# Metal-Enhanced S_1 and Alpha- S_1 Fluorescence: Effects of Far-Field Excitation Irradiance on Enhanced Fluorescence

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ABSTRACT: Metal-enhanced fluorescence (MEF) and delayed fluorescence (alpha α-S_1) studies of acriflavin have been undertaken using steady-state and time-resolved fluorescence measurements on silver island films (SiFs) supporting a thin poly(vinyl alcohol) (PVA) polymer film. Nearly a 3.5-fold enhancement in the far-field fluorescence intensity of acriflavin is observed coupled with a decrease in decay times and an improved fluorophore photostability. Our findings are consistent with our proposed model of the coupling and transferring of the excited state energies of fluorophores to silver surface plasmons. Further, an excitation power dependence of MEF is observed in these films, consistent with our postulate of MEF EVE (MEF excitation volumetric effect) and, ultimately, resulting in a ~7-fold enhancement in fluorescence intensity. Our findings suggest a significant opportunity for the development of shorter-lived, brighter, high photon flux luminescent materials for multifarious applications in the life-sciences.
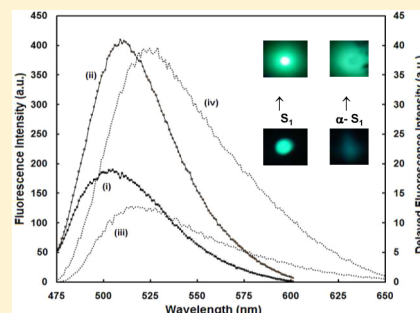
1.0. INTRODUCTION

In recent years, there has been growing interest in understanding the interactions of fluorophores with metallic particles.[1,2] Spectral properties of fluorophores are found to be dramatically changed near particulate surfaces due to near-field (<one λ of light) surface plasmon interactions.[3−5] A huge enhancement in fluorescence intensity coupled with an increased absorption, a decrease in decay times, an increase in photostabilities, and an angular-dependent emission is most frequently observed.[6,7] These effects have subsequently been named metal-enhanced fluorescence (MEF) by Geddes[6] in years past. The current interpretation of MEF has been underpinned by a radiating plasmon model (RPM),[8] whereby nonradiative energy transfer occurs from excited fluorophores to surface plasmons in noncontinuous films, due to fluorophore-plasmon near-field coupling. The surface plasmons, in turn, efficiently radiate the emission of the coupling fluorophores through the scattering mode of the nanoparticles extinction spectrum.[3,5] Our laboratories have both studied and demonstrated many applications of metal-enhanced fluorescence (MEF).[9] These have included the increased detectability and photostability of fluorophores,[10] chemiluminescent species,[11] and improved DNA detection,[12] drug discovery, and high-throughput screening[13] immunoassays,[14] singlet oxygen generation,[15] etc., to name but just a very few.

Nearly all previous MEF studies were carried out on metal nanoparticle-coated glass slides. Polymeric substrates may, however, provide a suitable, cheap, and versatile alternative to glass substrates. MEF from modified polycarbonate surfaces has been shown to produce nearly similar enhancements as compared to glass.[16,17] In the present paper we have studied the photophysics of acriflavin close-to silver nanostructures. Acriflavin is a well-known and important molecule, having

antibacterial and antibiotic[18−22] properties, and it is widely used in DNA studies within cells[23,24] and for sensing over a broad pH range.[25,26] It belongs to the acridine dye family, which shows delayed fluorescence (α-$S_1 \rightarrow S_0$) and phosphorescence ($T_1 \rightarrow S_0$) at room temperature, along with traditional fluorescence ($S_1 \rightarrow S_0$) in the wavelength range 475 to 700 nm. The long-lived fluorescence, i.e. delayed fluorescence of polyatomic molecules in rigid media, arises due to several different mechanisms.[27] The population of the excited singlet state (S_1) by thermal activation of the triplet state (T_1) is responsible for E type delayed fluorescence, whereas a triplet—triplet annihilation process gives rise to P-type delayed fluorescence. The third type of delayed fluorescence is attributed to the recombination of radical ions and matrix trapped electrons produced by photoionization of the molecules. Because of its long decay time, delayed fluorescence has been widely used to investigate the rotational diffusion time of biological macromolecules in membranes and also used to characterize metal oxide surfaces.[15] The photoionization of the acridine molecule in rigid glass has been known for some time; however, Lim et al.[28] reported the delayed fluorescence at 77K in acriflavin due to recombination of electrons with ions. Sato et al.[29] proposed the origin of long-lived delayed fluorescence due to the formation of an excimer in PVA sheets. Gangola et al.[30] explained the decrease in intensity and decay time with an increase in concentration as due to Coloumbic attraction between the charged cation and trapped electrons. However, recently the fluorescence resonance energy migration phenomena has explained the decrease in decay time and red shift in

delayed fluorescence, with the increase in concentration of acriflavin in a PVA film.[31,32] The efficiency of energy migration was also reported to be at a maximum in acriflavin doped PVA (protic matrix) with respect to an aprotic polymer matrix.[33]

The photophysical properties of long-lived luminescent species are found to be both drastically and favorably changed near the metallic nanoparticle surfaces in ways that provide an opportunity for the development of shorter-lived brighter higher photon flux luminescent materials that can be realized downstream as shown in Scheme I. Earlier, MEF studies from

Scheme I. Cartoon Depicting the MEF Effect for Long-Lived Luminescent Species[a]
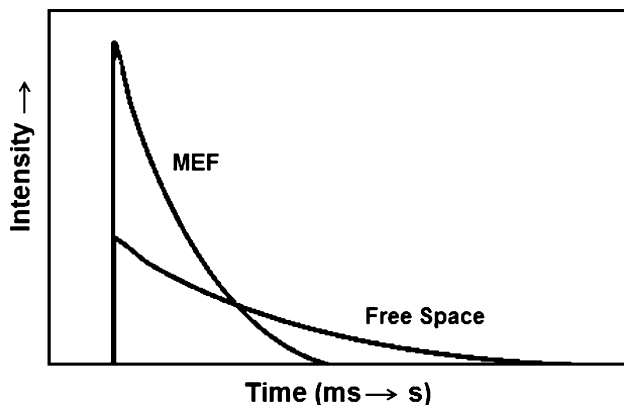
[a]The intensity increases along with a shorter decay time as compared to the free space condition. In essence, shorter-lived highly luminescent materials can be realized.

our group were exclusively focused on E and P type delayed emission of eosin[34] and the pyrene[35] dyes, respectively, on glass slides with a 2.5-fold far field enhancement in fluorescence reported. In this paper, a 3.5-fold far field enhancement is observed both in fluorescence ($S_1 \rightarrow S_0$) and delayed fluorescence (α-$S_1 \rightarrow S_0$) of acriflavine doped silver island films with respect to a control sample film (having no SiF's). Our findings are not only helpful for understanding the photophysics of acriflavin−metal interactions but also for the numerous practical applications that enhanced delayed fluorescence yields for the next generation of smart fluorescent polymers. Given the widespread use of MEF,[36−40] our approach to increase the absolute emission intensities of luminescent plastics while simultaneously reducing the decay time presents an opportunity for modulating (tuning) the photon flux of polymeric materials and even in the potential developments of energy efficient plastics.

2.0. EXPERIMENTAL SECTION

2.1. Materials. Acriflavin, Silane-prep glass microscope slides, Silver nitrate (99.9%), sodium hydroxide (99.996%), ammoniumhydroxide (90%), D-glucose, ethanol (HPLC/spectrophotometric grade), and poly(vinyl alcohol) (PVA) [molecular weight 70,000] were purchased from Sigma-Aldrich Chemical company (Milwaukee, WI, USA).

Silver colloids and SIFs were prepared according to previously published procedures.[9−12] The synthesis of silver colloids was undertaken using the following procedure: 2 mL of 1.16 mM trisodium citrate solution was added dropwise to a heated (90 °C) 98 mL aqueous solution of 0.65 mM silver nitrate while stirring. The mixture was heated for 10 min and

then cooled in ice until use. This procedure yields ca. 50 nm silver colloids as confirmed by TEM analysis. Three hundred microliters of acriflavin dissolved in 1.0 wt % PVA in 10 mL of water was dispersed over the SiFs coated glass slides.

2.2. Instrumentation. Absorption spectra of acriflavin on blank glass substrates and SiFs containing the PVA polymer films were collected using a single beam Varian Cary 50-Bio UV−vis spectrophotometer. Emission spectra were collected using a Varian Cary Eclipse fluorescence spectrophotometer having a pulsed xenon source for excitation. A front face sample geometry was used to undertake all the fluorescence measurements with a slit width of 5 nm, in both the excitation monochromator and emission monochromator channels. Fluorescence decays were measured using a Horiba Jobin Yvon Tem Pro fluorescence lifetime system employing the multiscale analysis technique, with a TBX picosecond detection module. The excitation source was a pulsed LED source of wavelength 372 nm having maximum repetition rate 1.0 MHz and pulse duration 1.1 ns (fwhm). The intensity decays were analyzed by decay analysis software (DAS) version 6.4 in terms of the multiexponential model:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \tag{1}$$

where α_i are the amplitudes and τ_i are the decay times, $\sum_i \alpha_i = 1.0$. The fractional contribution of each component to the steady state intensity is given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \tag{2}$$

The mean lifetime of the excited state is given by

$$\bar{\tau} = \sum_i f_i \tau_i \tag{3}$$

and the amplitude-weighted lifetime is given by

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \tag{4}$$

The values of α_i and τ_i were determined by nonlinear least-squares impulse reconvolution analysis with the goodness of fit judged by the residual, autocorrelation function and χ^2 values. The measurement error in decay time analysis was of the order of 0.01 ns. Photostability experiments where undertaken using a 405 nm laser line coupled with a neutral density filter and Ocean optics spectrophotometer HP2000. Real-color photographs of the fluorescence emission were taken through an emission filter with a Canon Power shot S50 Digital Camera.

3.0. RESULTS AND DISCUSSION

The absorption spectra of acriflavin doped in PVA controlled with and without SiFs are shown in Figure 1. The absorption maximum of acriflavin was 466 nm in PVA with an optical density ≈0.18, but in the presence of SiFs, the optical density increases nearly 2-fold. This effect can be explained as a result of the coupling of the fluorophore/lumophore dipole with the localized electromagnetic field of the metallic particles, efficiently increasing the absorption cross-section of the lumophore. It is well-known that conducting metallic particles can modify the free-space absorption condition in ways that increase the incident electric field, felt by fluorophores, effectively increasing the excitation rate of the fluorophore.[5] The enhanced absorption of dye molecules near metallic
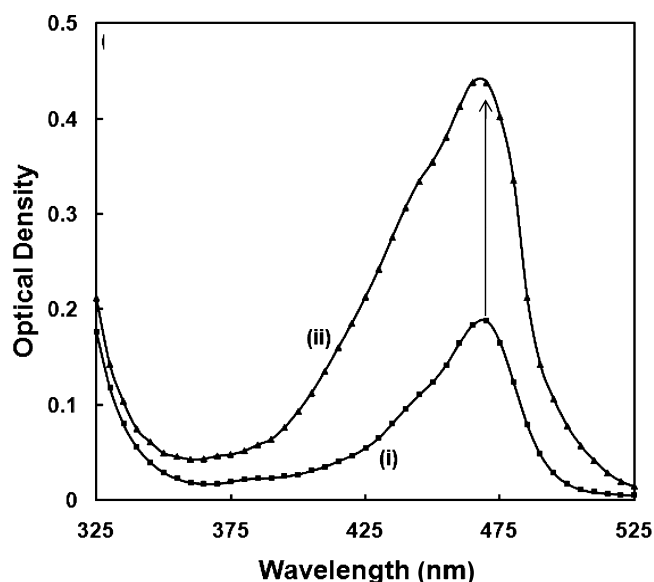
Figure 1. Absorption spectra of (i) PVA and (ii) SiFs deposited on PVA film (λ_{ex} = 550 nm).
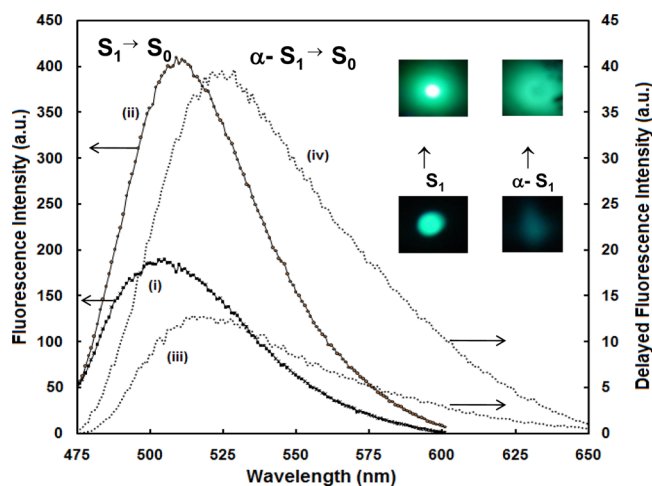

Figure 2. Fluorescence spectra of acriflavin in (i) PVA and (ii) SiFs doped PVA and the delayed fluorescence spectra of acriflavin in (iii) PVA and (iv) SiFs doped PVA, respectively (λ_{ex} = 450 nm). Enhancement factor ∼3.5. (Excitation source is a pulsed xenon lamp.)

Figure 3. Power dependence curve of acriflavin in (i) PVA and (ii) SiFs doped PVA (λ_{ex} = 473 nm laser). (iii) Dependence of enhancement factor on laser power, where the enhancement factor is the intensity observed from SiF's divided by that from an otherwise identical control sample containing no nanoparticles.

Figure 4. Excitation spectra of acriflavin in (i) PVA and (ii) SiFs deposited on PVA film (λ_{ex} = 550 nm).

surfaces was first reported by Glass et al.,[41] in 1980, and also confirmed by other groups.[5−8,42] The absorption spectra of acriflavin do not show any concentration dependence, which rules out the possibility of dimer or higher aggregate formation in the ground state in this particular polymer.

The fluorescence and delayed fluorescence spectra of acriflavin in PVA with and without SiFs are shown in Figure 2. Acriflavin shows a fluorescence maximum at 515 nm and a delayed emission at 525 nm in PVA. The delayed fluorescence is nearly 10 nm red-shifted as compared to the fluorescence band, and this shift is concentration dependent. Nearly a 3.5-fold enhancement is observed both in the fluorescence and in the delayed fluorescence emission from acriflavin in SiFs doped PVA films. The enhancement factor is defined as the ratio of intensity observed from SiFs (near-field) divided by that from a nonsilvered PVA control polymer film under otherwise identical conditions. The real color photographs in Figure 2 show the enhanced luminescence visually. Interestingly, we
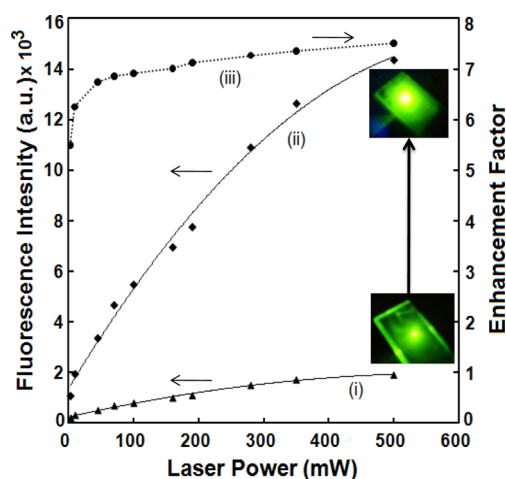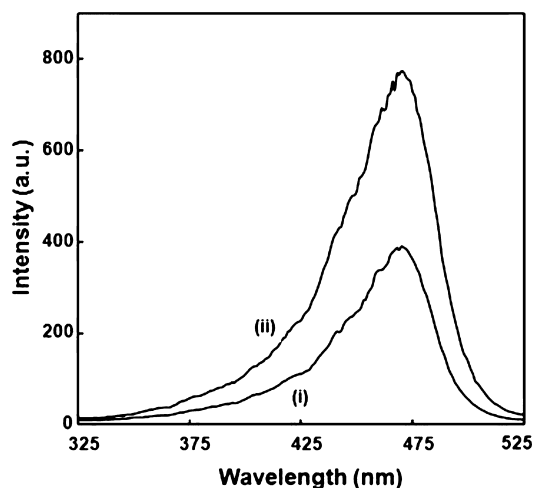
have also observed an excitation power dependence in fluorescence enhancement with SiFs as shown in Figure 3. The MEF factor increases exponentially ≈7 times, which is consistent with the recent work and theory from our laboratory[43] (i.e., MEF EVE), which explains an excitation power dependence in MEF being due to the near-field volume excited around the nanoparticles: the higher the excitation irradiance, the greater the excitation volume.[43] Given that this effect is a near-field effect around nanoparticles, further increased luminescence enhancements are observed at subsequently higher irradiances.[43] To the best of our knowledge, this is the first time this has been observed for alpha-emission. The shift in the delayed fluorescence spectra with respect to S_1 fluorescence (≈10 nm) suggests that the origin of this shift in the emission band is due to excited state excimer formation, by the recombination of acridine ions with trapped electrons.[30]

Further, to confirm the origin of the delayed fluorescence, excitation spectra were measured by monitoring the emission at the blue and the red edges of the fluorescence band, where we see no change in the excitation spectra, subsequently ruling out

Table 1. Time Resolved Decay Parameters of Acriflavin S_1 Fluorescence

sample	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	τ_3 (ns)	α_3 (%)	$<\tau>$ (ns)	$\overline{\tau}$ (ns)	χ^2
PVA	2.25	4	5.45	88	14.92	8	6.10	7.26	1.074
PVA + Ag	2.0	10	4.68	82	14.72	8	5.21	6.84	1.027

Table 2. Time Resolved Decay Parameters of Acriflavin α-S_1 Fluorescence[a]

sample	τ_1 (s)	α_1 (%)	τ_2 (s)	α_2 (%)	$<\tau>$ (s)	$\overline{\tau}$ (s)	χ^2
PVA	0.46	65	0.80	34	0.72	0.62	1.066
PVA + Ag	0.36	47	0.68	53	0.52	0.58	1.098

[a]$<\tau>$ is the amplitude weighted decay time, and $\overline{\tau}$ is the mean decay time.

the potential formation of any ground state complex. Further, again the excitation spectrum of acriflavin was determined in PVA alone and SiFs coated PVA films (Figure 4). In SiFs containing acriflavin polymer films, the intensity of the excitation band increases nearly twice with respect to the PVA film alone; this resembles the absorption spectra, as shown in Figure 1, and is again due to an effective increase in the coupled system absorption cross-section.

To understand the excited state dynamics of fluorescence and delayed fluorescence of acriflavin close to the metallic surface, time-resolved measurements were additionally undertaken using time-resolved lifetime analysis in the nanosecond ($S_1 \rightarrow S_0$) and second time (α-$S_1 \rightarrow S_0$) ranges. The analyzed decay data are given in Tables 1 and 2, respectively. The respective analyzed overlapped time-resolved decay curves are shown in Figure 5. The goodness of fit of the collected decay was judged by the distribution of residuals and associated chi-square values. It is observed that acriflavin decays triple-exponentially, with an average decay time of 7.26 ns and amplitude weighted decay time 6.10 ns in PVA; these values are found to decrease to 6.84 and 5.21 ns, respectively, in the presence of SiFs. The delayed fluorescence was found to decay biexponentially, having an average decay time of 0.62 s and an amplitude weighted value ≈ 0.72 s. These values are found to be reduced to 0.58 and 0.52 s, respectively, in SiFs deposited PVA films. These reductions in decay times in the presence of SiFs
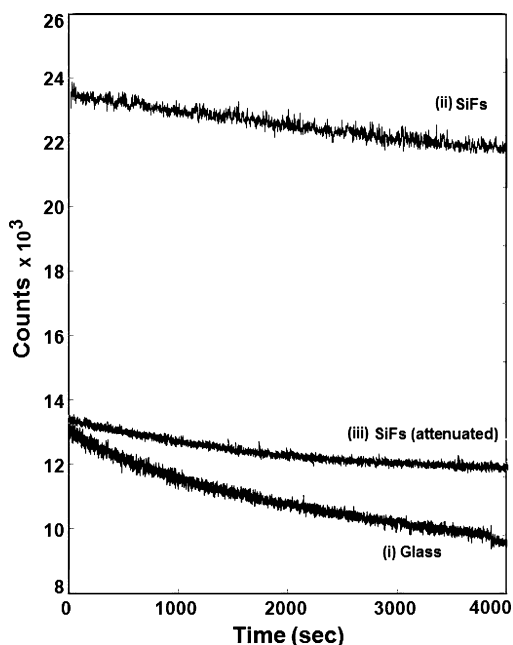

Figure 6. Intensity vs time (photostability) of acriflavin in PVA polymer film on a (i) glass slide and on (ii) SiFs. To demonstrate the benefit of SiFs, with regard to enhanced photostability, the laser power in one decay was attenuated (iii) to given the same initial steady-state intensity as was observed on glass. (λ_{ex} = 473 nm).

indicate the coupling of excited states of acriflavin with plasmons of the SiFs and the subsequent nonradiative energy transfer from acriflavin to metal particles, consistent with the MEF model postulated by Geddes.[9] In essence, the reduced lifetimes are due to the fact that the metal itself radiates the coupled quanta, with the very short plasmon lifetime decreasing
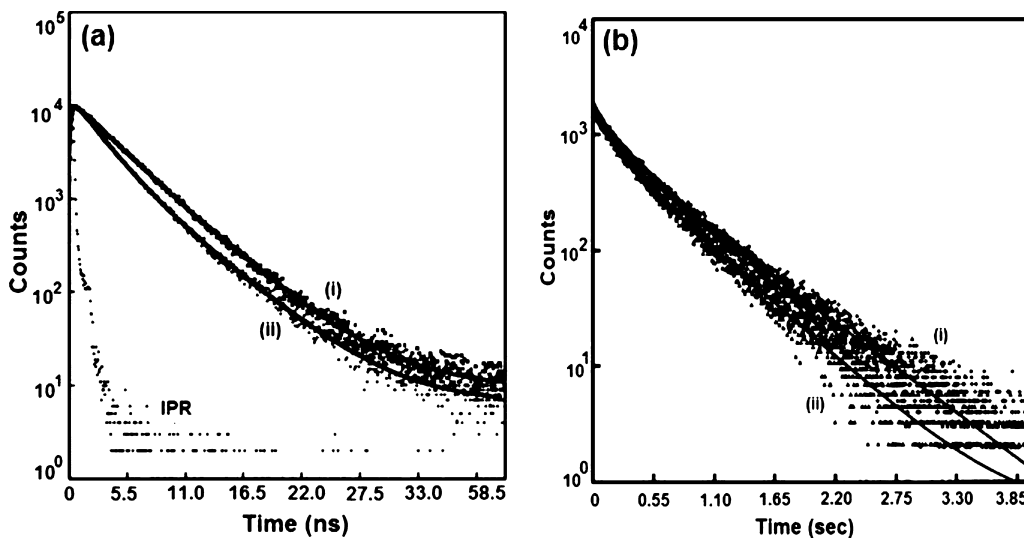

Figure 5. Time resolved decay curves of (a) fluorescence and (b) delayed fluorescence of acriflavin in (i) PVA and (ii) SiFs doped PVA polymer films (λ_{ex} = 455 nm).

the amplitude weighted heterogeneous sample excited-state lifetime.

In order to further understand metal-enhanced acriflavin fluorescence dynamics, we have also measured the photostability of acriflavin in PVA films with and without SiFs. These samples were exposed to a 473 nm laser line for about 300 s and the steady state fluorescence measured as a function of time (Figure 6). The photostability of the acriflavin is more pronounced on SiFs containing PVA films as compared to the PVA control; that is, more photons are observed per unit time (photon flux is proportional to the areas under the curves). This finding is consistent with the reduced decay time of acriflavin near-to SiFs (Table 1), e.g. a reduced decay time and enhanced fluorescence photostability.

Two distinct observations can therefore be made for fluorescent species in close proximity to plasmonic nanoparticles: (1) an increase in the fluorescence emission from the metal-fluorophore *unified system* with the spectral properties of the fluorophores maintained, and (2) a reduction in the fluorescence lifetime, giving rise to improvements in the photostability of the fluorophores; that is, fluorophores spending less time in an excited state are less prone to photo-oxidation and photodestruction. For acriflavin, we subsequently propose that there are two populations of adsorbed molecules on the SiFs containing PVA films: one population is composed of more isolated molecules far from the SiFs; the photophysical properties of these are similar to acriflavin in the far-field or free space condition. In the near-field, i.e. near-to-silver, an enhanced absorption and coupling to plasmons facilitates metal-enhanced acriflavin fluorescence and delayed fluorescence as well as an enhanced photostability. The photophysical properties of both fluorescence and delayed (long-lived) fluorescence are found to be both drastically and favorably changed near the metallic nanoparticle surfaces in ways that provide an opportunity for the development of shorter lived brighter luminescent materials where a higher photon flux can be realized. One particular application of these silver doped MEF plastics lies in energy efficient plastics for lighting and safety applications, where the decay time (length of luminescent glow) and absolute brightness can be potentially tuned (Scheme I), as we here show for acriflavin on SIFs.

4.0. CONCLUSION

In this paper, we have reported the near-field effects of metallic nanoparticles on the photophysics of acriflavin in a PVA polymer matrix. An enhanced absorption and photostability is observed along with a large enhancement in both the fluorescence and delayed fluorescence as compared to an identical control sample containing no silver, i.e. the far field condition. Our results show that near-field surface plasmons are observed to enhance both singlet and delayed singlet fluorescence emission, potentially providing for a new approach in the generation of energy efficient materials. In addition, we have observed a MEF EVE effect for both S_1 and alpha S_1 fluorescence, which has not *hither to* been reported.

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Notes
The authors declare no competing financial interest.

ABBREVIATIONS

MEF, metal-enhanced fluorescence
MEF EVE, metal-enhanced fluorescence excitation volumetric effects
SiFs, silver island films
PVA, poly(vinyl alcohol)
RPM, radiating plasmon model

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