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Steady State and Time Resolved Fluorescence Studies of Azadioxatriangulenium (ADOTA) Fluorophore in Silica and PVA Thin Films

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Abstract

A cationic azadioxatriangulenium (ADOTA) dye was entrapped in silica thin films obtained by the sol-gel process and in poly (vinyl) alcohol (PVA) thin films. Azadioxatriangulenium is a red emitting fluorophore with a long fluorescence lifetime of ~20 ns. The fluorescent properties of azadioxatriangulenium in silica thin films and PVA films were studied by means of steady-state and time resolved fluorescence techniques. We have found that the azadioxatriangulenium entrapped in silica thin film has a wider fluorescence lifetime distribution (Lorentzian distribution), lower fluorescence efficiencies, shorter lifetimes compared to Azadioxatriangulenium in a PVA film. The local environment of azadioxatriangulenium molecules in the silica thin film is rich with water and ethanol, which creates the possibility of forming excited state aggregates due to high concentration of dye within a small confined area. In contrast to the PVA matrices, the porous silica films allow restricted rotations of Azadioxatriangulenium molecules, which result in faster and complex fluorescence anisotropy decays suggesting energy migration among dye molecules.

Graphical Abstract

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Keywords

Azadioxatriangulenium; Fluorophore; Sol-gel process; Luminescence; Lorentzian lifetime distribution

1. INTRODUCTION

Over the past decade, the synthesis and characterization of hybrid materials based on silica, in which organic dyes were entrapped has attained considerable attention [1,2]. The use of semiconductor materials for entrapment of the organic fluorophores has been and will remain a very important way to obtain new materials for different applications, including, functional materials in optoelectronic devices, optical sensors,[3] optical components like solid state tunable lasers [4] and highly luminescent materials in medicine [5,6].

The initial investigation of entrapping optically active organic molecules with the sol-gel process was performed by Avnir et al.[7] in which they studied the fluorescence properties of rhodamine 6G in different stages of sol-gel development. Since then, various photoactive dyes have been incorporated and studied in the matrices obtained by the sol-gel process [8-10]. Moreover, new hybrid materials provide a great subject of extensive research in materials chemistry [11-13]. To obtain new hybrid materials, an efficient sol-gel process is used which combines the remarkable properties of inorganic and organic materials in a controlled way [14-18]. In particular, because of the good mixing of the starting compounds, it creates the possibility to obtain a homogenous hybrid material at relatively low temperatures [19]. During the sol-gel process, the host molecules are already present in the precursor solution and the conditions of the reactions are mild, therefore the optical properties of the fluorophore remain intact [20,21]. Also the entrapment of organic fluorophores in a gel matrix provides better protection of the molecule and higher stability than the free molecules in liquid media [22]. At the same time, silicon dioxide (SiO_2) is well-known as a material with good optical quality, thermal stability, mechanical strength and photochemical stability. Another property of silica, which makes it a good candidate as a host matrix for incorporation of organic fluorophores is the ability to modify the surface by amines, thiols and other coupling agents [23,24]. On the other hand, organic dyes incorporated into silica matrix, sometimes afford more interesting luminescence properties due to strong host-guest interactions. Therefore, preparation of new hybrid materials based on silica in which organic fluorophores are entrapped is suitable for the design of highly luminescent materials.

In the present study, we have introduced an azadioxatriangulenium dye into a silica thin film obtained by the sol-gel process and into a PVA film;[25-28] specifically the acid derivative *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA); the molecular

structure can be seen in scheme 1. ADOTA belongs to the class of azaoxa-triangulenium fluorophores which are planar and rigid in structure. Furthermore, ADOTA in water emits in the red region around 560 nm with a fluorescence lifetime of ~20 ns, which makes it the longest orange/red emitting organic fluorophore [29,30].

The spectroscopic properties of the material presented are discussed in this paper. In particular, we compared the absorption, fluorescence emission, steady state anisotropy, time resolved anisotropy and fluorescence lifetime of ADOTA in thin layers of silica and in a PVA film. The use of PVA provides a more rigid environment compared to silica matrix and hence the photophysical properties of the ADOTA can be studied in two different semi-solid matrices. In addition, the photophysical characterization of ADOTA in a heterogeneous environment can be efficiently studied over time by using time resolved fluorescence spectroscopy.

2. MATERIALS AND METHODS

All the starting materials for the preparation of silica thin films were of analytical grade. Tetramethoxysilane $\text{Si}(\text{OCH}_3)_4$ (TMOS) was purchased from Aldrich Co., methanol as a diluent and ammonia were purchased from POCH Company (Poland). Deionized (DI) water was obtained from a Hydrolab system. Polyvinyl alcohol (130, 000 MW) was purchased from Sigma Aldrich (Sigma Aldrich, St Louis, MO USA). *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) was prepared as previously described [27,31].

2.1. Sample preparation and AFM measurements

ADOTA/silica thin films were obtained by the sol-gel spin-coating method, summarized in Scheme 1. Firstly, tetramethoxysilane (TMOS) and methanol were mixed by vigorous stirring for 15 minutes. After several minutes (which is very important to reach homogeneity of the solution), ADOTA was dissolved in methanol and added to the precursor solution. In order to initiate the sol-gel process, an appropriate volume of water was added. The final molar ratio TMOS/water/methanol was 1:4:12, while the concentration of ADOTA in sol was $2 \cdot 10^{-3}$ M (a low concentration sample with an overall ADOTA concentration of $< 10^{-6}$ M was also prepared). The films were distributed over a clean piece of a microscopic slide using the spin-coating technique after 15 minutes after mixing all of the components. Microscopic glasses were cleaned in a mixture of 33% H_2O_2 and H_2SO_4 in molar ratio 2:1 over 24 h and rinsed with deionised water

The spin coating was done at 150 rpm for 60 s to disperse the sol. After that, the thin films were allowed to dry in air for 24 hours. Moreover, to prepare ADOTA in PVA films, the ADOTA was mixed in PVA solution and spread onto a thin glass slide to evenly coat the slide using spin coating.

Atomic Force Microscopy (AFM) micrographs were produced by using scanning atomic force microscopy on the NTEGRA Prima scanning probe microscope manufactured by NT-MDT (Moscow, Russia). Closed-loop feedback semi-contact mode has been used at rate 0.5 Hz. Scanning was controlled and images were analyzed with NOVA software by NT-MDT instrument manufacturer.

2.2. Absorption measurements

Absorption spectra were measured using a Cary 50 Bio UV-visible spectrophotometer (Varian Inc., Australia). Absorption spectra were scanned from 400 nm to 600 nm using bare glass slide as a baseline. Absorption spectra of silica thin films were corrected for the scattering by subtracting the scattering profile of silica thin films prepared without addition of the dye.

2.3. Steady-state fluorescence measurements

Steady-state fluorescence intensity measurements of all the samples were made using a Carry Eclipse spectrofluorometer (Varian Inc., Australia) by using the front face geometry as shown in scheme 2. In scheme 2, S represents the sample used for measurements, M is the mirror, F is a long pass filter before detector and L is the lens. The emission was scanned from 520 nm to 700 nm following a 470 nm excitation and using 495 nm long pass filter on the emission side. Steady-state excitation spectra were measured by observing the emission at 600 nm and excitation was scanned from 400 nm to 560 nm using a 570 nm long pass filter on the emission side. Steady-state fluorescence anisotropy of all the samples were measured using a Carry Eclipse spectrofluorometer (Varian Inc., Australia), with manual polarizers on both the excitation and emission side. Emission anisotropy was measured following 470 nm excitation with a 495 nm long pass filter along the emission side and manually operated parallel and perpendicular polarizers. Anisotropy was calculated using the following formula:

$$r = \frac{I_{VV} - I_{VH} G}{I_{VV} + 2I_{VH} G}$$

Where, I_{VV} is the fluorescence intensity measured with the parallel polarizer orientation on the observation path, I_{VH} is the fluorescence intensity at the perpendicular orientation of the polarizer on the emission side and G is the instrumental correction factor calculated by measuring the intensity in HV and HH polarizer orientation.

2.4. Fluorescence Intensity Decay

Fluorescence lifetimes of all the samples were measured using FluoTime 200 (PicoQuant, GmbH, Berlin, Germany) time resolved spectrofluorometer. This instrument contains a multichannel plate detector (Hamamatsu, Japan) and a 470 nm laser diode was used as the excitation source. The front face geometry as shown in scheme 2 was used for these measurements as well. The fluorescence intensity decays were measured under magic angle conditions and data was analyzed with FluoFit version 4.5.3 software (PicoQuant GmbH, Berlin, Germany) using both the exponential reconvolution procedure using non-linear regression (multiexponential deconvolution model) and by the lifetime distribution model (Lorentzian model) [32]. In the case of multiexponential analysis, the fluorescence decay was analyzed using:

$$I(t) = \int_{-\infty}^t \text{IRF}(t') \sum_i \alpha_i e^{-\frac{t-t'}{\tau_i}}$$

Where IRF (t') is the instrument response function at time t' , α is the amplitude of the decay of the i^{th} component at time t and τ_i is the lifetime of the i^{th} component. In case of Lorentzian lifetime distribution, all the data were analyzed using the following equation.

$$I(t) = \int_{-\infty}^{\infty} \rho(\tau) e^{-\frac{t}{\tau}} d\tau$$

Where,

$$\rho(\tau) = \sum_{i=1}^n \frac{A_i}{\pi} \frac{\frac{\Delta_{FWHM_i}}{2}}{(\tau - \tau_i)^2 + (\Delta_{FWHM_i}/2)^2}$$

Where A_i is the amplitude of the i^{th} component, τ_i is the central lifetime value of the i^{th} distribution. The use of the continuous distribution $\rho(\tau)$ minimizes the number of floating parameters in the fitting algorithms.

2.5. Time resolved anisotropy measurements

Excitation used for time resolved anisotropy measurement was 470 nm while emission was observed at 560 nm and 620 nm with vertical and horizontal polarizer position on the emission side using appropriate filters on both excitation and emission side. Anisotropy decays were analyzed with the exponential fitting model in the FluoFit 4.5.3 program from Pico-Quant, Inc. (Germany) using the following equation:

$$r(t) = r_{INF} + \sum_{i=1}^n r_i e^{-\frac{t}{\Phi_i}}$$

Where, r_i is the anisotropy of the i^{th} component at time t and Φ_i is the rotational correlation time of the i^{th} component. The quality of the fit in lifetime and anisotropy decay analysis was judged by the quality of the residuals and χ^2 square value.

3. RESULTS AND DISCUSSION

3.1. AFM micrographs of silica thin films

The surface topography of the silica thin film with *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) was studied using the AFM technique as shown in figure 1. From this micrograph, we can observe that the surface of the porous silica is rough, with maximum particle size close to 50 nm. The ADOTA-doped PVA films did not show any significant roughness (supplementary figure S1). In order to check how dye is distributed in the prepared silica or PVA layers, confocal fluorescence images were taken and found that

ADOTA dye is evenly distributed in all film on the scale of the optical resolution, about 400 nm (see supplementary information, Figure S2).

3.2. Absorption spectra

Figure 2 shows the absorption spectrum of ADOTA in silica thin films and in PVA films dried on a cover-glass. It can be seen from both the figures that the absorption spectra appears somewhat noisy. This is due to the small thickness/path-length (100-150 nm) and small absorptions recovered after correcting for the scattering arising in these samples. The absorption spectrum shape and peak absorption wavelengths did not change in the silica gel environment compared to the PVA film suggesting no significant perturbation in the immobilized ground state fluorophores population.

3.3. Steady state measurements

Figure 3 shows the normalized excitation and emission spectra of ADOTA entrapped in a silica thin film (red) and a PVA film (blue). Similar to the absorption data, we did not see any significant change in the excitation spectrum of the dye entrapped within the two different environments. However, a ~15nm red shift in the emission spectra was observed for the ADOTA in silica thin films.

When we examined the emission spectra of a low concentration ADOTA in the silica layer we found that the emission spectrum was similar to the emission spectrum of ADOTA in PVA film. ADOTA in silica layer shows concentration dependent red shift in emission spectrum. Supplementary information shows all the emission spectra along with measured Full Width Half Maxima (FWHM, supplementary figure S3). For high concentration of the dye in silica layer, the spectrum is red shifted compared to low concentration along with an increase in the FWHM value, suggesting dye aggregation/excimer formation. Although, we have already ruled out the possibility of ground state aggregation since there is no experimental evidence here in this case. However, we cannot rule out the possibility of excimer formation here as the dyes are packed very close to each other and do not require a significant diffusion to form aggregates in the excited state. The rapid formation of excimers is outside the measurement capabilities of our instrument and hence the rise time (negative exponent) cannot be observed. We ascribe the broadening of the emission spectrum from ADOTA in silica thin films to the heterogeneous environment and aggregation of dye molecules in the silica matrix. This is a very elaborate problem, because ADOTA entrapped in the pores of the matrix has a possibility to interact not only with itself but also with the surface of three-dimensional network. In the literature this problem is called as the “cage effect” [33]. In the present case, the local environment of the ADOTA molecule is rich with water and methanol. In other words, the cage is filled with solvent and this creates the possibility of forming heterogeneous environment within the confined area.

Steady state anisotropy measurements gave us the overall idea of the mobility of the fluorophore molecules in a silica film and PVA matrix. The steady state anisotropy data are presented in figure 4. ADOTA entrapped in a PVA matrix showed high anisotropy reaching a value close to 0.3, thus suggesting a very rigid environment. On the other hand, ADOTA incorporated into silica thin film showed moderate anisotropy of 0.05. This suggests that the

dyes are not rigidly linked to the matrix or that the anisotropy is lost due to energy transfer (HOMO-FRET) and that the pores are large on the molecular scale, making the silica matrix less rigid than PVA matrix. These results were confirmed by blowing the argon on to silica film for 1 hour and re-measuring the steady state anisotropy to exclude the possibility of rotational diffusion due to the hygroscopic nature of the silica film. Interestingly, the possibility of ADOTA being covalently linked to the matrices through the acid functional group is not explored and is a topic of further research in this area.

3.4. Time resolved intensity decay/fluorescence lifetime

The fluorescence lifetime of the ADOTA incorporated into a silica thin film was heterogeneous compared to fluorescence lifetime of the ADOTA incorporated into a PVA matrix. The lifetimes measured at 560 nm are shown in figure 5. The average lifetime was found to be ~ 12 ns in the silica matrix and ~20 ns in the PVA matrix. Three components are needed to fit the data at 560 nm for the silica thin film, whereas only two components are needed for the sample in the PVA film (table 1). The shorter and heterogeneous nature of the decay in silica suggests different environments to which dye molecules are exposed to [28]. Hence, we decided to measure the lifetimes at a longer wavelength (620 nm) which will further allow us to know more about the silica network environment. Figure 6 shows the lifetime decays at 620 nm wavelength. One can see that the lifetime of ADOTA in the PVA matrix did not change significantly and stayed approximately 20 ns. However, the fluorescence lifetime of ADOTA (at 620 nm) incorporated into the silica matrix is significantly longer than the fluorescence lifetime measured at 560 nm. This could be due to the presence of the dye molecules in the confined space in the silica matrix. Stacking of dyes leads to the restricted motion and less vibrational/torsional losses and hence the longer lifetime at 620 nm observation. Table 1 shows the lifetime values from the data analyzed using multiexponential reconvolution model.

We also analyzed the fluorescence intensity decays using a Lorentzian lifetime distribution model that takes into consideration the average number of emitting species in different environments. The FWHM values of the distribution shows the degree of distribution of the emitting molecules which are much higher in silica thin films than in the PVA films (figure 7). The Lorentzian distributions (table 2) for ADOTA in PVA films are very narrow, about 3 ns at 560 nm observation, and about 2.45 ns at 620 nm observation, which suggests a very uniform environment and lack of interactions between fluorescent molecules. In contrast, the Lorentzian distributions in silica are very wide. In case of ADOTA in silica thin films, the FWHM value at 560 nm is about 15 ns and at 620 nm about 13ns. These higher FWHM values show the broader distribution of the emitting molecules in silica thin films.

3.5. Time resolved anisotropy

Time resolved anisotropy measurements presented in figure 8 and 9 shows some interesting information. As expected in the case of the PVA matrix, initial anisotropy was high (0.3) with a very long correlation time (>500ns) (correlation time $\gg$ fluorescence lifetime) at both observation wavelengths (560 and 620nm). However, in case of silica gel matrix the recovered initial anisotropy values are 0.21 and 0.19 for 560 and 620nm observation and the measured correlation times and r_{INF} values are not significantly different (Table 3). The

heterogeneous nature of the correlation times is due to the populations of the dyes in different types of matrix environment. Therefore, it will not be surprising if a part of the dye population is immobilized completely and a part of it has a moderate freedom than the others. It is suggested in the literature that the hydrogen bonding among silica mesh and the dye molecules undergoes reshuffling in excited state and it may give rise to such correlation times [7]. Moreover, the estimated steady state anisotropy values at these wavelengths are 0.069 and 0.042. The drop in the initial recovered anisotropy, in case of anisotropy decay, and steady state anisotropy values suggests energy migration (if the dye molecules are sitting together below 40 Å distances which is the approximate Forster distance for such homo-transfer and is a possibility we cannot ignore). Although energy migration is a rapid process which takes place over the lifetime of the fluorophore despite having a relatively long fluorescence lifetime in both matrices.

4. CONCLUSIONS

In the present work, the organic fluorophore, *N*-(ω-butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) was successfully incorporated into silica thin films by the sol-gel process as well as in PVA films. The PVA matrix was chosen to study fluorescence properties of ADOTA in a more rigid environment. Silica matrix provides an unusual environment for the entrapped fluorophores and affects the dye properties in many different ways. A red shift of 15 nm was observed in the emission spectra of ADOTA in silica thin film. In addition, the fluorescence lifetime of ADOTA at 560 nm observation in silica matrix was 12 ns compared to 20 ns in PVA film. However, when the wavelength of observation was placed at 620 nm, the fluorescence lifetimes of ADOTA entrapped in both the matrices are almost similar. The Lorentzian lifetime distribution shows the presence of broad distribution of the emitting species in silica thin layer compared to PVA film. To sum up, considering all the observations such as lower fluorescence efficiencies, shorter lifetimes and concentration dependent red shifted emission suggests aggregation of dye molecules in the excited state. Ultrafast spectroscopy experiments will further support our observations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- ➤ The silica matrix favors the formation of aggregates of the entrapped fluorophore.
- ➤ Azadioxatriangulenium dye in the silica layer shows a 15 nm red shift.
- ➤ The fluorescence lifetime at 560 nm observation in silica was 11.84 ns.
- ➤ The fluorescence lifetime at 560 nm observation in PVA was 19.95 ns.
- ➤ Low anisotropy in silica compared to PVA film.

Scheme 1.

Flow chart of preparation of ADOTA doped silica thin films. Insert: molecular structure of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA).

Figure 1.
AFM showing the surface topography of the silica thin layer prepared by the sol gel process.

Figure 2.

Top panel: absorption spectrum of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film. Bottom panel: absorption spectrum of ADOTA in PVA film.

Scheme 2.

Schematic of the front face arrangement used for steady state and time resolved fluorescence measurements. In this scheme, S represents the sample used for measurement, M is the mirror, F is long pass filter before detector, L is lens.

Figure 3. Normalized excitation and emission spectrum of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film (red) and in PVA film (blue).

Figure 4.

Top panel: fluorescence emission spectrum (red line) and anisotropy (blue circle) of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film. Bottom panel: fluorescence emission spectrum (blue line) and anisotropy (blue circle) of ADOTA in PVA film.

Figure 5.

Top Panel: fluorescence intensity decay of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film (Ex: 470nm, Obs: 560nm). Bottom panel: fluorescence intensity decay of ADOTA in PVA film (Ex: 470nm, Obs: 560nm).

Figure 6.

Top panel: fluorescence intensity decay of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film (Ex: 470nm, Obs: 620nm). Bottom panel: fluorescence intensity decay of ADOTA in PVA film (Ex: 470nm, Obs: 620nm).

Figure 7.

Lifetime distribution (Lorentzian Model) of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film and PVA film. (Top Panel) This figure represents the fluorescence lifetime distribution when observed at 560 nm. (Bottom Panel) This figure represents fluorescence lifetime distribution observed at 620 nm. ADOTA is more heterogeneous at 560 nm (Silica Thin Film_{FWHM} = 15.05 ns, PVA Film_{FWHM} = 3.22 ns) compared to observation at 620 nm (Silica Thin Film_{FWHM} = 12.71 ns, PVA Film_{FWHM} = 2.45 ns)

Figure 8.

Top panel: Fluorescence anisotropy decay of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film (Ex: 470nm, Obs: 560nm). Bottom panel: fluorescence anisotropy decay of ADOTA in PVA film (Ex: 470nm, Obs: 560nm).

Figure 9.

Top panel: Fluorescence anisotropy decay of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) in silica thin film (Ex: 470nm, Obs: 620nm). Bottom panel: fluorescence anisotropy decay of ADOTA in PVA film (Ex: 470nm, Obs: 620nm).

Analysis of *N*-(ω-butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) fluorescence intensity decay using multi-exponential model of in Silica thin film and PVA thin film.

Table 1

Sample	Observation (nm)	Lifetime (ns)			Amplitudes			Average lifetime (ns)		Chi square
		τ ₁	τ ₂	τ ₃	α ₁	α ₂	α ₃	τ _{AMP}	τ _{INT}	
ADOTA Silica thin film	560	25.14	10.10	2.004	0.28	0.41	0.31	11.84	18.60	0.955
	620	26.98	9.33	–	0.65	0.35	–	20.89	20.24	0.892
ADOTA PVA film	560	21.01	13.42	–	0.86	0.14	–	19.95	20.30	0.997
	620	20.46	–	–	1	–	–	20.46	20.46	0.811

Where,

$$\tau_{AMP} = \sum_i \alpha_i \tau_i$$
$$\tau_{INT} = \sum_i f_i \tau_i$$
$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i}$$

Table 2

Lorentzian analysis of fluorescence intensity decays of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA).

	Observation (nm)	Lifetime, τ (ns)	FWHM (ns)	Chi square(χ^2)
ADOTA silica thin film	560	13.5	15.1	0.98
	620	21.8	12.7	0.92
ADOTA PVA film	560	20	3.2	0.98
	620	20.4	2.45	0.93

Table 3

Analysis of *N*-(ω -butanoic acid)-azatriangulenium tetrafluoroborate (ADOTA) anisotropy decay using multiexponential model of on Silica thin film and PVA film.

Sample	Observation (nm)	Anisotropy			Correlation time (ns)	
		r_1	r_2	r_{INF}	Φ_1	Φ_2
ADOTA Silica thin film	560	0.088	0.095	0.03	0.81	7.37
	620	0.081	0.093	0.017	6.02	0.58
ADOTA PVA film	560	0.30	–	–	504.4	–
	620	0.26	–	–	1000	–