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# The excited states interaction of safranin-O with low generation carboxyl terminated PAMAM dendrimers in aqueous medium

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## Abstract

The interaction of the singlet and triplet excited states of the synthetic dye safranin-O with carboxyl-terminated poly(amidoamine) (PAMAM) dendrimers was investigated in buffer solution at pH 8. Low half-generation PAMAM dendrimers (G -0.5; G +0.5; G 1.5) were employed. UV-vis absorption spectrum of the dye presents only a very small red shift in the presence of dendrimers. Fluorescence quenching was detected and it was interpreted by a static mechanism in terms of the association of the dye to the dendrimer. Laser flash photolysis experiments were carried out and the transient absorption spectra of the triplet and radicals were obtained. The triplet state is quenched by the dendrimers with rate constants well below the diffusional limit. The quenching process was characterized as an electron transfer process and the quantum yield of radicals was estimated. It was found that radicals are formed with a high efficiency in the triplet quenching reaction.

## INTRODUCTION

Dendrimers are a special class of molecules characterized by a highly branched compact structure of great regularity and they may contain a large number of reactive end groups. Their molecular structure comprises a central core around which successive layers of repeating units of monomer, called generations, are covalently bound. Dendrimers applications range from drug delivery<sup>1</sup> to molecular encapsulation and gene therapy<sup>2</sup> and building blocks for nanostructures.<sup>3</sup> Dendrimers of lower generations (0, 1, and 2) have highly asymmetric shape and possess more open structures as compared to higher generation dendrimers. As the chains growing from the core molecule become longer and more branched, dendrimers adopt a globular structure and they are frequently described as unimolecular micelles. Poly(amidoamine) (PAMAM) dendrimers are among the most commonly used and they are commercially available with different terminal groups such as amino, carboxyl or hydroxyl. PAMAMs full generation are when primary amino are terminal groups, while half generation PAMAMs are termed in the case of carboxyl terminal groups.

The use of synthetic dyes as probe molecules in organized systems is a long standing practice and has been successfully applied to investigate dendrimer properties.<sup>4</sup> In addition, the encapsulation of dye molecules in dendrimers is of interest for its biological applications<sup>5</sup> and the study of the dye-dendrimer interaction have been the subject of several papers.<sup>6,7</sup> At the same time, the photoreactions of dyes in dendritic domains arose particular interest.<sup>8,9</sup> The electron transfer quenching of excited states of dyes by dendrimers has been previously reported.<sup>10,11</sup> The interaction of the xanthene dye eosin Y with full generation PAMAM dendrimers was the subject of previous publications by our group.<sup>12,13</sup> The photoinduced triplet state electron transfer reactions of the dye with PAMAM as an electron donor was characterized and the yield of radicals was determined.<sup>13</sup>

Interest has arisen in recent years for the use of dendrimers in macrophotoinitiator systems of photopolymerization.<sup>14</sup> In particular, the use of dendrimers as co-initiators of vinyl photopolymerization seems to be promising in order control structural features of the resulting polymer. In this sense, PPI dendrimers were employed as co-initiators in photoinitiated radical polymerization.<sup>15</sup> The polymerization was carried out with UV

radiation employing benzophenone and thioxanthone as sensitizers. The researchers suggested the presence of stretched polymer chains around the dendrimer as the structure of the resulting polymers.

Macrophotoinitiators were also prepared using DAB dendrimers and thioxanthone as sensitizer in the UV.<sup>16</sup> These initiators were found suitable for use in aqueous systems, and it was therefore of interest to investigate the extension of their use to the visible zone of the spectrum. Synthetic dyes are an adequate choice for use as visible sensitizers in aqueous media. We have previously investigated several of these photoinitiating systems operating in the visible region.<sup>17</sup> In particular, safranin-O ( $SF^+$ ) was widely employed as a visible sensitizer of vinyl polymerization with aliphatic amines as electron donors.<sup>18</sup> The photochemistry of this photoinitiating system was well characterized.

Previously we have investigated the interaction of  $SF^+$  with whole generation PAMAM dendrimers in methanolic solution.<sup>10</sup> In this paper we present a study of the interaction of the dye with low generation carboxyl-terminated PAMAM dendrimers in aqueous media, with particular emphasis on the triplet excited state processes.

(For structures see **Scheme 1**)

## EXPERIMENTAL

Safranine-O ( $\text{SF}^+\text{Cl}^-$ ) was purchased from Aldrich ( $\geq 85\%$ ) and was recrystallized from ethanol. It was checked that its photophysical properties coincided with those reported in the literature.<sup>19</sup> The ground state absorption coefficient at the wavelength maximum (520 nm) was determined as  $26000 (\pm 500) \text{ M}^{-1}\text{cm}^{-1}$ . The final analytical concentration of the dye was *ca.*  $5 \times 10^{-6} \text{ M}$  in fluorescence determinations and *ca.*  $1 \times 10^{-5} \text{ M}$  in laser flash photolysis experiments, as determined by its absorption coefficient. The dendrimers were also from Aldrich and were obtained as methanolic solution in the case of PAMAM generation 0.5 and 1.5 (PAMAM G +0.5 and PAMAM G 1.5) and solid in the case of generation -0.5 (PAMAM G -0.5). Unless otherwise stated all experiments were carried out with borate buffer (0.025 M) at pH 8.

Absorption spectra were obtained by using a Hewlett Packard 6453E diode array spectrophotometer. Steady state fluorescence experiments were carried out with a Spex Fluorolog spectrofluorometer. Fluorescence lifetime measurements were done with the time correlated single photon counting technique using Edinburgh Instruments OB-900 equipment. The fluorescence measurements were carried out with air-equilibrated solutions. Transient absorption spectra and triplet quenching were determined by laser flash photolysis with a Spectron SL400 Nd:YAG laser generating 532 nm laser pulses (20 mJ per pulse, 18 ns FWHM). The laser beam was at right angle of the sample cell and was defocused in order to cover all the path length (10 mm) of the analyzing beam from a 150 W Xe lamp. The detection system comprises a Hamamatsu R666 PM tube coupled to a PTI monochromator. The signal was acquired by a digitizing scope (Hewlett-Packard 54504) where it was averaged and then transferred to a computer. For the laser photolysis experiments the solutions were de-oxygenated by bubbling during 30 min with solvent saturated high purity argon and all determinations were performed at  $20 \pm 1^\circ\text{C}$ .

## RESULTS AND DISCUSSION

### Absorption and fluorescence spectra

Safranine ground state is in its monocationic form in the pH range 2-11 and there are not changes in the absorption spectrum in this pH range.<sup>20,21</sup> The UV-vis absorption spectrum of the dye is practically unaltered by the presence of the dendrimers. Only a small red shift can be observed at the higher concentrations employed. In Figure 1 it is shown the absorption spectrum of safranine-O in the presence of the PAMAM G 1.5 at 2.8 mM.

According to the chemical structure of carboxylated PAMAM dendrimers there are subsets of protonation sites, i.e. the internal tertiary amine groups and the terminal carboxylates. To establish the protonation state of the molecule requires a knowledge of the equilibrium constant for each protonation site and this is difficult to assess due to the energy cost for charging neighbor sites. Nevertheless, it is safe to assume that the terminal carboxylate groups are deprotonated at the working pH 8. Assuming that the inner tertiary amine groups are protonated in a similar fashion to those in amino terminal PAMAMs it may be concluded that at pH 8 they are in its neutral form.<sup>22</sup>

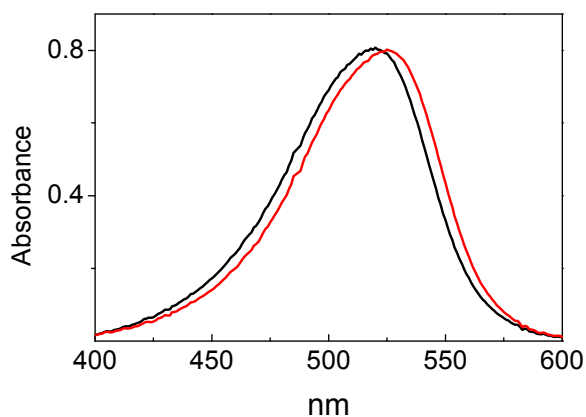
In Figure 2 are shown the fluorescence spectra of SF<sup>+</sup> in the presence of increasing concentration of PAMAM G 1.5. The shape and position of the spectra are scarcely affected by the presence of the dendrimer, even at the higher concentration investigated. However, a quenching of intensity occurs. The fluorescence quenching proceeds by a static mechanism, since the excited singlet lifetime of the dye, 1.3 ns, is practically unaffected by the highest dendrimer concentration used in all the three cases. Stern Volmer plots are shown in **Figure 3**. The solid lines were adjusted to a binding model (see below). A summary of the absorption and fluorescence parameters are presented in **Table 1**.

**Table 1:** Absorption and fluorescence parameters and binding constants

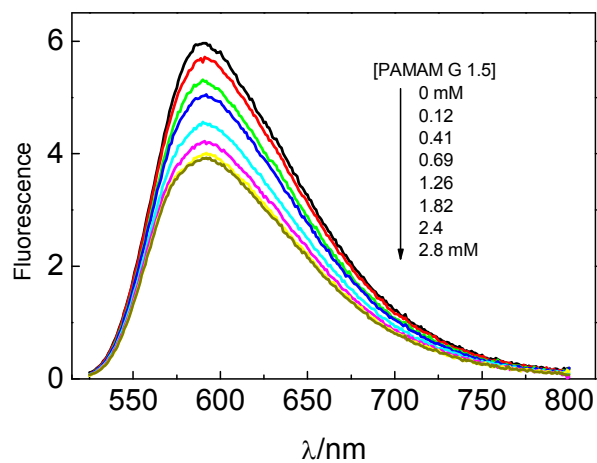
	$\lambda_{\max}(\text{abs}) / \text{nm}$	$\lambda_{\max}(\text{flu}) / \text{nm}$	$K_{\text{bind}} / \text{M}^{-1}$
Buffer pH 8	520	588	---
G -0.5	522	589	11
G +0.5	522	589	71
G 1.5	525	589	606

Estimated error of  $K_{\text{bind}} \pm 10\%$

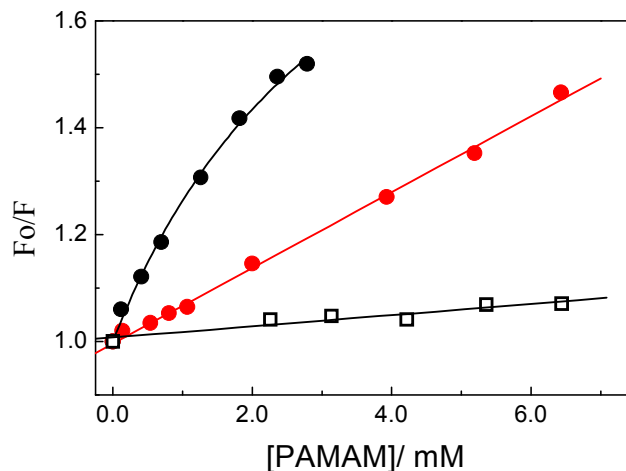




**Figure 1:** Absorption spectrum of SF<sup>+</sup> in the absence (black) and the presence of G 1.5 (2.8 mM red)



**Figure 2:** Fluorescence spectra of SF<sup>+</sup> as a function of G 1.5 concentration



**Figure 3:** Stern-Volmer plots for the quenching of SF fluorescence by PAMAM dendrimers G -0.5 (□), G +0.5(●), G 1.5 (●)

## Binding

From a simple equilibrium model between SF<sup>+</sup> and PAMAM dendrimers, assuming a 1:1 stoichiometry for the SF/dendrimer complex (SFPm) a binding constant may be written as

$$K_{bind} = \frac{[SFPm]}{[SF^+]_f [Pm]_f} \quad (1)$$

Where  $[Pm]$  stands for the PAMAM concentration and the subscript  $f$  indicates the not bound species.

Equivalent and independent binding sites were assumed in Eq. (1). The same assumptions have been made by other authors to determine  $K_{bind}$  values of PAMAM with

several drugs.<sup>29, 30</sup> Under these conditions the fraction of  $SF^+$  molecules that are bound to the dendrimer  $\alpha$  can be expressed in terms of the fluorescence intensity as follows<sup>23</sup>

$$\alpha = \frac{[SFPm]}{[SF^+]_t} = \frac{F_0 - F}{F_0 - F^*} \quad (2)$$

where  $[SFPm]_t$  is the total analytical concentration of the dye, and  $F_0$  and  $F$  stand for fluorescence intensity in the absence and presence of dendrimer.  $F^*$  is the residual emission when hypothetically all the dye would be bonded to dendrimer. In terms of the binding constant  $K_{bind}$  the dependence of the fluorescence intensity with the PAMAM concentration can be written as

$$F^0 - F = \frac{K_{bind} [Pm](F^0 - F^*)}{1 + K_{bind} [Pm]} \quad (3)$$

Note that in this equation  $[Pm]_f$  has been replaced by  $[Pm]$ , the total dendrimer concentration. This assumption is reasonable because the dendrimer concentration was at least 200-fold greater than that of the dye for all generations used.

Equation (3) may be rearranged to a form similar to a Stern-Volmer plot,  $F^0/F$ .

$$\frac{F^0}{F} = \frac{1 + K_{bind} [Pm]}{1 + (F^*/F^0)K_{bind} [Pm]} \quad (4)$$

Eq. (4) reduces to a linear form in absence of residual emission ( $F^*=0$ ), and a Stern-Volmer linear plot of  $F^0/F$  results, with  $K_{bind} \equiv K_{SV}$  as is the case for G -0.5 and G +0.5.

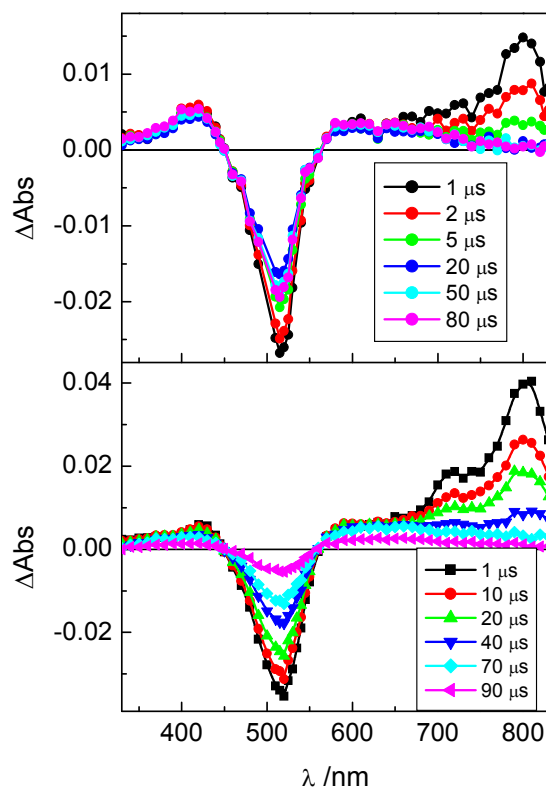
The values of the binding constants obtained by the fitting of the data to eqn (4) are also collected in **Table 1**. In spite of the opposite charge of the dye molecules and the carboxylate groups, the binding constants are much lower than those for the case of a xanthene dye, eosin Y, interacting with full generation amino terminal PAMAM dendrimers.<sup>13</sup> Thus in the case of eosin Y- PAMAM G1  $K_{bind} = 1160 \text{ M}^{-1}$  and  $5600 \text{ M}^{-1}$  for

the case of G 2. These values are near one order of magnitude higher than those for the binding of SF<sup>+</sup> to PAMAM dendrimers with the same number of terminal groups, with the difference of them being carboxylate instead of amino groups. The high value of the binding constant was also observed for other xanthene dyes.<sup>24</sup> Moreover, for the fluorescence quenching of Eos by PAMAM G0 and G1 the SV plots present a noticeable downward curvature, which is absent for the low fractional generation PAMAM in its interaction with SF<sup>+</sup>. It must be noted that both dyes are of comparable molecular size. This effect is probably related to specific effects in the interaction of the dyes with PAMAM dendrimers. When similar experiments were done on the fluorescence quenching of SF<sup>+</sup> by PAMAM G 2 (data not shown) the apparent  $K_{bind}$  was of the same order of magnitude than for G 1.5. Therefore, the differences must be ascribed to the dyes and not to a general effect of the PAMAM structure when whole generation are compared with half generation dendrimers of similar size. This result is of importance in relation to the application of PAMAM dendrimer as drug delivery agents.

### Triplet state

The effect of PAMAM G -0.5 to G 1.5 on the triplet state of the dye was investigated by laser flash photolysis. Ground state absorption and fluorescence spectra of safranin do not show any change in the presence of the dendrimers at the concentrations used in the triplet state experiments. The absorption and emission maxima at 521 nm and 588 nm respectively, are not altered by the presence of PAMAM of generations -0.5 to 1.5. Since absorption and fluorescence spectra of SF<sup>+</sup> are highly dependent on solvent polarity,<sup>25</sup> this can be taken as an indication that the dye is not incorporated to the inner dendrimer structure, even for the higher generation explored in this experiments, and in spite of the opposite charge of the dye-dendrimer system. This is further confirmed by the transient absorption spectrum in the presence of dendrimers following excitation of the dye at 532 nm. In Figure 4 the spectra in the absence and the presence of PAMAM G 1.5 are shown. It can be seen that in the absence of the dendrimer the absorption decays

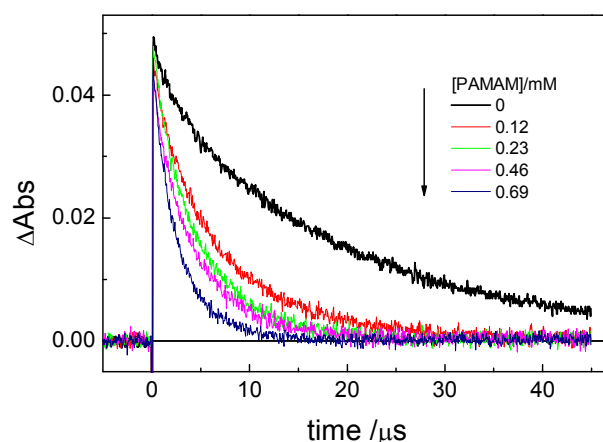
monotonically in all the spectral region of interest, while in the presence of PAMAM a long lived absorption remains in the region of 400 nm.



**Figure 4:** Transient absorption spectrum of  $\text{SF}^+$  in the presence of PAMAM G 1.5 ( $7 \times 10^{-4} \text{ M}$ ) top panel, and in the absence (lower panel) in buffer pH 8. Insets: Time in microseconds after the laser pulse.

The spectrum extrapolated at  $t = 0$  is practically the same in the presence and the absence of PAMAM for all generations (data not shown) and corresponds to the T-T absorption of the dye.<sup>19</sup> A strong pH dependence of the triplet–triplet transient absorption spectra of  $\text{SH}^+$  has been reported.<sup>20</sup> In buffer at pH 8 the absorption around 800 nm can be assigned to the monocationic form of the triplet state of the dye,  $^3\text{SF}^+$ .

For the dye alone, in buffer pH 8, the triplet decays in tens of microseconds, mainly by self-quenching, to the semireduced and semioxidized forms of the dye. In the presence of the dendrimers, the decay time becomes shorter and follows a first order kinetics. The decays are shown in **Figure 5** for the case of PAMAM G 1.5.

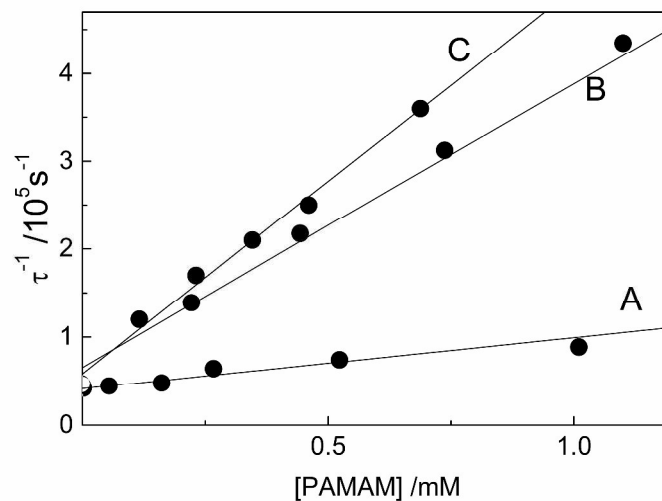


**Figure 5:** Triplet decay at 800 nm as a function of PAMAM G1.5 concentration.

Triplet quenching rate constants were determined by monitoring the decay of the absorption at 800 nm as function of PAMAM concentration according to eqn (5)

$$\tau^{-1} = \tau_o^{-1} + k_q [Pm] \quad (5)$$

where  $\tau_o$  and  $\tau$  stand for the triplet lifetime in the absence and the presence of the dendrimer  $Pm$ , respectively. Linear plots were obtained for the three PAMAM investigated, **Figure 6**, and the rate constants are collected in Table 2. At difference of what is observed in the triplet quenching of  $SF^+$  by amino terminal PAMAMs in methanol where a downward curvature resulted, here linear plots were obtained in all cases.



**Figure 6:** SF<sup>+</sup> triplet quenching by PAMAM G -0.5 (A); G +0.5 (B); G 1.5 (C)

**Table 2.** Rate constants for the quenching of the excited triplet state of SF<sup>+</sup> by carboxyl terminated PAMAM dendrimers

PAMAM	$k_q / M^{-1}s^{-1}$
G -0.5	$5.9 \times 10^7$
G +0.5	$3.2 \times 10^8$
G 1.5	$4.4 \times 10^8$

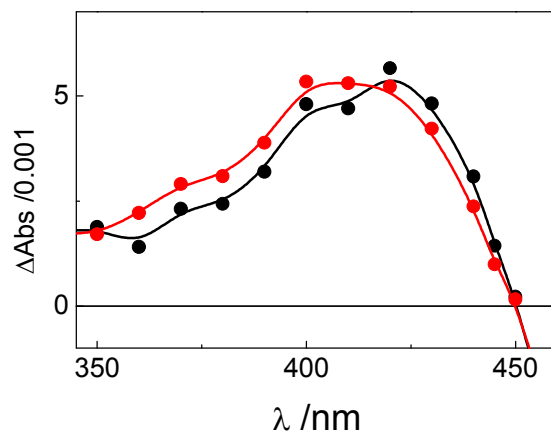
The quenching process may be assigned to the one-electron reduction of the dye. It is well known that the triplet is efficiently quenched by aliphatic amines<sup>26</sup> and by amino terminal PAMAM dendrimers in methanol. In the present case, there are not terminal primary amino groups and the quenching most likely process by collisional electron transfer from the secondary and tertiary amino groups of the dendrimer. Although there is an increase of the bimolecular quenching rate constant, this is not in accordance with the number of secondary and tertiary amine groups in the dendrimer. Besides, the rate constants are, in the most favourable case, one order of magnitude lower than the diffusional limit in water ( $\sim 5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ). Some specific effect of the dendrimer structure is playing a role in the quenching mechanism, precluding all amino groups to be accessible for quenching.

### Radical yields

As above mentioned, it is of interest to explore the possible use of the system  $\text{SF}^+$  - PAMAM half generation dendrimers as co-initiator of vinyl polymerization. A point of relevance related to this application is the radical yield of the system. In order to make feasible the use of the  $\text{SF}^+$ -PAMAM in aqueous media we choose the negative carbonyl terminated dendrimers to make sure of the solubility of both the dye and the co-initiator in this environmental friendly condition. In this sense, we have here undertaken the determination of radical yields in the triplet quenching of  $\text{SF}^+$  by carboxyl terminated PAMAM dendrimers. For this we assume that the yield of the semireduced form of the dye is a valid quantum counter of the total radical yield in the system.

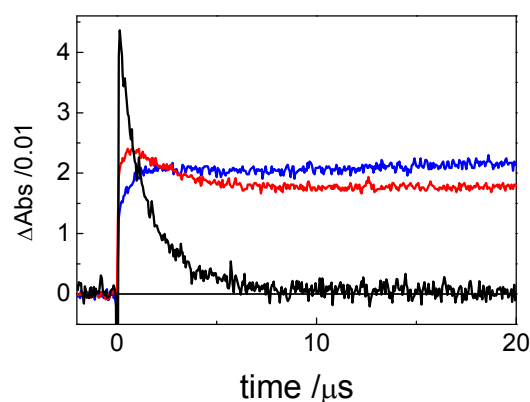
From the spectrum in **Fig. 4** it can be seen that in the presence of the dendrimer a long-lived absorption remains in the region of 400 nm. In **Figure 7**, an enlargement of the absorption in the region of 350-450 nm in the presence of PAMAM G 1.5 is shown. The initial spectrum matches the T-T absorption with an apparent maximum at 420 nm.<sup>27</sup> It is clear that the spectrum at 80  $\mu\text{s}$  after the laser pulse presents essentially the same difference absorbance, but a clear blue shift is apparent with a maximum ca. 400 nm. Guha and Mittal<sup>21</sup> found by pulse radiolysis experiments that the monoprotonated semireduced form of the dye has a peak at 400 nm. Therefore, the absorption at this wavelength may be useful to determine the radical yield in the quenching reaction.





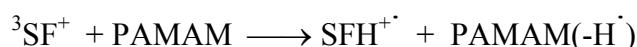
**Figure 7:** Transient absorption spectrum of  $\text{SF}^+$  in the presence of PAMAM G 1.5 0.7 mM at  $t = 0$  (black) and  $80 \mu\text{s}$  (red)

In **Figure 8** it is shown an example of the determination of the radical quantum yield for the quenching of the triplet state by PAMAM G +0.5.



**Figure 8:** Absorbance profiles after the laser pulse of  $\text{SF}^+$  in the presence of PAMAM G +0.5 (3.9 mM) at 800 nm (black), 400 nm (blue), 420 nm (red).

The time profile of the absorption at 420 nm shows an initial growth that follows the triplet decay at 800 nm. Initially the absorption overcomes that at 400 nm, and afterwards decays to a lower level. This changes may be explained by the initial formation of the neutral radical  $SF^{\cdot}$  with absorption maximum at 400 nm and its subsequent protonation to  $SFH^{+\cdot}$  with absorption maximum at 400 nm.<sup>21</sup> Therefore, the quenching process may be written as



assuming that after the initial electron transfer quenching reaction a fast proton transfer process takes place yielding the active PAMAM based radical and the protonated semireduced form of the dye.

From the trace at 400 nm, with the extinction coefficient for the protonated semireduced species,  $6900 \text{ cm}^{-1}\text{M}^{-1}$  from ref 21, the radical quantum yield can be obtained according to<sup>28</sup>

$$\Phi_R = \frac{\Delta A_R \varepsilon_T}{\Delta A_T \varepsilon_R} \Phi_T \quad (6)$$

Here  $\Delta A_R$  is the long time absorption after the triplet decay in the presence of PAMAM measured at 400 nm,  $\Delta A_T$  is the prompt T-T transient absorption measured at 800 nm immediately after the laser pulse,  $\varepsilon_R$  and  $\varepsilon_T$  are the respective absorption coefficients and  $\Phi_T$  is the triplet quantum yield in the absence of the quencher ( $\varepsilon_T = 16200 \text{ cm}^{-1}\text{M}^{-1}$ ;  $\Phi_T = 0.28$  from ref 19).

The radical quantum yields are collected in **Table 3**. Also in the table is the fraction of triplet states intercepted by the quenchers at the concentration employed in the radical yield determinations, according to:

$$f = \frac{k_q[Pm]}{k_o + k_q[Pm]} \quad (7)$$

where  $k_q$  is the triplet quenching rate constant as in Table 2 and  $k_o$  is the first order decay rate constant of  $SF^+$  in the absence of the dendrimer,  $5 \times 10^4 \text{ s}^{-1}$ .

**Table 3:** Radical quantum yields in the triplet quenching of  $SF^+$  by PAMAM dendrimers of intermediate generation

PAMAM	f	$\Phi_R$
G -0.5	0.94	0.28
G + 0.5	0.96	0.30
G 1.5	0.86	0.16

Taken into account the experimental errors of the laser flash photolysis absorption measurements<sup>29</sup> and the uncertainty in the literature values of the absorption coefficients,<sup>20,21</sup> it may be assure that every quenching event leads to active radicals in the case of PAMAM G -0.5 and G +0.5, while only a fraction close to 70 % is effective in the case of G 1.5. This may be related to the more complex structure of the dendrimer and a more likely retention of the initially formed radicals favouring the back recombination of them.

## Conclusions

The quenching of the singlet excited state of safranin by low generation carboxyl terminated PAMAM dendrimers follows a static mechanism and the dye-dendrimer binding

constant could be evaluated from Stern-Volmer plots of the fluorescence intensity. The binding constant are much lower than those observed for the xanthene dye eosin-Y with amino terminated PAMAM with the same number of peripheral groups. On the other hand, the triplet quenching follows an electron transfer kinetics with rate constants that increase with the generation of the dendrimer. From laser flash photolysis experiments the radical quantum yield could be estimated and it results in a high efficiency for the triplet quenching process. This renders these systems as possible candidates to be used in photoinitiating systems of vinyl photopolymerization.

### **Acknowledgments**

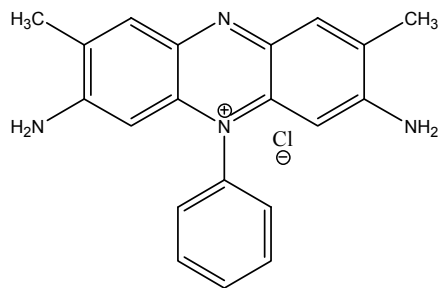
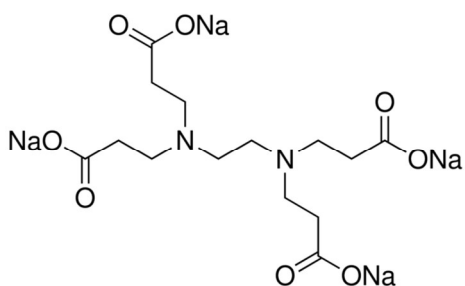
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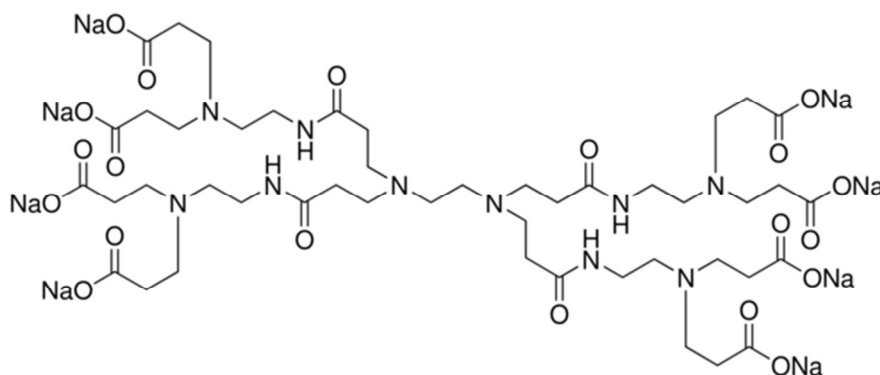
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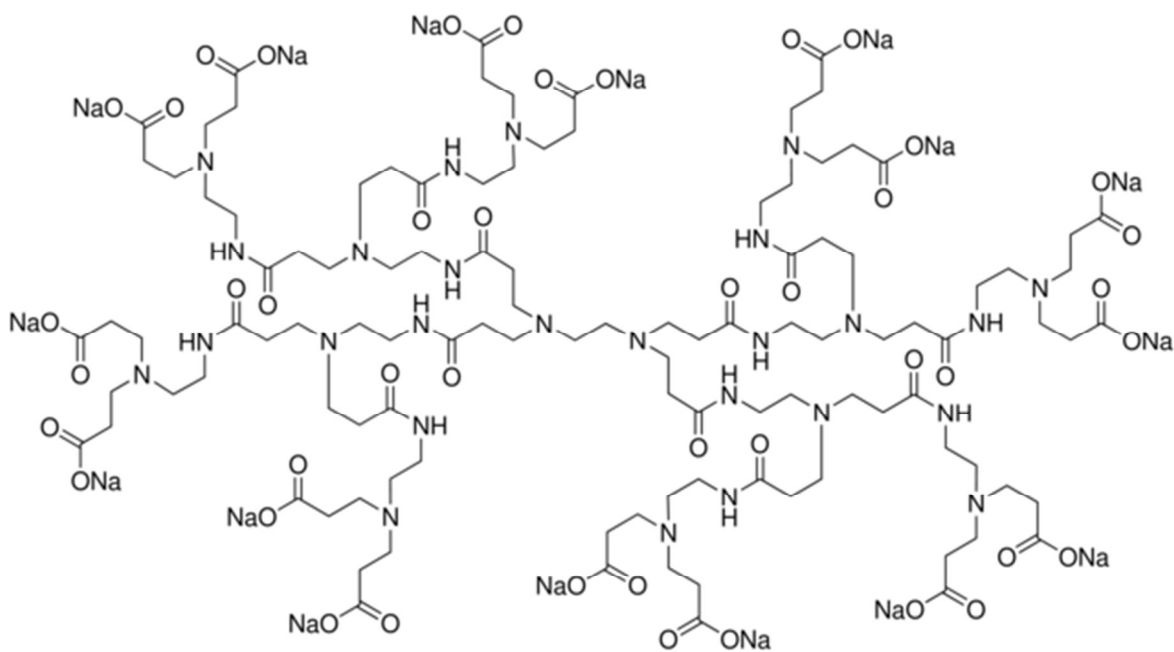
G -0.5

Safranin-O

G +0.5

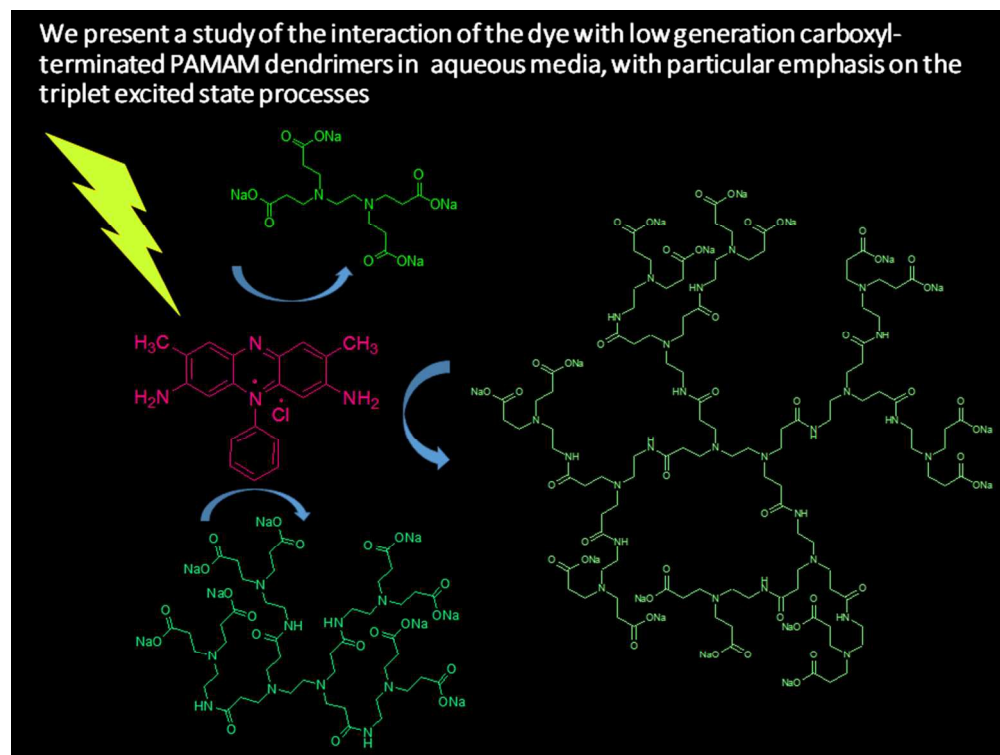


G 1.5



**Scheme 1:** Half-generation poly(amidoamine) (G-0.5; G +0.5 and G1.5 PAMAM) dendrimers and safranin-O structures





254x190mm (96 x 96 DPI)