

## Quenching of the triplet state of safranine-O by aliphatic amines in AOT reverse micelles studied by transient absorption spectroscopy

Gabriela V. Porcal, Carlos A. Chesta, M. Alicia Biasutti, Sonia G. Bertolotti and Carlos M. Previtali\*

Received 15th August 2011, Accepted 12th October 2011

DOI: 10.1039/c1pp05252g

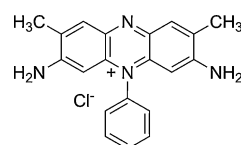
The photophysics of Safranine-O (3,6-diamino-2,7-dimethyl-5-phenyl phenazinium chloride) ( $\text{SfH}^+\text{Cl}^-$ ) was investigated in reverse micelles (RMs) of AOT (sodium bis(2-ethylhexyl)sulfosuccinate) with special emphasis on the triplet state processes. The triplet is formed in its monoprotonated form, independently of the pH of the water used to prepare the RMs. While the intersystem crossing quantum yields in RMs are similar to those in organic solvents, the triplet lifetime is much longer. Since the pH in the water pool of AOT RMs is close to 5 and the triplet state of the dye is subjected to proton quenching, the long lifetime indicates that the dye resides in a region where it cannot be reached by protons during its lifetime. All the measurements indicate that the dye is localized in the interface, sensing a medium of micropolarity similar to EtOH : water (3 : 1) mixtures. The quenching by aliphatic amines was also investigated. While the quenching by the hydrophobic tributylamine is similar to that in methanol, the hydro-soluble triethanolamine is one order of magnitude more effective in RMs than in homogeneous solution. In the latter case the quenching process is interpreted by a very fast intramicellar quenching, the overall kinetics being controlled by the exchange of amine molecules between RMs. Semireduced dye is formed in the quenching process in RMs in the di-protonated state with a comparable quantum yield to the monoprotonated state formed in homogeneous solvents. The results point to the advantage of the reverse micellar system for the generation of active radicals for the initiation of vinyl polymerization, since a much lower concentration of amine can be employed with similar quantum yields.

### Introduction

Reverse micelles (RMs) and microemulsions have attracted considerable interest in recent years because they can provide “nano-sized reactors” for chemical, photochemical and biological reactions.<sup>1</sup> Water encapsulated in such aggregates is thought to mimic water in biological membranes and proteins.<sup>2</sup> Aerosol OT (AOT, sodium bis(2-ethylhexyl)sulfosuccinate) is probably the surfactant most extensively used to form RMs. It can solubilize large amounts of water without the aid of a co-surfactant, up to 30% depending on the non-polar medium surrounding the RMs. The solubilized water exhibits peculiar properties<sup>3</sup> owing to interactions with the polar heads of surfactant and the counter-ions. Several investigations have shown a transition region at molar ratios  $w = 6$ – $10$  (where  $w = \text{moles H}_2\text{O}/\text{moles AOT}$ ) between a reverse micellar state (where water is involved in the hydration of surfactant and sodium ion) and a microphase domain corresponding to a water-in-oil microemulsion (where part of the solubilized water is apparently free).<sup>4</sup>

The reactivity of substrates incorporated in RMs depends strongly on their location in the microphases of the system. Several

molecular probes have been used to characterize the properties of the interface in AOT RMs.<sup>5</sup> Most of these studies are based on the study of the photophysical properties of synthetic dyes and their dependence with the water : surfactant ratio ( $w$ ). Safranine-O (3,6-diamino-2,7-dimethyl-5-phenylphenazinium chloride) ( $\text{SfH}^+\text{Cl}^-$ , Scheme 1) is a synthetic dye that was widely employed to characterize normal micelles,<sup>6</sup> to investigate the kinetics of electron transfer reactions in these media<sup>7</sup> and in studies DNA-dye interactions.<sup>8</sup> In RMs absorption and fluorescence emission spectra of safranine-O were determined in order to understand the localization of the dye in the microheterogeneous domains and to determine the properties of the microenvironment where the dye is located. Most of these studies were carried out in AOT-heptane solutions. Since the interfacial region is composed by the negative heads of the surfactant, it is expected that the positive dye will remain either



Safranine-O

Scheme 1

Departamento de Química, Universidad Nacional de Río Cuarto, 5800, Río Cuarto, Argentina. E-mail: cprevitali@exa.unrc.edu.ar

close to the hydrated heads or oriented toward the bulk organic phase. From fluorescence studies Bose *et al.*<sup>9</sup> suggest that at low  $w$  values, SfH<sup>+</sup> does not penetrate into the reverse micellar core; rather it binds at the interfacial region. Also, the fluorescence quenching of SfH<sup>+</sup> by AgCl nanoparticles has been investigated in the W/O microemulsion medium at different [H<sub>2</sub>O]/[AOT] ratios by Pramanik *et al.*<sup>10</sup>

The fluorescence quenching of Safranin-O by the inorganic ions Fe<sup>2+</sup>, [Fe(CN)<sub>6</sub>]<sup>3-</sup> and Cu<sup>2+</sup> was studied in AOT RMs and microemulsions in various non-polar solvents.<sup>11</sup> Chaudhuri *et al.*<sup>12</sup> studied the luminescence behaviour of the related dye phenosafranin in RMs of AOT in heptane. They concluded that the photophysical properties of the dye do not reach those in pure water even at high  $w$ . However, the interpretation of these results may be biased by high local concentration of the dye and the consequent dimerization (aggregation). The temperature dependence of the absorption spectrum of SfH<sup>+</sup> at  $6.6 \times 10^{-5}$  M in water suggest that even at such a low concentration there is dimerization of the dye.<sup>13</sup>

All these studies of the photophysics of SfH<sup>+</sup> in RMs have been based on the absorption and emission spectra and excited singlet lifetime measurements. To our knowledge the triplet state properties of SfH<sup>+</sup> in RMs have not received attention. The effect of microheterogeneous media on the triplet state of the dye was investigated by Pastre and Neumann.<sup>14</sup> The triplet state of the dye is responsible for many useful applications of the dye, one of the most important being its use as photoinitiator of the vinyl polymerization.<sup>15,16</sup> RMs have been extensively employed as a medium for controlled polymerization.<sup>17</sup> Since the applications of the dye in photopolymerization systems are based on the electron transfer reaction from electron donors to the excited triplet of the dye, we decided to study these processes in RMs. In the present paper we present a study of the photophysics of SfH<sup>+</sup>, with special emphasis on the triplet state processes in AOT RMs. In particular, the effect of the organized medium on the triplet quenching by aliphatic amines and the quantum yield of radicals was investigated.

## Experimental

### Materials and methods

Safranin-O (3,6-diamino-2,7-dimethyl-5-phenylphenazinium chloride) (SfH<sup>+</sup>Cl<sup>-</sup>) from Aldrich ( $\geq 85\%$ ) was recrystallized from ethanol. Sodium 1,4-bis(2-ethylhexyl)sulfosuccinate (AOT) from Sigma was dried under vacuum over P<sub>2</sub>O<sub>5</sub>. n-Heptane and ethanol were from Sintorgan (HPLC grade) and used as received. Water was purified through a Millipore Milli-Q system. Reverse micelles solutions were prepared by the addition of a small amount of SfH<sup>+</sup>Cl<sup>-</sup> dissolved in water to a 0.2 M AOT in n-heptane. The final analytical concentration of the dye was *ca.*  $10^{-5}$  M. The mean occupation number of the dye was less than 0.1. The water micelle content,  $w = [\text{H}_2\text{O}]/[\text{Surfactant}]$ , was varied by adding water at neutral pH. Triethanolamine (TEOA) and tributylamine (TBA) both from Aldrich, were purified by vacuum distillation before use. In the quenching experiments the TEOA was added as a concentrated aqueous solution and as the pure compound in the case of TBA. Due to the buffer-like action of AOT reverse

micelles<sup>18</sup> the addition of the amine are not expected to change the pH of the water pool, and it will remain close to 5.

Absorption spectra were obtained by using a Hewlett Packard 6453E diode array spectrophotometer. Fluorescence spectra were measured with a Spex Fluoromax spectrofluorometer in air equilibrated solutions. Fluorescence lifetimes were determined by using the time-correlated-single-photon-counting technique with a FL 900 Edinburgh Instruments equipped with a PicoQuant subnanosecond pulsed LED emitting at 498 nm. Transient absorption measurements were carried-out by excitation at 532 nm using a laser flash photolysis equipment as previously described.<sup>19</sup> The samples were deoxygenated by continuous bubbling with high purity argon. Steady state fluorescence anisotropy measurements  $\langle r \rangle$  were determined with a Hitachi 2500 spectrofluorometer, with Glan-Thomson polarizers. Fluorescence anisotropy value was obtained using the expression

$$\langle r \rangle = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where  $I_{VV}$  and  $I_{VH}$  are the vertically and horizontally polarized emission components after excitation by vertically polarized light and  $G$  is the sensitivity factor of the detection system. All measurements were carried out at 25 °C.

## Results and discussion

The absorption and fluorescence parameters of the dye in homogeneous media and in AOT/heptane reverse micelles are collected in Table 1.

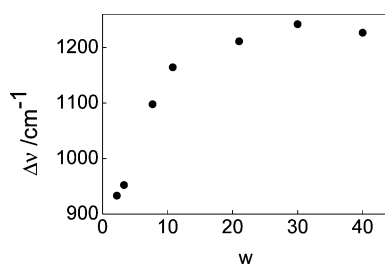
In Fig. 1 the Stoke's shift ( $\Delta\nu$ ) is plotted as a function of  $w$ .  $\Delta\nu$  increases with increasing  $w$  until a plateau at  $w \sim 20$  is reached. Interestingly, the Stoke's shift at  $w > 20$  ( $\sim 1200$  cm<sup>-1</sup>) is similar to that observed in EtOH : water (3 : 1) and much lower than that in pure water (2200 cm<sup>-1</sup>).<sup>20</sup> From Fig. 1 and the data in Table 1 it is apparent that as  $w$  increases the dye perceives a more polar environment; although the medium sensed does not resemble that of pure water, even at the larger values of  $w$  studied. These results agree in part with previous studies<sup>9-12</sup> which concluded that SfH<sup>+</sup> locates near the hydrated AOT negative polar heads at the reverse micelle interface.

Measurements of the fluorescence anisotropy can give an indication of the rigidity of the medium surrounding a fluorophore. The anisotropy  $\langle r \rangle$  was determined for the dye at different values of  $w$  and it is shown in Fig. 2. Since the dye is located in the micellar interface, the observed changes in the emission anisotropy can be taken as an indication of a slight decrease of the rigidity of the

**Table 1** Absorption and emission maxima wavelength, fluorescence lifetime and Stokes' shift ( $\Delta\nu$ ) of SfH<sup>+</sup>Cl<sup>-</sup> in different media

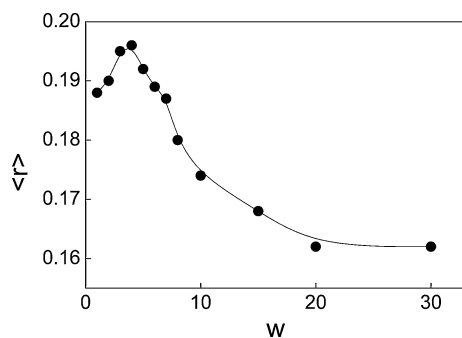
Medium	$\lambda_{\text{max}}(\text{abs})/\text{nm}$	$\lambda_{\text{max}}(\text{fl})/\text{nm}$	$\tau/\text{ns}$	$\Delta\nu/\text{cm}^{-1}$
Water	520	588	1.30	2230
MeOH	529	564	2.60	1170
EtOH : H <sub>2</sub> O (3 : 1)	533	570	2.68	1220
AOT $w = 5$	532	564	2.40	1070
AOT $w = 20$	531	568	2.15	1230

The estimated errors are  $\pm 0.05$  ns in fluorescence lifetimes and  $\pm 60$  cm<sup>-1</sup> in  $\Delta\nu$ .



**Fig. 1** Stoke's shift ( $\Delta\nu$ ) of  $\text{SfH}^+\text{Cl}^-$  fluorescence in AOT/heptane reverse micelles as a function of the water content.

interface with increasing size of the water pool. The dependence of the anisotropy as a function of  $w$  was investigated for the related dye phenosafranine by Chaudhuri *et al.*<sup>12</sup> and by Bose *et al.*<sup>9</sup> In both works it was also observed that the anisotropy levels-off for  $w > 20$  at a value much higher than that observed in pure water. Nevertheless the results differ in the initial portion of the graph. While Chaudhuri *et al.* find an initial growth of the anisotropy and a maximum near  $w = 10$ , this behaviour is not observed by Bose *et al.* We also observed that the anisotropy of  $\text{SfH}^+\text{Cl}^-$  initially increases and afterwards there is an abrupt decrement and a plateau is attained at  $w > 20$ . The singlet lifetime decreases by 15% on going from  $w = 5$  to 20. The longer lifetime correspond to the higher value of the anisotropy and if this is taken in consideration the differences observed in Fig. 2 should be slightly larger. This indicates that the changes in the rigidity of the interface sensed by the dye are complex. In principle, a change of  $w$  from 2 to 30 should radically affect the properties of the micellar interface. In a study of the microviscosity of AOT RMs, Hasegawa and Kitahara<sup>21</sup> found that the water pool is highly viscous at lower  $w$  values and then the microviscosity rapidly decreases approaching  $w = 10$ . Afterwards it decreases slowly, until  $w = 50$ . This is in line with the results shown in Fig. 2. However, since in the present case the change in anisotropy with increasing size of the water pool is relatively small, the results suggest that the dye is probably located in the inner region of the interface, close to the organic phase and not in the proximity of the hydrated heads of AOT. This is further confirmed by the results of the triplet properties of the dye in the reverse micelles (*vide infra*).



**Fig. 2** Fluorescence anisotropy of  $\text{SfH}^+\text{Cl}^-$  as a function of  $w$  in AOT/heptane reverse micelles.

### Triplet state properties

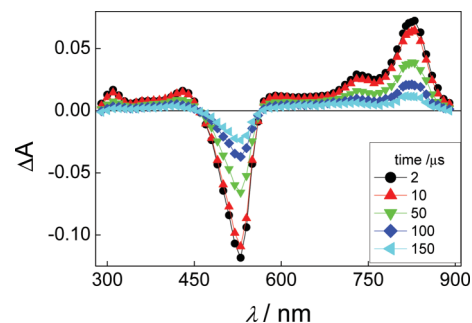
Laser excitation at 532 nm of  $\text{SfH}^+$  in the micellar media causes the immediate appearance of a strong transient absorption in the

**Table 2** Absorption maximum ( $\lambda_{\text{max}}$ ), absorption coefficient ( $\epsilon$ ), lifetime ( $\tau$ ) and quantum yield ( $\Phi_{\text{T}}$ ) of the triplet state and intersystem crossing rate constants of safranine-O in homogeneous and AOT/heptane reverse micelle media

Medium	$\lambda_{\text{max}}/\text{nm}$ ( $\epsilon/\text{M}^{-1} \text{cm}^{-1}$ )	$\tau/\mu\text{s}$	$\Phi_{\text{T}}$	$k_{\text{isc}}/10^8 \text{s}^{-1}$
Water	805 (16200)	60	0.28 <sup>20</sup>	2.15
MeOH	730 (7300), 822 (19500)	18	0.31 <sup>20</sup>	1.19
EtOH : water (3 : 1)	730 (9700), 825 (25500)	41	0.34 <sup>20</sup>	1.23
AOT $w = 20$	730, 828	103	0.31 <sup>a</sup>	1.14
AOT $w = 5$	740, 842	114	0.33 <sup>b</sup>	1.04

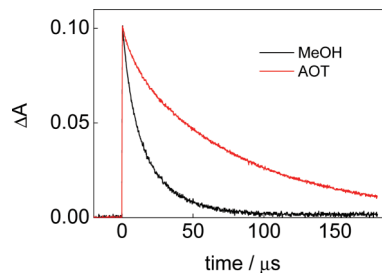
The uncertainty in triplet lifetime is less than 5%. Estimated errors in absorption coefficients, triplet quantum yield and intersystem crossing rate constants are  $\pm 20\%$ .<sup>a</sup> With T-T molar absorption coefficients assumed to be those in EtOH:water (3 : 1) (from ref. 20). <sup>b</sup> With T-T molar absorption coefficients assumed to be those in EtOH (from ref. 20).

region 300–900 nm. The temporal evolution of this absorption at  $w = 20$  is shown in Fig. 3. The spectrum shows prominent maxima in the 700–850 nm wavelength range along with the bleaching of the ground state ( $\sim 530$  nm). This spectrum is typical of the triplet excited state of the dye in its monoprotonated form,  $^3\text{SfH}^+$ . At this  $w$  the absorption maximum ( $\sim 830$  nm) is red shifted compared to the value observed in water and similar to that detected in EtOH : water (3 : 1).<sup>20</sup> For comparison the properties of the triplet state of  $\text{SfH}^+$  in different media are summarized in Table 2.



**Fig. 3** Triplet–triplet absorption spectrum of  $\text{SfH}^+\text{Cl}^-$  in AOT/heptane at  $w = 20$ .

The triplet decay measured at 830 nm in the AOT micelles follows a first order kinetics and the lifetime in micellar media is noticeable longer than in pure organic solvents ( $\tau = 18 \mu\text{s}$  in MeOH) as shown in Fig. 4.

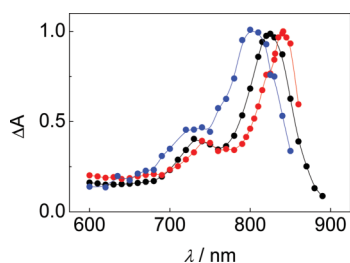


**Fig. 4** Decay profiles of  $\text{SfH}^+\text{Cl}^-$  in AOT/heptane reverse micelles ( $w = 20$ ) and in MeOH at 825 nm.

The spectral characteristics are practically insensitive to the pH of the water added to form the water pool (data not shown). This

is not surprising, since according to Hasegawa<sup>22</sup> AOT has a buffer-like effect and the pH of the water pool is close to 5 regardless of the pH adjustment procedures (NaOH/HCl or buffers). From the observed lifetime of <sup>3</sup>SfH<sup>+</sup>, it is apparent that the dye is not sensing such pH. In effect, the rate constant for the quenching of <sup>3</sup>SfH<sup>+</sup> by protons in water is  $\sim 1.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ .<sup>23</sup> Accordingly, the expected lifetime of <sup>3</sup>SfH<sup>+</sup> at pH = 5 should be around 6  $\mu\text{s}$ . However, and independently of the pH used to form the water pool, the observed decay time in the micellar media is always longer than 100  $\mu\text{s}$ . This clearly indicates that the dye resides in a region where it cannot be reached by protons during its lifetime.

The T-T absorption characteristics are also a function of the water content. In Fig. 5 the spectra in water, AOT/heptane  $w = 5$  and AOT/heptane  $w = 20$  are shown. The T-T absorption maximum undergoes a clear blue shift with increasing  $w$ , from 845 nm at  $w = 5$  to 830 nm at  $w = 20$ . This is a further indication of an increase in the polarity of the microenvironment of the dye with  $w$ .



**Fig. 5** Normalized T-T absorption spectrum in water (blue), AOT  $w = 20$  (black) and  $w = 5$  (red).

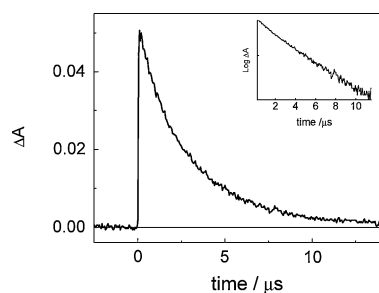
### Triplet quantum yield

Triplet quantum yields were estimated assuming that the absorption coefficient at 830 nm in AOT RMs ( $w = 20$ ) and in EtOH : H<sub>2</sub>O (3 : 1) is the same, *ca.*  $25\,500 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>20</sup> Solutions of matched absorbances at 532 nm were employed and the T-T absorption at the maximum, extrapolated at  $t = 0$ , was taken as measured of the triplet yield. The triplet quantum yields in different media are also collected in Table 1. From the singlet lifetime and triplet quantum yield, the intersystem crossing rate constant  $k_{\text{isc}}$ , can be obtained (Table 1). It can be seen that in RMs  $k_{\text{isc}}$  follows the same trend as in homogeneous solvents, decreasing when the polarity of the medium decreases.<sup>20</sup> The lower value at  $w = 5$  and the red shift in the T-T absorption spectrum ( $\lambda_{\text{max}} = 842 \text{ nm}$  at  $w = 5$ ;  $828 \text{ nm}$  at  $w = 20$ ) indicates that the dye is in a lower polarity environment at the lower water content.

### Triplet quenching by amines

Triethanolamine (TEOA) and tributylamine (TBA) have been used as electron donor in the polymerization of vinyl monomers photoinitiated by safranin/amine systems.<sup>16</sup> Since in these cases the generation of active radicals involves the quenching of the triplet state of the dye by the amine, we decided to investigate this process in reverse micelles.

In RMs the triplet decays faster in the presence of the amines with a first order kinetics. A typical decay of the triplet in the presence of TEOA is presented in Fig. 6.



**Fig. 6** Triplet decay of SfH<sup>+</sup>Cl<sup>-</sup> in AOT/heptane reverse micelles ( $w = 20$ ) in the presence of TEOA 0.01 M.

The apparent quenching rate constant ( $k_q$ ) was obtained from the triplet lifetime (measured by the T-T absorption at 825 nm) as a function of the amine concentration according to

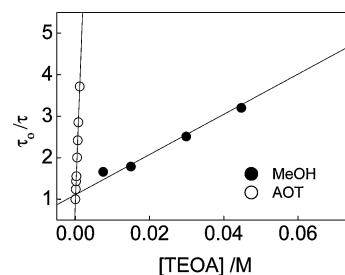
$$\tau^{-1} = k_o + k_q[Q]_{\text{T}} \quad (1)$$

where  $\tau$  is the triplet lifetime,  $k_o$  is the first order rate constant for the triplet decay in the absence of the amine and  $[Q]_{\text{T}}$  is the analytical concentration of the amine.

In Fig. 7 a plot of  $\tau_o/\tau$  vs. the analytical concentration of TEOA is presented in AOT and in MeOH. From the slopes and triplet lifetimes, apparent quenching rate constants  $k_q = 2.9 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  and  $3.8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  were calculated for the micellar medium and MeOH, respectively. The higher quenching in RMs can be understood in terms of an exchange mechanism between micelles. TEOA is a hydrophilic molecule (the octanol-water partition coefficient is 0.1)<sup>24</sup> and it is expected to be totally incorporated into the micellar pseudophase partitioning between the water pool and the polar interface. The aggregation number of AOT in heptane at  $w = 20$  is 230 and the micellar concentration at 0.2 M AOT is  $9 \times 10^{-4} \text{ M}$ .<sup>25</sup> Since, the TEOA concentration in the quenching experiments varies from  $1 \times 10^{-4}$  to  $1 \times 10^{-3} \text{ M}$ , the mean occupation number by AOT is  $\leq 1$ . The molar concentration of the dye is of the order of  $10^{-5} \text{ M}$  and the occupation number is very low. A quenching kinetics scheme in reverse micellar system for a quencher totally incorporated to the micelles leads to a non-exponential decay for the excited state. The decay kinetics of the excited state can be shown to be depicted by eqn (2)–(5)<sup>26,27</sup>

$$[T] = [T]_0 \exp[-C_1 t - C_2(1 - \exp(-C_3 t))] \quad (2)$$

$$C_1 = k_o + \frac{nk_{\text{qm}}k_{\text{ex}}[M]}{k_{\text{qm}} + k_{\text{ex}}[M]} \quad (3)$$



**Fig. 7** Stern-Volmer plot for the triplet quenching of SfH<sup>+</sup>Cl<sup>-</sup> in AOT/heptane RMs ( $w = 20$ ) (○) and in methanol (●).

$$C_2 = \frac{nk_{qm}^2}{(k_{qm} + k_{ex}[M])^2} \quad (4)$$

$$C_3 = k_{qm} + k_{ex}[M] \quad (5)$$

where  $k_{qm}$  is the unimolecular intramicellar quenching rate constant,  $k_{ex}$  is the rate constant for the exchange of solute between micelles and it will depend on the diffusion of two micelles in the organic pseudophase,  $n$  is the mean occupation number and  $[M]$  is the micellar concentration. In the limit of

$$k_{qm} \gg k_{ex}[M] \quad (6)$$

eqn (2) reduces to

$$[T] = [T]_0 \exp[-(k_0 + k_{ex}[Q])t - n(1 - \exp(-k_{qm}t))] \quad (7)$$

and after an initial fast decay the kinetics becomes first order with a lifetime

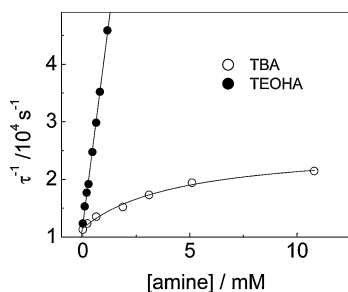
$$\tau^{-1} = k_0 + k_{ex}[Q] \quad (8)$$

Condition (6) is fulfilled in the present case if  $k_{qm} \geq 10^6 \text{ s}^{-1}$ , which is not an unreasonable value for the intramicellar quenching by a neutral quencher.

This interpretation of the quenching mechanism,  $k_q = k_{ex}$ , is further confirmed by the value we find for  $k_q$ , which is of the same order of those reported in the literature for the exchange rate constant between AOT reverse micelles in heptane<sup>26</sup> and isooctane.<sup>27</sup>

The quenching by TBA was also investigated. In homogeneous media this amine behaves as a better quencher of the dye triplet state than TEOA.<sup>28</sup> However, as shown in Fig. 7, TBA is much less efficient quencher in the micellar medium, always in terms of the analytical concentration. This can be explained taking into account the molecular structure of the amines. In a study of the partition of amines in AOT/hexane RMs Zingaretti *et al.*<sup>29</sup> concluded that tertiary amines remain mostly in the organic phase. In fact, the partition coefficients were too small and could not be determined. Therefore, the low quenching efficiency observed for TBA in RMs could be a consequence of the poor access of the amine to the interface where the dye resides.

Interestingly, as can be seen in Fig. 8 the quenching by TBA shows a downward curvature. A similar behaviour was previously observed for the quenching of phenosafranine by aliphatic amines in methanol.<sup>30</sup> It was explained by a quenching mechanism with the participation of a reversible proton transfer step in the triplet excited state. An alternative explanation in the present case is a

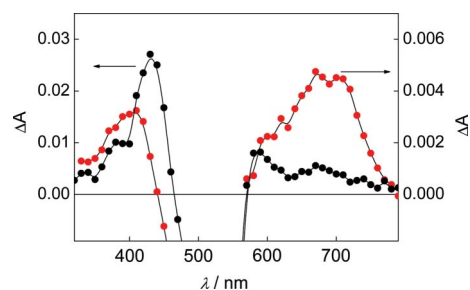


**Fig. 8** Triplet quenching of  $\text{SfH}^+\text{Cl}^-$  triplet by TEOA and TBA in AOT/heptane ( $w = 20$ ).

binding process of the amine to the interface. In this case the downward curvature in the quenching by TBA could be due to the partial saturation of the interface by the amine.<sup>31</sup>

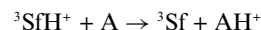
### Transient species in the quenching of $^3\text{SfH}^+$ by TEOA

In Fig. 9 the transient absorption spectra of the dye in the presence of TEOA in AOT/heptane ( $w = 20$ ) and in EtOH :  $\text{H}_2\text{O}$  (3 : 1) are shown. As discussed above, in the latter solvent the photophysical properties of the singlet state and the maximum of the T-T absorption spectrum are similar to those in AOT RMs. Therefore, the polarity of the microenvironment in the RMs may be considered similar to those in the homogeneous mixture. These spectra are taken at the same initial absorption of the dye at 532 nm and with concentration of TEOA that in both cases produces near total quenching of the triplet at the time at which the spectra are taken. At these times, the remaining absorption has not decayed. Nevertheless, as can be seen in Fig. 8 the long time absorption spectrum in RMs differs in both position and intensity from that in the homogeneous solvent mixture.



**Fig. 9** Transient difference absorption spectrum at 10  $\mu\text{s}$  after the laser flash in EtOH :  $\text{H}_2\text{O}$  (3 : 1 w/w) (black) and at 40  $\mu\text{s}$  in AOT/heptane at  $w = 20$  (red). TEOA concentrations are 0.1 M and 0.01 M respectively.

In Table 3 are given the spectral characteristics of the transient species formed in AOT reverse micelles together with those in homogeneous solvents for comparison. From the data in the table the transient species in the different media can be recognized. The spectrum in EtOH: $\text{H}_2\text{O}$  mixture presents two maxima at  $\sim 420$  and  $\sim 580$  nm which can be ascribed to the monoprotonated form of the semireduced dye  $\text{SfH}^+$  by analogy with the reported absorption spectra of this species in aqueous solution.<sup>32</sup> The protonated semireduced dye is formed by a proton transfer process to an amine (A), yielding the unprotonated form of the triplet dye, which in turn undergoes an electron transfer reaction with a second amine molecule.



**Table 3** Summary of the absorption characteristics of the semireduced safranine-O

Medium	Species	$\lambda_{\text{max}}/\text{nm}$	$\epsilon/\text{M}^{-1} \text{ cm}^{-1}$
$\text{H}_2\text{O}$ (pH = 11)	$\text{SfH}^+$	420, 590 <sup>32</sup>	15500, 3300
$\text{H}_2\text{O}$ (pH = 6)	$\text{SfH}_2^{++}$	400, 650 <sup>32</sup>	6900, 9900
EtOH : $\text{H}_2\text{O}$ (3 : 1)	$\text{SfH}^+$	430, 580 [this work]	11600, 4000
AOT $w = 20$	$\text{SfH}_2^{++}$	410, 680 [this work]	6700, 7000

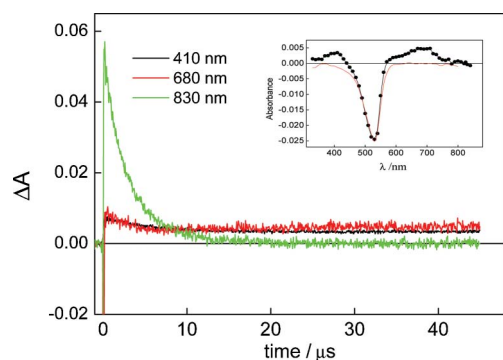


The last step involves a fast in-cage proton transfer from the amine radical cation to the unprotonated radical of the dye.<sup>28</sup>

On the other hand, in AOT RMs the transient absorption spectrum shows two maxima at ~410 and ~670 nm. The diprotonated semireduced form of the dye SfH<sub>2</sub><sup>••</sup> may be responsible for these bands by comparison with the spectrum of this species in water at pH 6 with maxima at 400 and 650 nm.<sup>32</sup> It may be originated in a proton donation from the amine radical cation, yielding SfH<sub>2</sub><sup>••</sup> and the neutral amine radical.



Fig. 10 shows the time profile of the transient absorption in the presence of TEOA.



**Fig. 10** Transient absorption in AOT RMs  $w = 20$  in the presence of TEOA 0.01 M. Inset: spectrum observed at 40  $\mu\text{s}$  showing depletion of the dye ground state.

The semireduced dye quantum yield can be estimated from the transient absorption and the absorption coefficients listed in Table 2. Radical quantum yields were obtained according to

$$\Phi_{\text{R}} = \frac{\Delta A_{\text{R}} \varepsilon_{\text{T}}}{\Delta A_{\text{T}} \varepsilon_{\text{R}}} \Phi_{\text{T}} \quad (9)$$

Here  $\Delta A_{\text{R}}$  is the long time absorption remaining after the triplet decay in the presence of TEOA measured at 410 nm,  $\Delta A_{\text{T}}$  is the prompt T-T transient absorption measured at 830 nm immediately after the laser pulse,  $\varepsilon_{\text{R}}$  and  $\varepsilon_{\text{T}}$  are the respective absorption coefficients and  $\Phi_{\text{T}}$  is the triplet quantum yield in the absence of the amine.

In order to determine  $\Phi_{\text{R}}$  by means of eqn (9), the absorption coefficients of the radical in all media are necessary. They were determined by the ground state depletion (GSD) method.<sup>33</sup> The transient negative difference absorbance at the maximum wavelength of the ground absorption,  $\Delta A_{\text{G}}$ , was compared with the absorption at the maximum of the spectrum of the semireduced dye,  $\Delta A_{\text{R}}$ , with the aid of eqn (10).

$$\varepsilon_{\text{R}} = \left( \frac{\Delta A_{\text{R}}}{\Delta A_{\text{G}}} \right) \varepsilon_{\text{G}} \quad (10)$$

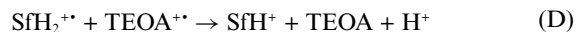
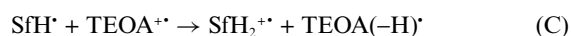
In eqn (10)  $\varepsilon_{\text{R}}$  and  $\varepsilon_{\text{G}}$  are the molar absorption coefficients of the radical and ground state respectively, at the wavelengths of maximum absorbance.

As an example, the transient absorption spectrum of the radical generated in AOT RMs and the normalized ground-state absorption spectrum are shown in the inset of Fig. 10. It can be seen

that the bleaching in the region around the maximum matches the ground-state absorption, as required for the appropriated usage of the GSD method.

In this way values of 0.14 for SfH<sub>2</sub><sup>••</sup> in AOT at  $w = 20$  and TEOA 0.01 M and 0.31 for SfH<sup>•</sup> in EtOH : H<sub>2</sub>O (3 : 1) with TEOA 0.1 M were obtained. The lower radical yield in the reverse micellar system may be due to the predominance of the recombination reaction, in view of the positive charge of the radicals that retains them in the interface at a high local concentration. In spite of the quantum yield being lower in the reverse micellar system it must be considered that the amine concentration used is ten times lower. It is important to point out that the yields actually measured are for the semireduced form of the dye and it is assumed that active alkyl radicals originated from the amine are formed with the same yield.

Summing up the results, the quenching mechanism by TEOA in AOT reverse micelles can be represented by the following scheme:



Reactions (C) and (D) are intramicellar processes. The last step is a back reaction included in order to explain the low value of the radical yield compared with the triplet quantum yield.

In summary, in AOT RMs the mono protonated triplet of safranin-O is formed with similar quantum yield than in organic solvents. A remarkable difference is observed in the quenching by aliphatic amines, while the quenching by hydrophobic tributylamine follows a similar trend as in methanol, the hydrosoluble tri-ethanolamine is one order of magnitude more effective in the RMs than in homogeneous solution. In this case semireduced dye is formed in different protonation state in the RMs but with a comparable quantum yield at a concentration of amine ten times lower. In homogeneous photopolymerization an inhibitory effect of the amine coinitiator at high concentrations was observed.<sup>34</sup> The present results point to the advantage of the reverse micellar system for the generation of active radicals for the initiation of vinyl polymerization.

## Acknowledgements

Financial support from the Agencia Nacional de Promoción Científica (PICT 32351/05, 2213/07) and Universidad Nacional de Río Cuarto is gratefully acknowledged. G.V.P. thanks CONICET for a post-doctoral research fellowship.

## References

- 1 P. L. Liusi and B. E. Straubs, (ed.), *Reverse Micelles*, Plenum Press, New York, 1984; T. K. De and A. Maitra, Solution behaviour of Aerosol OT in non-polar solvents, *Adv. Colloid Interface Sci.*, 1995, **59**, 95–193; A. Mallick, P. Purkayastha and N. Chattopadhyay, Photoprocesses of excited molecules in confined liquid environments: An overview, *J. Photochem. Photobiol., C*, 2007, **8**, 109–127.

- 2 W. D. Van Horn, M. E. Ogilvie and P. F. Flynn, Reverse Micelle Encapsulation as a Model for Intracellular Crowding, *J. Am. Chem. Soc.*, 2009, **131**, 8030–8039.
- 3 M. Wong, J. K. Thomas and T. Nowak, Structure and state of water in reversed micelles, *J. Am. Chem. Soc.*, 1977, **99**, 4730–4736; B. Baruah, J. M. Roden, M. Sedgwick, N. M. Correa, D. C. Crans and N. E. Levinger, When Is Water Not Water? Exploring Water Confined in Large Reverse Micelles Using a Highly Charged Inorganic Molecular Probe, *J. Am. Chem. Soc.*, 2006, **128**, 12758–12765; B. Baruah, L. A. Swafford, D. C. Crans and N. E. Levinger, Do Probe Molecules Influence Water in Confinement?, *J. Phys. Chem. B*, 2008, **112**, 10158–10164.
- 4 M. Zulauf and H. F. Eicke, Inverted micelles and microemulsions in the ternary system water/aerosol-OT/isooctane as studied by photon correlation spectroscopy, *J. Phys. Chem.*, 1979, **83**, 480–486; E. Keh and B. Veleur, Investigation of water-containing inverted micelles by fluorescence polarization. Determination of size and internal fluidity, *J. Colloid Interface Sci.*, 1981, **79**, 465–478; E. Bandez, E. Monnier and B. Valeur, Dynamics of excited-state reactions in reversed micelles. 2. Proton transfer involving various fluorescent probes according to their sites of solubilization, *J. Phys. Chem.*, 1985, **89**, 5031–5036; J. Peyrelasse and C. Boned, Study of the structure of water/Aerosol OT/dodecane systems by time domain dielectric spectroscopy, *J. Phys. Chem.*, 1985, **89**, 370; M. P. Pileni, B. Hickel, C. Ferradini and J. Pucheault, Hydrated electron in reverse micelles, *Chem. Phys. Lett.*, 1982, **92**, 308–312.
- 5 I. Capek, Fate of excited probes in micellar systems, *Adv. Colloid Interface Sci.*, 2002, **97**, 91–149.
- 6 S. C. Bhattacharya, H. Das and S. P. Moulik, Quenching of the fluorescence of safranin T by inorganic ions in aqueous and non-ionic micellar media, *J. Photochem. Photobiol., A*, 1994, **84**, 39–44; P. Ray, S. C. Bhattacharya and S. P. Moulik, Spectroscopic studies of the interaction of the dye safranin T with Brij micelles in aqueous medium, *J. Photochem. Photobiol., A*, 1997, **108**, 267–272; H. W. Gao, Q.-S. Ye and W.-G. Liu, Langmuir aggregation of Nile blue and safranin T on sodium dodecylbenzenesulfonate surface and its application to quantitative determination of anionic detergent, *Anal. Sci.*, 2002, **18**, 455–459.
- 7 Maged A. El-Kemary, Rehab A. Khedr, Safaa El-Din and H. Etaiw, Fluorescence decay of singlet excited-state of safranin T and its interaction with ground-state of pyridinthiones in micelles and homogeneous media, *Spectrochim. Acta, Part A*, 2002, **58**, 3011–3019.
- 8 G. Zhang, Y. Pang, S. Shuang, C. Dong, M. M. F. Choi and D. Liu, Spectroscopic studies on the interaction of Safranin T with DNA in  $\beta$ -cyclodextrin and carboxymethyl- $\beta$ -cyclodextrin, *J. Photochem. Photobiol., A*, 2005, **169**, 153–158.
- 9 D. Bose, D. Sarkar, A. Girigoswami, A. Mahata, D. Ghosh and N. Chattopadhyay, Photophysics and rotational relaxation dynamics of cationic phenazinium dyes in anionic reverse micelles: Effect of methyl substitution, *J. Chem. Phys.*, 2009, **131**, 114707.
- 10 S. Pramanik, S. C. Bhattacharya and T. Imae, Fluorescence quenching of 3,7-diamino-2,8-dimethyl-5-phenyl phenazinium chloride by AgCl and Ag nanoparticles, *J. Lumin.*, 2007, **126**, 155–159.
- 11 K. M. Glenn and R. M. Palepu, Fluorescence probing of aerosol OT based reverse micelles and microemulsions in n-alkanes (C6–C16) and quenching of Safranin-T in these systems, *J. Photochem. Photobiol., A*, 2006, **179**, 283–288.
- 12 R. Chaudhuri, P. K. Sengupta and K. K. Rohatgi Mukherjee, Luminescence behaviour of phenosafranin in reverse micelles of AOT in n-heptane, *J. Photochem. Photobiol., A*, 1997, **108**, 261–265.
- 13 A. Niazi, A. Yazdanipour, J. Ghasemi and M. Kubista, Spectrophotometric and thermodynamic study on the dimerization equilibrium of ionic dyes in water by chemometrics method, *Spectrochim. Acta, Part A*, 2006, **65**, 73–78.
- 14 I. A. Pastre and M. G. Neumann, The effect of microheterogeneous domains in the protonation of the triplet state of safranin-T, *J. Colloid Interface Sci.*, 1996, **179**, 227.
- 15 C. M. Previtali, S. G. Bertolotti, M. G. Neumann, I. A. Pastre, A. M. Rufs and M. V. Encinas, Laser Flash Photolysis Study of the Photoinitiator System for Vinyl Polymerization: Safranin T - Aliphatic Amines, *Macromolecules*, 1994, **27**, 7454–58.
- 16 M. V. Encinas, A. M. Rufs, M. G. Neumann and C. M. Previtali, Photoinitiated Vinyl Polymerization by Safranin T/triethanolamine in Aqueous Solution, *Polymer*, 1996, **37**, 1395–1398; M. L. Gómez, V. Avila, H. A. Montejano and C. M. Previtali, A mechanistic and laser flash photolysis investigation of acrylamide polymerization photoinitiated by the three component system safranin-T/triethanolamine/diphenyliodonium chloride, *Polymer*, 2003, **44**, 2875–2881; M. L. Gómez, H. A. Montejano, M. Bohorquez and C. M. Previtali, Photopolymerization of acrylamide initiated by the three component system safranin/triethanolamine/diphenyliodonium chloride. The effect of aggregation of the salt, *J. Polym. Sci., Part A: Polym. Chem.*, 2004, **42**, 4916–4920; M. L. Gómez, C. M. Previtali and H. A. Montejano, Phenylonium salts as third component of the photoinitiator system safranin O/triethanolamine: A comparative study in aqueous media, *Polymer*, 2007, **48**, 2355–236.
- 17 E. R. Macias, L. A. Rodríguez-Guadarrama, B. A. Cisneros, A. Castañeda, E. Mendizábal and J. E. Puig, Microemulsion polymerization of methyl methacrylate with the functional monomer N-methylolacrylamide, *Colloids Surf., A*, 1995, **103**, 119–126; I. Capek, On the role of oil-soluble initiators in the radical polymerization of micellar systems, *Adv. Colloid Interface Sci.*, 2001, **91**, 295–334; K. Trickett and J. Eastoe, Surfactant-based gels, *Adv. Colloid Interface Sci.*, 2008, **144**, 66–74; B. Schulz, I. Orgzall, I. Diez, B. Dietzel and K. Tauer, Template mediated formation of shaped polypyrrole particles, *Colloids Surf., A*, 2010, **354**, 368–376.
- 18 M. Hasegawa, Buffer-like Action in Water Pool of Aerosol OT Reverse Micelles, *Langmuir*, 2001, **17**, 1426–1431.
- 19 H. A. Montejano, M. Gervaldo and S. G. Bertolotti, The excited-states quenching of resazurin and resorufin by p-benzoquinones in polar solvents, *Dyes Pigm.*, 2005, **64**, 117–124.
- 20 M. L. Gómez, C. M. Previtali and H. A. Montejano, Photophysical properties of safranin O in protic solvents, *Spectrochim. Acta, Part A*, 2004, **60**, 2433–2439.
- 21 M. Hasegawa and A. Kitahara, Microviscosity in water pool of Aerosol-OT reversed micelle determined with viscosity-sensitive fluorescence probe, auramine O, and fluorescence depolarization of xanthene dyes, *J. Phys. Chem.*, 1994, **98**, 2120–2124.
- 22 M. Hasegawa, Buffer-like Action in Water Pool of Aerosol OT Reverse Micelles, *Langmuir*, 2001, **17**, 1426–1431.
- 23 C. D. Borsarelli, S. G. Bertolotti and C. M. Previtali, Thermodynamic changes in the photoinduced proton-transfer reaction of the triplet state of safranin-T, *Photochem. Photobiol. Sci.*, 2002, **1**, 574–580.
- 24 J. Sangster, *Octanol-water Partition Coefficients: fundamentals and physical chemistry*, John Wiley & Sons, Chichester (1997) p. 161.
- 25 D. Grand, Electron Transfer in Reverse Micellar Solutions: Influence of the Interfacial Bound Water, *J. Phys. Chem. B*, 1998, **102**, 4322–4326.
- 26 S. S. Atik and J. K. Thomas, Transport of Photoproduced Ions in Water in Oil Microemulsions: Movement of Ions from One Water Pool to Another, *J. Am. Chem. Soc.*, 1981, **103**, 3543–3550.
- 27 D. M. Togashi and S. M. B. Costa, Excited state quenching kinetics of zinc meso-tetrakis (N-methylpyridinium-4-yl) porphyrin by methyl viologen in AOT reverse micelles, *Phys. Chem. Chem. Phys.*, 2002, **4**, 1141–1150.
- 28 M. V. Encinas, C. M. Previtali, S. G. Bertolotti and M. G. Neumann, The interaction of the excited states of safranin T with aliphatic amines in organic solvents, *Photochem. Photobiol.*, 1995, **62**, 65–70.
- 29 L. Zingaretti, N. M. Correa, L. Boscatto, S. M. Chiacchiera, E. Durantini, S. G. Bertolotti, C. R. Rivarola and J. J. Silber, Distribution of amines in water/AOT/n-hexane reverse micelles: influence of the amine chemical structure, *J. Colloid Interface Sci.*, 2005, **286**, 245–252.
- 30 M. F. Broglia, S. G. Bertolotti and C. M. Previtali, Proton and Electron Transfer in the Excited State Quenching of Phenosafranin by Aliphatic Amines, *Photochem. Photobiol.*, 2007, **83**, 535–541.
- 31 J. J. Silber, A. Biasutti, E. Abuin and E. Lissi, Interactions of small molecules with reverse micelles, *Adv. Colloid Interface Sci.*, 1999, **82**, 189–252.
- 32 S. N. Guha and J. P. Mittal, Pulse radiolysis study of one-electron reduction of safranin T, *J. Chem. Soc., Faraday Trans.*, 1997, **93**, 3647.
- 33 R. Bonneau, I. Carmichael and G. L. Hug, Molar absorption coefficients of transient species in solution, *Pure Appl. Chem.*, 1991, **63**, 289–299.
- 34 M. V. Encinas, E. A. Lissi, A. M. Rufs and C. M. Previtali, Polymerization photoinitiated by carbonyl compounds. X. Methyl, methacrylate polymerization photoinitiated by fluorenone in the presence of triethylamine, *J. Polym. Sci., Part A: Polym. Chem.*, 1994, **32**, 1649–1655; C. M. Previtali, S. G. Bertolotti, M. G. Neumann, I. A. Pastre, A. M. Rufs and M. V. Encinas, Laser flash photolysis of the photoinitiator system for vinyl polymerization: safranin T - aliphatic amines, *Macromolecules*, 1994, **27**, 7454–58.