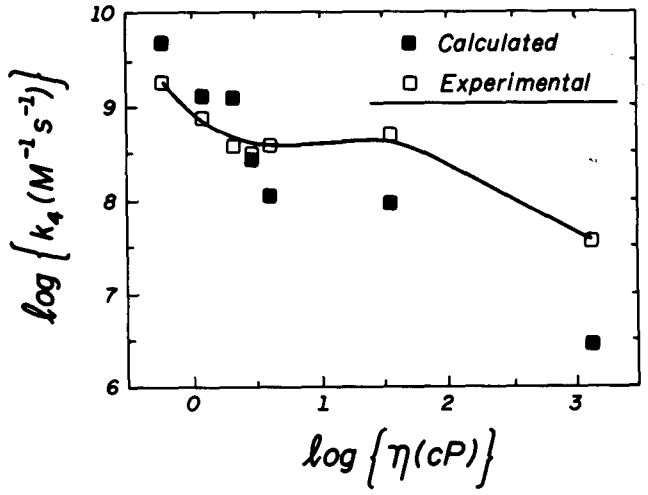


Figure 6. Double-log plot of the ratio between experimental and calculated rate constants for the protonation of triplet Safranine as a function of viscosity. [Ref.18]

$$[DH^\bullet]_{mic} + R^{\bullet+} \longrightarrow [DH^+]_{mic} + [R]_{mic} \qquad (4)$$

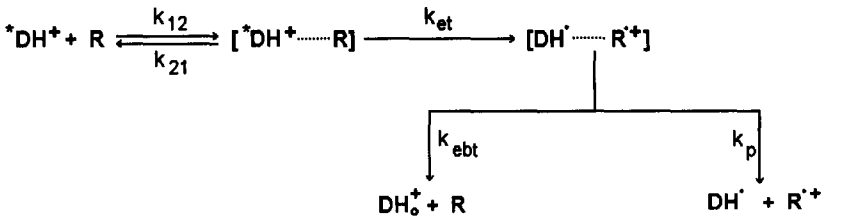
The rate of this recombination can be correlated with the potential at the interface, the degree of dissociation of the detergents in the micelle, and its aggregation number [20]. Another way to determine the aggregation numbers of surfactants in micelles is by comparing the uni-and bimolecular decay rates of the triplet states of basic dyes [21].

The (micro)-viscosity inside the microheterogeneous systems is another property of organized systems that can be readily studied using basic dyes. Two methods can be used: fluorescence depolarization (using static methodologies), and rotational relaxations, that require dynamic methodologies [22]. These measurements provide information related to the microviscosity at the site where the probes are placed, as well as of the interactions between the sites and the probe [23].

BASIC DYES AS PROBES FOR THEORIES

As the reduction potentials of most dyes are relatively low, a large number of oxidants can reduce them in the excited state. The electron transfer to, or from, an excited state (singlet or triplet), involves the formation of encounter complexes, eventually leading to ions in polar solvents (See Scheme 2).

In general, reactions leading to the semireduced forms of the dyes, originate from the triplet state. Reactions from the singlet state, that could also end up in these forms, generally lead to the quenching of the excited state to the ground state,



Scheme 2

as a result of the electron back-transfer within the encounter complex.

According to the Marcus theory [24], the electron transfer rate for the Scheme 2 is given by

$$k_{et} = \kappa.\upsilon_{23}.\exp\left\{-\frac{\lambda}{4RT}.\left[1+\frac{\Delta G}{\lambda}\right]^2\right\} \qquad (5)$$

where κ is the transmission coefficient (1 for adiabatic reactions), υ_{23} is a frequency factor, and λ is the solvent reorganization energy. Using the mechanism above and Rehm and Weller's approximation [25], it is possible to check the theoretical relationship between the specific rate constant for electron transfer k_{et} and the free energy change for the process ΔG. In cases when only one dye is used and the reductants are all derived from the same aromatic system, a dependence can also be found between k_{et} and the Hammett parameter[26] σ. (See Figure 7)

An interesting application of dyes as probes for theories is related to the eventual difference in reactivity of singlet and triplet states. Studies toward these ends were performed for reactions with excited dyes, comparing the dependence of the rate constants with the ΔG values for various quenchers and reductants. The use of dyes eliminates one of the most important source of errors, namely, the uncertainty in the values of the reduction and oxidation potentials of the reactants. When using the Rehm and Weller equation and assuming the change in free energy to be

$$\Delta G^o = E_{(D/D^+)} - E_{(A/A^-)} - E^* + \frac{Z_1.Z_2}{D.r_{12}} \qquad (6)$$

the same value for the reduction potentials $E_{(A/A^-)}$, can be used for both the triplet and singlet systems (independent of they being the correct ones or not!), so that only an adjustment for the excitation energy of the excited state E^* has to be done. These energies can be determined, practically without error, from spectroscopic experiments. In addition, by using the same dye, the distance between the reactants in the precursor complex will be kept constant, eliminating another possible source of errors. Results obtained using Safranine demonstrate that the reactivities of both states are equal, with no dependence on the multiplicity of the states [27], as shown in Figure 8.

The fact that dyes can exist in several protonation states and originate excited states with different amount of charge on them, makes them very appropriate probes to test the difference in reactivity due to the presence of these charges. According to Marcus theory, (see eqn 5), the λ-parameter is related to the solvent reorganization energy when going from the state of reagents to that of activated complex [28]. Obviously, the value of this parameter will depend on the solvent as well as of the charges on the species at their different states. A larger difference between the charges in reactants and activated complex will result in larger solvent reorganization energy.

$$*DH^+ + R \longrightarrow [DH^{\delta+} \ldots {}^{\delta+}R]^{\neq} \longrightarrow DH^{\bullet} + R^{\bullet+} \qquad (7)$$

$$*D + R \longrightarrow [D^{\delta-} \ldots {}^{\delta+}R]^{\neq} \longrightarrow D^{\bullet-} + R^{\bullet+} \qquad (8)$$

In reaction 7, little solvent reorganization will occur, as the charge on the protonated dye is quite delocalized, not very different from that in the activated complex. On the other hand, in reaction 8, a charge separation will occur, giving rise to a reordering of the solvent around the complex.

Once more, the use of basic dyes, which do retain their basic geometry, independent of the degree of protonation, can help towards the elucidation of this problem. Experiments of quenching of the fluorescence of Neutral Red and Thionine, in acid and basic solutions [29], made it possible to prove that the reactions with the singlet monoprotonated dyes (eqn 7)

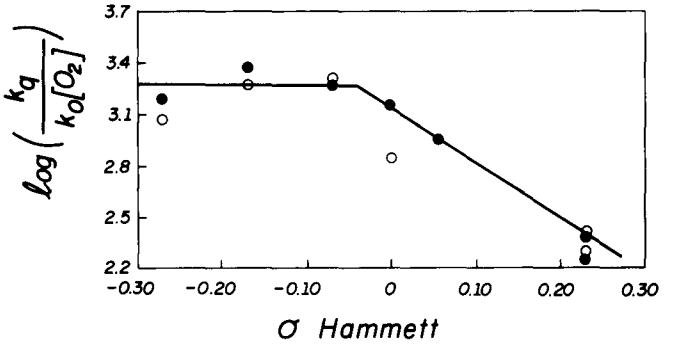


Figure 7. Hammett plots for the relative fluorescence quenching rate of triplet Safranine by substituted anilines (○) and anilinomethanesulfonates (●). [Ref.26]

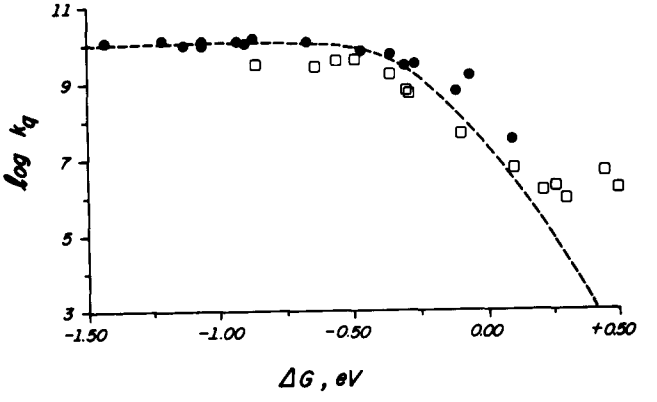


Figure 8. Rehm and Weller plots for the quenching of singlet and triplet Safranine by substituted phenols and amines. The broken line corresponds to the theoretical behaviour according to eqn 5. [Ref.27]

have lower reorganization energies than the neutral species (eqn 8).

Basic dyes are also quite useful to probe energy transfer theories.

$$D^* + A \longrightarrow D + A^* \qquad (9)$$

In the processes of energy transfer, the interesting reaction is the non-radiative energy transfer process, and specially that due to dipole-dipole coupling. Förster [30] developed a theory to explain the relationship between the distance between the energy exchanging species and experimentally determined parameters

$$R_o^6 = \frac{8.8 \times 10^{-25} \cdot \kappa^2 \cdot \Phi_D}{n^4} \cdot \int_0^\infty I_D(\nu) \cdot E_A(\nu) \frac{d\nu}{\nu^4} \qquad (10)$$

where R_o is the distance between acceptor and donor for which the transfer efficiency is 50%, κ^2 is an orientation factor (2/3 for random orientations), Φ_D is the fluorescence quantum yield of the donor, $I_D(\upsilon)$ is the normalized emission spectrum of the donor, $E_A(\upsilon)$ is the extinction coefficient of the acceptor at the wavelength corresponding to υ, and n is the refraction index of the solvent.

Energy transfer occurs only when there is a significant overlap between the donor's emission spectrum and the acceptor's absorption spectrum. This is the case of most pairs of basic dyes. This fact, combined with the high extinction coefficients of the dyes, gives values of 10^{-10} to 10^{-11} $cm^6.M^{-1}$ for the overlapping integral in eqn 10, which means a high energy transfer efficiency, corresponding to Förster radii up to 150 Å [31] . (See Table 4).

Table 4. Experimental and calculated Förster radii for energy transfer between basic dyes. [Ref.31]

Donor/Acceptor	R_{expti} (Å)	R_{calc} (Å)
Acridine Yellow/Safranine	132	156
Proflavine/Safranine	138	153
Proflavine/Basic Blue 3	102	98
Proflavine/Thionine	109	123
Safranine/Thionine	125	124
Proflavine/Methylene Blue	106	84

BASIC DYES AS PROBES IN POLYELECTROLYTES

Interactions between dyes and polyelectrolytes have been studied for a long time, specially due to its interest in the dying industry, as well as for its importance in the biological field. This importance arises from the fact that biopolymers, like proteins and DNA, are really polyelectrolytes, and their interactions with dyes and other small molecules are similar to those of synthetic polyelectrolytes [32].

The main characteristic of the interaction of basic dyes with polyelectrolytes and biopolymers is a change in the absorption spectra (defined as *metachromasy*), due to the binding of the dyes to the polymer chain and to the induced formation of dye aggregates. The latter effect is known to occur in concentrated aqueous solutions, and its extent depends on several factors like dye structure, temperature, solvent, presence of polyelectrolytes, etc. Several alternative, or concurrent mechanisms have been suggested to explain these effects: additive van der Waals forces [33], intermolecular hydrogen bonds [34], hydrophobic interactions [35] when aggregations occur in water, Π-electron interactions [36], etc.

These effects are enhanced in solutions containing polyelectrolytes, where aggregation can be observed at very low dye concentrations ($<10^{-5}$M), where no aggregation occurs in pure aqueous solutions. The larger tendency to aggregation has been ascribed to the partial neutralization of the electrostatic repulsion between the dye molecules by the charges on the polyelectrolytes, as well as an ordering effect of the polyelectrolyte chain, forcing the dye molecules into a pattern appropriate for Π electron interactions [37].

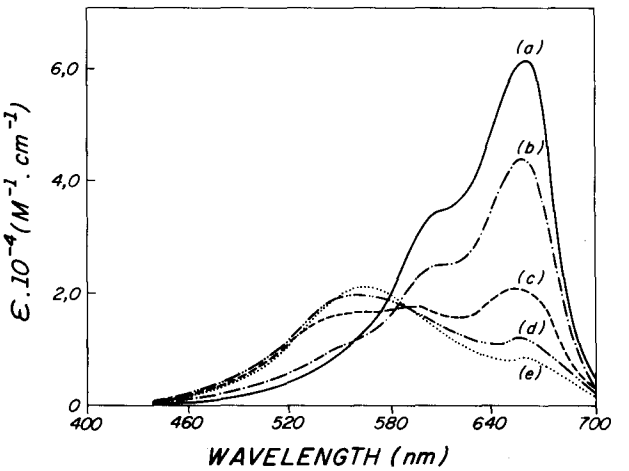


Figure 9. Absorption spectra of Methylene Blue in the presence of poly(styrene sulfonate). S/D ratios: (a) 0.00; (b) 0.17; (c) 0.52; (d) 0.69; (e) 1.21 . [Ref.6]

For most of the basic dyes in the presence of polyelectrolytes, the maximae of the absorption bands are shifted toward shorter wavelengths, and at the same time, the absorption at the monomer maximum decreases (See Figure 9). New bands at shorter wavelengths are due to the dimers, trimers and higher aggregates, which are formed when the *local* dye concentrations are increased. On the other hand, when the amount of poylectrolyte increases over a certain limit, these shifts are reverted and the maximum goes again to longer wavelengths, usually about 10 nm higher than that corresponding to the maximum of the monomer in aqueous solution. This effect corresponds to a redistribution of the dye molecules along the chain (dilution), leading to the dyes molecules too far apart from each other to interact. Based on this effect, Vitagliano et al.[38], developed a spectroscopic method to determine the number of anionic sites on a poly(styrene sulfonate), by titration with basic dyes (mainly, Acridine Orange and Pyronine G). The same procedure has been used for evaluating anionic sites in DNA [39,40] and weak polyelectrolytes, such as poly(acrylic acid) [41] and poly(metacrylic acid) [42].

Models for the configuration of aggregates have been proposed considering the stoichiometry of the dye-polyelectrolyte associations and interactions in systems with different polyelectrolytes [43]. (See Figure 10)

The emission characteristics of some dyes can also be used to study properties of polymers and polyelectrolytes. The dye Auramine O does fluoresce very weakly in aqueous solution. When placed in a medium in which the rotation of the phenyl groups is restricted, as in the case of polymers, its fluorescence increases dramatically. Therefore, conformational transitions [44] and other intrinsic properties of polymer macromolecules [45] can be studied with this probe.

The energy transfer between singlet states of dyes, as described above, can also be used to get information about macromolecular conformations. The efficiency of the process depends on the localization of the probes. Basic dyes have

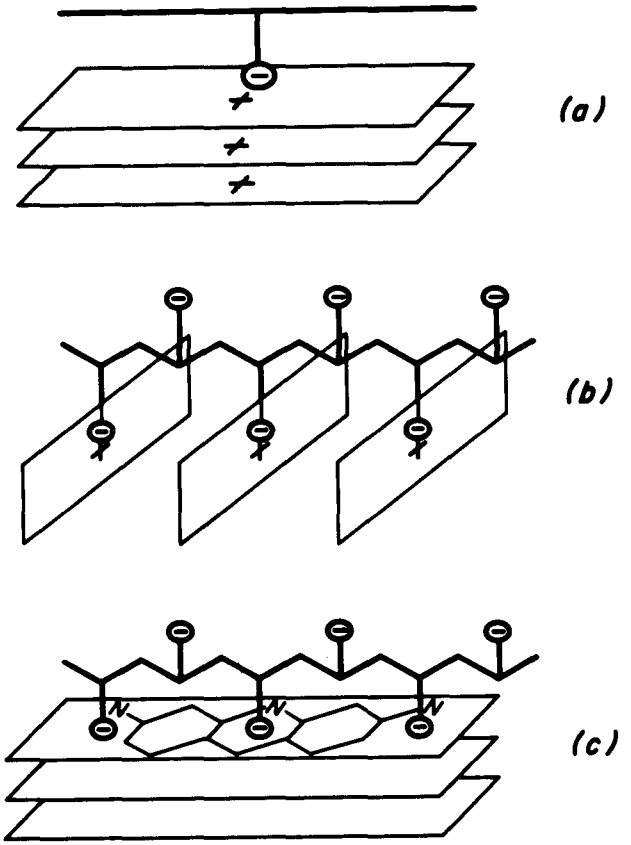


Figure 10. a) Aggregation of three dye molecules on polyelectrolyte anionic sites;b) Conventional model for polyelectrolyte induced aggregation; c) model for induced aggregation by a syndiotactic-type polyelectrolyte. [Ref.43]

been used as probes for anionic polyelectrolytes, *e.g.*, Rhodamine-B and Rhodamine-6G, to study the difference in hydrophobicity of various polyelectrolytes [46].

BASIC DYES IN BIOLOGICAL SYSTEMS

The use of dyes in biological applications started at the end of the last century, when basic and acid dyes (*e.g.*, Methylene Blue and Methyl Green and Eosine, respectively) were used to identify different regions in animal tissue [47]. Gram's test was developed in 1884, after the observation that some basic dyes, like Crystal Violet, can stain irreversibly some bacteriae, whereas some others would remain uncoloured. This is the origin of the classification of bacteriae as gram-positive and gram-negative [48].

At present, basic dyes are used in various applications in the field of biology. The labeling of bacteriae, protozoaries and other microorganisms by dyes, associated with the use of fluorescence microscopy techniques, configure a powerful method for the investigation of the morphology of cells and tissues, as well as for the diagnosis of diseases such as tuberculosis, diphtheria, malaria, etc.[49]

Nucleic acids have a highly acid character conferred by the presence of phosphate groups (pK_a~2.0), whereas proteins, having mostly carboxyl groups (pK_a~5.0) are more basic. Therefore, in the range of pH 2-5 only nucleic acids will be ionized and behave as polyanions. Thus, basic dyes such as Proflavine, Acridine Orange and Methylene Blue, are often used to stain nucleic acids in these conditions, in order to differentiate them from proteins.

The mechanism of the interaction between DNA and basic dyes is now accepted to proceed via intercalation of the planar dye molecules between a pair of nucleic bases, interacting electrostatically with the phosphate groups of the DNA [50], as depicted in Figure 11 [51]. This intercalation, which produces the staining, allows the characterization, localization and evaluation of the nucleic acids. In addition, the models for intercalation processes have also been used to explain the mutagenic and pharmacological activities of the dyes [52]. On the other hand, the intercalation model of basic dyes in DNA has also be used to explain the damage-protecting effect of these dyes in the presence of excited carbonyl compounds [53].

Acridine Orange is one of the most used fluorescent dyes in biological studies. From its interactions with biological systems, information can be obtained about molecular structure, conformation and environment of nucleic acids in cells. Nucleic acids and nucleoproteins can be characterized by their binding with Acridine Orange, measuring the ratio between the 530 nm and 650 nm fluorescence intensities [54]. The first emission corresponds to the dye intercalated in DNA, whereas the latter to the dye bound to RNA. The interaction of the dye with nucleic acids permits also studies of the secondary structures, differentiating native double-stranded DNA from single-stranded and denatured DNA [55].

Recently, Acridine Orange has been used to investigate configurational changes of DNA during gel electrophoresis [56] and as a general indicator for intercellular pH gradients [57]. Pyronine and Methyl Green are used to differentiate between DNA and RNA. The former stains selectively RNA and the latter stains selectively DNA [58]. Ethidium Bromide and Propidium Iodide are other intercalating basic dyes that allow the identification of different kinds of DNA [59], as well as its detection in gels [60]. Other basic dyes, such as the Rhodamines [61] and Acridine Orange [62] have been used to study cellular organelles.

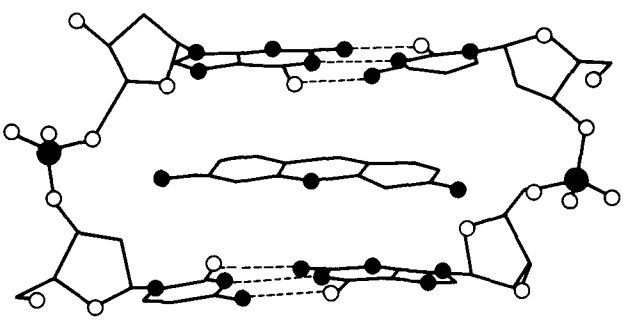


Figure 11. Intercalation of Proflavine in a self-complementary dinucleotide. [Ref.51]

ACKNOWLEDGEMENTS

The authors thank FAPESP, CNPq and FINEP for financial support for their investigations mentioned in this review.

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This special issue of Química Nova is dedicated to Prof. G. Cilento on the occasion of his 70th birthday and is financed by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).