Time-resolved photoluminescence of 6-thienyl-lumazine fluorophores in cellulose acetate nanofibers for detection of mercury ions

Noura Al-Shamsi^a, Sergey P. Laptanok^b, Muna S. Bufaroosha^a, Yaser E. Greish^a, Na'il Saleh^{a,*}

^a Chemistry Department, College of Science, United Arab Emirates University, P.O. Box 15551, Al Ain, United Arab Emirates

^b Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, P.O. Box 4700, Thuwal 23955-6900, Saudi Arabia

ARTICLE INFO

Article history:

Received 15 February 2019

Received in revised form 21 May 2019

Accepted 26 May 2019

Available online xxx

Keywords:

Lumazine

Nanofibers

Time-resolved photoluminescence

ESPT

Fluorescence quenching

ABSTRACT

Time-resolved photoluminescence measurements were used to characterize the photophysical properties of 6-thienyllumazine (TLM) fluorophores in cellulose acetate nanofibers (NFs) in the presence and absence of mercuric acetate salts. In solution, excited-state proton transfer (ESPT) from TLM to water molecules was investigated at pH from 2 to 12. The insertion of thienyl group into lumazine introduces *cis* and *trans* conformers while keeping the same tautomerization structures. Global and target analyses were employed to resolve the true emission spectra of all prototropic, tautomeric, and rotameric species for TLM in water. The results support the premise that only the *cis* conformers are related to the ESPT process. However, no ESPT from TLM to a nearby water molecule was observed in NFs. The addition of NFs increases the excited-state lifetime of TLM in the solid state because of combined polarity/confinement effects. The solid-state fluorescence of TLM (in NFs) was quenched by mercuric acetate through different mechanisms—dynamic and static—depending on the applied pressure—atmospheric and vacuum, respectively. The new solid-state sensor is simple, ecofriendly, and instantly fabricated. TLM-loaded NFs can detect mercuric ions at a concentration of 50 picomolar. The formation of non-fluorescent ground-state complex between TLM molecules and mercuric ions inside the pores of NFs was achieved under vacuum condition.

© 2019.

1. Introduction

For so many years, there have been numerous reports dealing with the utilization of nanofibers in broad range of applications, such as chemical sensing, filtration, drug delivery, tissue engineering, oil industry, and energy research [1–3]. Of interest, the utilization of the large surface area-to-volume ratios of those electrospun nanofibers [4].

The characterizations of nanofibers by steady-state [5] (PL) and time-resolved photoluminescence (TRPL) techniques [6,7] have been reported by many researchers for many applications. In the TRPL measurements, which have recently come to fore, the time dependence of the fluorescence intensity or spectrum is monitored after excitation with a brief light flash, reflecting directly the rate constants of the depopulating processes of the excited states. The excited-state lifetime is, then, the time when the initial population of the excited state reaches $1/e$ its initial value [8]. It provides information on how the energy, orientation and movement of a chromophore change relative to the framework of the surrounding microenvironment. Our specific aspiration comes from the fact that such TRPL techniques offer the ability to obtain more information about the local environments when compared to the stationary PL measurements, coupled with the fact that the fluorescence lifetime is an absolute measure, whereas the steady-state just provides relative and average information [8].

Exploring the properties of lumazine (Lm) derivatives at both the ground and the excited states should help in explaining the properties of structurally related flavins and pterins (Scheme 1) in their surrounding microenvironment. This motivates us to specifically select 6-thienyllumazine (TLM in Scheme 1) as the chromophore to probe cellulose acetate nanofibers (NFs, Fig. 1) [9]. The spectrophysics of Lm in a series of solvents with varying dielectric constants and hydrogen bonding properties was studied by fluorescence spectroscopy and density functional theory calculation. The analysis revealed that the hydrogen bond acceptance ability of the solvent is the most important parameter, which characterizes the excited state behavior of Lm [10]. Moreover, Lm contains both acidic and basic groups in proximity and, thus, may undergo intramolecular proton transfer (tautomerization) in the excited state. Intermolecular excited state proton transfer (ESPT) to a solvent has also been widely researched for many years [11–18]. The ESPT process enables those chromophores to produce protons at pH value, upon excitation, lower than that in the ground state. For example, such photoacid behaviour of Lm has been specifically studied by Huppert research group utilizing TRPL spectroscopy in water [17,18] and of other photoacids when adsorbed on cellulose [19], starch [20], and other biomaterials [21].

In the present work, the ESPT phenomenon is taken into consideration when TLM is used to probe cellulose acetate nanofibers (NFs, Fig. 1), which were obtained by electrospinning in our laboratory [9]. The TRPL of TLM molecules are then measured to decide which factors inside the NFs affect their proximity to mercury ions. Electrospinning is one simple technique to fabricate nanofibers [22]. It is

* Corresponding author.

Email address: n.saleh@uaeu.ac.ae (N. Saleh)

(6-Thienyllumazine, TLm)

(Lumazine, LM)

(Pterins)

(Flavins)

Scheme 1. 6-Thienyllumazine (6-TLm) and other similar pteridines.

Fig. 1. Cellulose acetate nanofibers (NFs), and the two possible loading of TLm in NFs under vacuum (vac) and atmospheric (atm) conditions. Cyan and blue colors of TLm reflect the static quenching by mercury ions (red ball) under vacuum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

based on exposing a solution of the polymers to a high voltage. The nanofibers have special advantages because of their good mechanical properties that could sustain reversible sensing and high surface area for water absorbing [4].

In the present study, not only we are motivated to report more information about the structure of NFs, but also, we seek to fabricate a solid-state sensor for Hg^{2+} . Lm compounds are known to form coordination bonds with biologically essential metals in some metalloen-

zymes [23]. In fact, TLm itself was designed and prepared by our research group [24] as selective fluorescent sensors for pH [25] and heavy metal ions, such as Cd^{2+} and Hg^{2+} in pure water [24,26]. Thus, our second aim is to fabricate TLmNFs as a fluorescence-based solid sensor to detect micromoles or less of Hg^{2+} ions, urged by the increasing global concern on water pollutions [27]. In our approach, the fabrication of TLmNFs was established by stirring directly in aqueous solution, instead of electrospinning, the mixture of the fluores-

cent probe and the precursor polymer. The later procedure was used for the detection of several metal ions using nanofibers, such as Cu^{2+} , Cr^{3+} , Fe^{3+} and Hg^{2+} ions [5,28]. However, we were inspired to consider the former procedure, which allows TLM to distribute freely throughout the NFs system. We also aim to benefit from the superiority of TRPL techniques for detecting mercury ions, when compared to the stationary methods [5,28]. In fact, the belated loading of many fluorescent probes on cellulose-related biomaterials has also been employed by many researchers. For example, Kaur et al published a dual colorimetric and fluorescent sensor for Hg^{2+} and Cu^{2+} , which is simply made of phenothiazine-bound TLC plates/test papers. The detection limit of the sensor was determined to be 1.5 nM for Hg^{2+} and 3 nM for Cu^{2+} [29].

Putting all together, the present work is aimed at understanding the mechanism of fluorescence quenching utilizing TLM and mercuric acetates as model guests for optimizing the performance of the selected nanofibers for sensing application. The results should also help in expanding the utilization of nanofibers for other applications in biotechnology, nanotechnology, and beyond.

2. Results and discussion

2.1. Conformation and tautomerization effects on the photoacid behaviour of 6-thienyl-substituted lumazine chromophore in aqueous solution

The first part of the present study is aimed at investigating the spectrophysics of TLM in water before mixing it with the NFs to unfold its structure and dynamics under this condition alone. Various cations and anions in the structure of TLM may occur in various tautomeric forms because of protonation at the nitrogen atom sites and deprotonation at the oxo sites. Fortunately, Afaneh and Schreckenbach [30] computationally investigated the most stable prototropic structures of TLM in gas and aqueous phases at the ground state taken into consideration all possible tautomers and conformers, as illustrated in Chart 1. The authors concluded that insertion of thienyl group into the carbon atom at position 6 introduced two different conformers (*cis* and *trans*). The calculated pK_a values of TLM at the ground state for the two subsequent deprotonation equilibria in Chart 1 were also in good agreement with our experimental values [25]. Moreover, their calculations for the relative stability of the 13-N and 38-N tautomers (Chart 1) were different than those calculated for Lm tautomers. Accordingly, separating the effects of both tautomeric and rotameric equilibria on ESPT of TLM in aqueous solution are motivated. We have, therefore, measured the TRPL properties of TLM in water solutions at different pH values. Specifically, global and target analyses [31] are used to analyze the experimental data and extract the true emission spectra for all possible tautomers and conformers, as illustrated in detail in the Supporting Information, Part S1.

Fig. 2A shows the steady-state emission spectra of TLM in aqueous solution at different pH values (2, 9, and 12). The sample was excited at 375 nm, which is rationalized by virtue of the absorption spectra of all forms (inset of Fig. 2A). The peaks of the lowest energy absorption bands were at ~360, 380, and 420 nm for the N, A, and D forms, respectively. One broad band with a maximum around ~452 and a shoulder at ~390 nm are observed at pH 2, which we attribute to the A* and the N* forms of TLM, respectively. At pH of 12, the dianionic D* form emits at around ~445 nm. In a precedent study [25], we have measured UV-vis absorption spectra of TLM in several aqueous solutions at different pH values from 3 to 12, from which two subsequent pK_a values for the deprotonation processes were ex-

tracted from the titration plots at about 350 and 300 nm; $\text{pK}_a = 6.84 \pm 0.07$ and 10.8 ± 0.1 , respectively.

Table 1 and Fig. 2B summarize the global and target analysis of TRPL data at different pH values (see part S1 in the Supporting Information). It transpires that the two species at pH lower than the first pK_a ~5–6 (Chart 1), whose emissions appear below 418 nm are attributed to the different neutral tautomers—13-N and 38-N (390 and 405 nm for *cis* and *trans* 13-N and 415 nm for 38-N), whereas the two corresponding anionic forms, whose emission appears above 418 nm, belong to the 3-A and 1-A forms (440 and 450 nm for *trans* and *cis*-3-A and 420 and 430 nm for *cis* and *trans*-1-A). The D form has an emission maximum at 435 nm. The most stable neutral and anionic tautomers and conformers, which were calculated in aqueous solution by Afaneh and Schreckenbach [30], were assumed to give the decay-associated spectra (DAS) amplitudes and the excited-state lifetime values in Table 1. Because strong acids are known to significantly decrease lifetime constants, the two conformers of the minor species 38-N (3.0 ns) were not resolved by our instrument at pH 2–4. However, *cis*-13-N versus *trans*-13-N have emission peak and lifetime of 390 and 250 ps versus 405 and 470 ps, in agreement with theoretical prediction that the *trans* form is red-shifted with respect to the *cis* form [32]. The subsequent formation (with no time evolution) of 3-A and 1-A species from 38-N and 13-N, respectively, are expected at pH above the pK_a values of 5, 8 and 10 (Chart 1), which correspond to pH 4.3, 6.6 and 8.5 (Table 1). The results in the present work highlight the true emission spectra and excited-state lifetime for each conformer, not only tautomer of TLM; and for both anions. Noticeably, both the *trans* and *cis*-3-A forms have longer excited-state lifetime values than their counterparts *trans* and *cis*-1-A forms (7.3 and 7.9 ns versus 3.7 ns and 550 ps, respectively), thus dominating the steady emission spectra at about 452 nm (Fig. 2A). It is worth to mention that only the decay of the short-wavelength species *cis*-13-N (250 ps) and *cis*-1-A (550 ps) begins to fit the rise time of anionic species *cis*-3-A (7.9 ns), and D (2.2 ns) forms, respectively, with a concomitant increase in the DAS's negative amplitude (see Part S1 in the Supporting information). Generally, the possibility of having overlapped species in those experiments cannot be perfectly ruled out, however the equilibrium shift between those species across the different set of independent experiments has interestingly resulted in a similar observed spectral shift, which ensures the integrity of our analysis.

In their article, Presiado et al. [18] reported the emission properties of Lm using steady-state and TRPL techniques, concluding that the deprotonation time of neutral Lm to form the corresponding anion is about ~35 ps at the excited state. They interpreted the photophysical properties of Lm at different pH values taken into consideration the various tautomeric species at both the ground and excited states. The authors also concluded that the neutral form of Lm is an irreversible mild photoacid, and that only one of the two tautomer has ESPT activity [18]. We also conclude that ESPT (with a deprotonation time of ~350 ps) occurs only from *cis* forms, confirming the significant role played by conformation, not only tautomerization. The conformation effects on the emission properties of TLM, which distinguish the present work, have also been theoretically addressed by Afaneh and Schreckenbach [32]. The authors calculated the emission maxima of TLM to be 439 nm (for *cis*-TLM) and 441 nm (for *trans*-TLM) in agreement with our experimental results.

In summary, we have characterized the photophysical properties of TLM compound in water. Our results explain its ESPT behaviors as a photoacid using quantitative TRPL tools and considering the excited state population kinetics of its tautomers and conformers. All neutral and anionic forms of TLM have overlapped absorption and emission, yet their different acidities were best exploited in the present work to separate the spectra of all possible tautomers and con-

Chart 1. TLM neutral (N), anionic (A), and dianionic (D) tautomers present in aqueous solution. The corresponding calculated acid-dissociation constants ($\text{p}K_{\text{a}}\text{S}$) at ground states are also included (in parenthesis) as adapted from reference 30. Only major conformational structures are shown (*cis*). λ_{em} is the emission maximum from the extracted true spectra in the present study and τ is the corresponding fluorescence lifetime for all possible tautomers and conformers (see Fig. 2B and Table 1). The numbers in the 38-N, 13-N, 3-A, and 1-A notations are numbers of atom where the hydrogens are attached.

formers. Comprehensive, global and target kinetic analysis of excited-state population transfer were performed, in which the true emission spectra and excited-state lifetimes of all prototropic, tautomers and conformers were sorted out in the pH range from 2 to 12. The experimental results support the premise that both conformers (*cis*-13-N and *cis*-1-A forms) are related to the ESPT process. Thus, photoprotolytic processes are strongly influenced by both tautomerization and conformation effects.

2.2. Spectrophysics of TLM in cellulose acetate nanofibers (confinement vs. polarity effects)

In the second part of the present work, the steady and TRPL spectral properties (position, intensity and excited-state lifetime) of TLM upon stirring with NFs in aqueous solution were monitored. Taken into consideration the spectrophysical properties of TLM in water, as described above, we have opted to stir the TLM with NFs under pH of 2, at which the neutral TLM species should persist. The ESPT process, which produces mono-anionic species, is also expected to occur at this pH. The NFs was then filtered out and used for analyses

Fig. 2. A) Emission spectra of different prototropic species of TLM excited at 375 nm; neutral (N), anionic (A), and dianionic (D) in aqueous solutions at room temperature. The inset shows that all three species can be excited simultaneously at 375 nm. B) The extracted true emission spectra for all possible tautomers and conformers from the global/target analysis.

Table 1

The extracted excited-state lifetimes of all tautomers and conformers of TLM in water from the global/target analysis of TRPL data at different pH values.

	13-N		38-N	3-A	1-A		D
	cis	trans		trans	cis	cis	trans
λ_{em}/nm	390 nm	405 nm	415 nm	440 nm	450 nm	420 nm	430 nm
pH 1.8	232 ps	ND	ND		3.1 ns		
	[565 ps]				[1.3 ns]		
pH 2.4	254 ps	ND	ND		4.7 ns		
	[918 ps]				[933 ps]		
pH 2.8	253 ps	ND	ND		5.9 ns		
	[937 ps]				[1.0 ns]		
pH 3.4	257 ps	ND	ND		6.5 ns		
	[925 ps]				[974 ps]		
pH 3.9	259 ps	ND	ND		6.7 ns		
	[1.0 ns]				[1.0 ns]		
pH 4.3 ^a	ND	387 ps	2.7 ns	7.3 ns			
pH 4.5	ND	427 ps	3.0 ns	7.4 ns			
pH 5.6	ND	298 ps	2.5 ns	7.3 ns			
pH 6.0	ND	394 ps	3.0 ns	7.4 ns			
pH 6.6 ^b	ND	470 ps			7.6 ns		3.7 ns ^d
pH 7.1	ND	478 ps			7.6 ns		3.9 ns ^d
pH 7.6	ND	512 ps			7.2 ns		3.6 ns ^d
pH 8.5 ^c	ND	ND			7.6 ns	559 ps	3.2 ns
pH 8.9	ND	ND			7.6 ns	532 ps	2.6 ns
pH 9.5	ND	ND			7.6 ns	620 ps	2.4 ns
pH 10.6					7.5 ns	184 ps	ND
pH 11.0					7.8 ns	198 ps	ND
pH 11.5					7.5 ns	186 ps	ND
pH 11.8					6.8 ns	146 ps	ND
							2.1 ns
							2.1 ns
							2.1 ns
							2.1 ns

Species whose lifetimes are displayed in bold fonts were formed by ESPT. Minor species due to nonexponential behavior are shown in parentheses. Also, the *cis*-13-N is blue-shifted with respect to *trans*-13-N as predicted by theory [32]. ND means the species were quenched by either basic or acidic media, consequently their excited-lifetimes were not detected due to the low time-resolution of our instrument. ^apH around the pK_a associated with the protonation-deprotonation equilibrium between 38-N and 3-A (Chart 1). ^bpH around the pK_a associated with the protonation-deprotonation equilibrium between 13-N and 3-A (Chart 1). ^cpH around the pK_a associated with the protonation-deprotonation equilibrium between 13-N and 1-A (Chart 1). ^dSpecies *trans*-1-A appeared with low amplitudes in the pH range from 6.6 to 8.5.

by SEM and EDS (see the Experimental Section). The EDS confirms the presence of N and S in the structure of TLMNFs (Fig. 3).

The steady-state fluorescence spectra in Fig. 4A clearly confirm that an interaction has occurred between TLM solid and NFs, leading to a shift in the emission maximum from 420 nm to 441 nm in the case of the TLMNFs, which was prepared under atmospheric condition, whereas a shift from 420 nm to 407 nm was observed for the film, which was prepared under vacuum condition. The blue shift of the emission maximum and the enhancement of its intensity and excited-state lifetime (see below) when TLM molecules was mixed with

NFs under vacuum conditions can be best explained by polarity effects (non-radiative rate law). Inside the pores of nanofibers and away from the acetate polar moiety (Fig. 1), TLM is experiencing a less polar environment, which destabilizes the energy of its excited state. The destabilization of this energy, in turns, should decrease the non-radiative deactivation pathways, leading to an enhancement in its emission and an elongation of its excited-state lifetime. Contrarily, the TLM solids when mixed with NFs under atmospheric condition remain attached to the exterior part of the fibres (Fig. 1). The NFs alone is non-emissive.

Fig. 3. The EDS spectra and SEM images for cellulose acetate nanofibers (NFs) with (A and C) and without (B and D) stirring in aqueous solution of TLm under atmospheric conditions.

Fig. 4. Photoluminescence (PL) spectra in the solid state for TLm before and after stirring with NFs under atmospheric (TLmNFs(atm)) and vacuum pressure (TLmNFs(vac)) at room temperature (A); and their fluorescence decay traces monitored at 400 nm (B) and 450 nm (C) after 375 nm excitation. The real photos for all solids are shown in D. Colored dots represent the raw data and colored solid lines correspond to the fits derived from the global analysis, see Fig. S19, S20 and S21 in the Supporting information.

By analogy to the results in solution, we have analyzed the emission decays measured for solid TLm, TLmNFs(vac), and TLmNFs(atm) at different emission wavelengths across the entire emission spectra (Figs. S19, S20 and S21 in the Supporting information, Part S2). It transpires that the data are best fitted utilizing the global

method, which assumes parallel unimolecular kinetic model. This confirms the lack of ESPT process inside the NFs, which agrees with Huppert recent findings on carbohydrates' surface for similar Lm photoacids [19–21]. The authors attributed their results to the lack of water molecule inside the NFs in the presence of Lm photoacids. In

contract to data in solution, each DAS peak in these figures appears to be associated with a distinct structure of TLM (in NFs) because we could not fit all data simultaneously. Thus, interpretation of each emission maximum and the associated lifetime constant requires a pre-knowledge on the solid-state structure of TLM and TLM-NFs. This needs X-ray crystallographic studies, which is not available to us. However, it transpires from the trend in these data that distinct electronic states are observed depending on their emission maxima—400 nm (blue-shifted band) and 450 nm (red-shifted band)—and excited-state lifetime constants. While one specie emits at 400 nm, the species emit at the red side can be further sorted out as short-lived, long-lived, and very-long-lived species. In Fig. 4 (B,C), the monitored emission decays at 400 and 450 nm for TLM solids before and after stirring with NFs films confirmed a considerable elongation of the excited-state lifetime. The extended excited-state lifetime reflects a major role played by the confinement/rigidity factor, which restricts the movement of TLM inside the fibres. It must be noticed that because the very-long lived specie appears only under atmospheric condition at 450 nm, it contributes to the additional elongation of the excited-state lifetime monitored at 450 nm (compare Fig. 4B versus 4C). Overall, both steady and TRPL measurements confirmed the interactions of TLM with NFs.

2.3. Fluorescence detection for mercuric acetate in solid state and nanofibers (static vs. dynamic quenching)

In the third part of the present study, we have tested the effects of adding mercuric acetate on the emission properties in the solid state of TLM (in NFs). The fluorescent sensing of TLM for Hg^{2+} ions in aqueous solution has already been reported experimentally by one of us [26] and theoretically by others [32], from which it was concluded that the coordination with mercury ion occurs through the atoms sulphur, oxygen, and nitrogen with a 2-to-1 stoichiometry between ligand and metal [32]. The mechanism of fluorescence quenching was also explained in detail through the molecular orbital calculations [32]. One expects two possible limiting cases for the fluorescence quenching—dynamic and static [8]. In the dynamic case, the observed fluorescence lifetime of TLM decreases with the increase in the concentration of the quencher because of the opening of a new deactivation channel, whereas in the static fluorescence quenching the observed lifetime is unaffected because the formed non-fluorescent associated complex in the ground state only decreases the number of emitters without an associated quenching molecule.

The PL and TRPL (Fig. 5) were measured in solid state for TLM-NFs(atm) and TLMNFs(vac) at different $[\text{Hg}^{2+}]$ concentrations—nM, μM , and mM. The number of moles for TLM was kept at $20\mu\text{mol}$. The PL spectra appear not to correlate with the amount of the added

Fig. 5. Photoluminescence (PL) spectra (A and C) of solid TLMNFs(vac) and TLMNFs(atm) before and after addition of mercuric ions at various TLM/ Hg^{2+} molar ratios (as indicated in the graph), and fluorescence decay traces (B and D) of the same solid films monitored at 450 nm after excitation at 375 nm. The number of moles for TLM was $20\mu\text{mol}$. Colored dots represent the raw data and colored solid lines correspond to the fits derived from the global analysis, see Figs. S22, S23, S24, S25, S26, and S27 in the Supporting Information in Part S2.

ions under either condition (Fig. 5A and C) because of the involvement of multiple species, which confirms that steady-state data are not an absolute measure and are unreliable for creating a standard calibration sensing curve in the solid state [8]. Not to mention the intensity is also affected by the position of laser exposure to the measured film.

However, adding Hg^{2+} ion to TLM decreases the excited-state lifetime monitored at 450 nm for the case of NFs, which was prepared under atmospheric, not vacuum conditions (Fig. 5D versus Fig. 5B). We have, therefore, measured the PL and TRPL in solid state for TLMNFs(atm) with additional $[\text{Hg}^{2+}]$ concentrations in picomolar (pM)—100, 250, 300 and 500 pM—to clarify the mechanism of fluorescence quenching by mercuric ions and determine the linear sensing range (Table S1, S2, and S3 in the Supporting Information in Part S3). The results in Fig. 6 confirms that the average excited-state lifetime monitored at three wavelength: 450, 460 and 470 nm and presented as mean $\pm$ standard deviation (Tables S1, S3, and S3 in the Supporting Information), linearly correlate ($R=0.92$ with a slope of 0.005 pM^{-1}) with the concentration of Hg^{2+} ions (from 60 pM to 500 pM). It must be said that the excited-state lifetime values associated with the DAS peak at 450 nm, not 400 nm (compare Fig. 5D with Fig. S28 in the Supporting Information, Part S2) are more sensitive to mercuric concentrations. The average lifetime for TLMNFs(atm) was calculated 20 times. Then, the standard deviation was estimated for these data to be ± 0.30 (Tables S1, S2, and S3 in the Supporting Information). The low detection limit (LOD) was then calculated by dividing the standard deviation over the slope of the linear plot in Fig. 6 (0.0050). The LOD value was estimated to be 60 picomolar.

Putting all together, the calibration curve constructed from excited-state lifetime data in the solid state utilizing TRPL measurements is, therefore, more accurate and quantitative, when compared to stationary data. The decrease in the excited-state lifetime value at 450 nm under atmospheric condition can be related to a predominant dynamic quenching, whereas a pure static mechanism governs the quenching under vacuum condition. The results, thus, provide accurate sensing data in the solid state with high sensitivity and selectivity (picomolar). Thus, the present results have a higher economic and environmental value and a wider adaptability to domestic users when compared to results in solution [33–36].

Fig. 6. The dependence of the mean average excited-state lifetimes (τ) monitored at 450, 460 and 470 nm (Tables S1, S2, and S3 in the Supporting Information, Part S3) for TLMNFs(atm) on the concentration of Hg^{2+} in picomolar. τ_0 =unquenched lifetime.

2.4. Complex formation of TLM with mercuric acetate in the solid state

The FT-IR measurements (Fig. S37 in the Supporting Information in Part S4) for the prepared TLM— Hg^{2+} complex in solid state has confirmed the formation of the complex and the possible occurrence of static quenching inside NFs. The peaks associated with stretching frequencies of carbonyl and imine bonds were shifted to lower wavenumbers with the addition of mercuric acetate. In addition, no change in the excited-state lifetime constants was observed with the addition of mercuric ions (1.2 versus 1.0 ns in Table S1 in the Supporting Information in Part S3). Moreover, the appearance of isosbestic points in the UV–vis absorption spectra upon titrating TLM with Hg^{2+} in aqueous solution (Fig. S38 in the Supporting Information in Part S4) indicates the formation of non-fluorescent complex at the ground state in the solid state and supports the static quenching observed above for the reaction of TLM with mercuric acetate inside the pores of NFs under vacuum condition (Fig. 1).

2.5. Conclusions

TRPL measurements have unfolded the confirmation effects on the ESPT process in the excited state of TLM in water. Only the *cis*-conformers are involved in the ESPT process. The results, also, revealed, the mechanism of fluorescence quenching by mercuric ions of TLM fluorophore inside NFs in the solid state. Static quenching has occurred when TLM was loaded in NFs under vacuum, not atmospheric pressure. Despite the large number of reported sensors for mercuric ions in solid and solution, the results in the present work highlight the advantage of TRPL over steady-state emission methods for detection of picomolar of Hg^{2+} ions in the solid state.

3. Experimental section

3.1. Reagents and synthesis procedures

TLM was synthesized as reported previously [24]. All reagents were purchased from Sigma-Aldrich (St. Louise, MO, USA) with purity >99%. Millipore water had conductivity <0.05 μS . The pH values of the solutions were adjusted (± 0.2 units) by adding adequate amounts of HCl or NaOH. Cellulose acetate nanofibers (NFs) were prepared by electrospinning. Solution of cellulose acetate was prepared in a solvent mixture of acetone and dimethyl acetamide, with 12% weight of the polymers to the volume of solvent. Then, it was warmed at temperature of 30–35 $^{\circ}\text{C}$ until solid completely dissolved [9].

3.2. Interactions of TLM with cellulose acetate nanofibers (NFs)

The interaction of TLM solids with NFs was established by stirring a mixture of TLM with acetate moieties (in NFs) in aqueous solution with a $\sim 1:2$ M ratio under atmospheric pressure, in ethanol with a $\sim 1:3$ M ratio under atmospheric pressure, or in aqueous solution with a $\sim 2:1$ M ratio under vacuum pressure. The NFs was assumed to contain 39.6% acetate content in calculating the number of moles. The exact number of moles, depending on media, were as follows; For TLM, $n=32$ and $21 \mu\text{mol}$ in water under atmospheric and vacuum condition, respectively, whereas, $n=21 \mu\text{mol}$ in ethanol under atmospheric condition; For the acetate moieties (in NFs), $n=78$ and $10 \mu\text{mol}$ in water under atmospheric and vacuum condition, respectively, whereas $n=70$ in ethanol under atmospheric condition. The pH of the formed suspension was adjusted to 2.1. The TLM/NFs mixture

was immersed in water overnight with stirring in darkness under fixed pH. The resulted TLmNFs films were then washed with deionized water and left to dry for 48 h under ambient air.

3.3. Interactions of TLmNFs with mercuric acetate.

TLmNFs films of around 4 mm² area prepared either under atmospheric or vacuum conditions were immersed in mercuric acetate solutions in water (4 mL, pH2) having the following concentrations: 1 mM, 1 μ M, 1 nM, 500 pM, 300 pM, 250 pM, and 100 pM. After shaking the suspension for overnight, the final nanofibers were left to dry completely for 48 h.

3.4. Preparation of TLmHg²⁺ complex in solid state

The complex was prepared by grinding method, in which a 1:1 ratio of TLm/mercuric acetate mixture was grounded in solid state for about 20 min before washing the new composite by acetone.

3.5. Techniques

3.5.1. Absorption/steady-state fluorescence spectroscopy

The UV–visible absorption spectra were measured on Cary-300 instrument (Agilent, Santa Clara, CA, USA) at room temperature, between 200 and 600 nm. Fluorescence spectra measurements were scanned at room temperature, between 400 and 700 nm on a Cary-Eclipse fluorimeter. Slit widths were 5 nm for both excitation and emission monochromators, unless otherwise specified. The pH values were recorded using a pH meter (WTW 330i equipped with a WTW SenTix Mic glass electrode). Quartz cuvettes (1.0 cm, 4.0 mL) were used in all spectroscopic measurements and were obtained from Starna Cells Inc. (Atascadero, CA, USA).

3.5.2. Fourier transform infrared (FTIR) spectroscopic measurements

Structural composition of the electrospun fibrous sorbents was carried out using Fourier transform infrared spectrometer (FTIR) (IR-Prestige-21, Shimadzu, Tokyo, Japan) in a transmission mode.

3.5.3. Scanning electron microscopy (SEM)

The morphology of the fibrous sorbents was evaluated using a scanning electron microscope (SEM) (Oxford Inca Energy EDS System, Oxford, UK) after gold coating.

3.6. The pH-titration studies

The pH titration by UV–visible absorption and fluorescence spectroscopic method was accomplished by measuring the pH values of about 3 mL-solution in a rectangular quartz cuvette with 1 cm optical path length, and the absorption spectra were then recorded. To adjust pH, microliter volumes from 0.01 and 0.1 M NaOH and HCl solutions were pipetted consecutively to achieve the indicated pH values. The pK_a value was determined finally from fitting the titration data at a selected wavelength to a sigmoidal formula derived from Henderson-Hasselbalch and Beer-Lambert laws. The fitting algorithm was provided by SigmaPlot software (version 6.1; SPSS, Inc., Chicago, IL, USA).

3.7. Time-resolved photoluminescence (TRPL) measurements

The emission decays of TLm in water at different pH values were collected using time-correlated single photon counting (TCSPC) technique implemented on a LifeSpec II spectrometer (Edinburgh Inc., Edinburgh, UK). The excitation was set at 375 nm by using Ed-

inburgh's diode laser with a repetition rate at 20 MHz. A red-sensitive high-speed PMT (H5773-04, Hamamatsu, Japan) was used for fluorescence detection. The time resolution of the system is on the order of 30 ps. Emission decays were collected every 2 nm (unless otherwise specified) over the entire emission spectra of TLm in aqueous solution at certain pH value with a dwell time of 10 s at each wavelength.

3.7.1. Kinetic (global and target) analysis

The data were globally analyzed using publicly available software Glotaran [31]. In the global analysis, the data measured at all wavelengths are fitted simultaneously by a sum of exponential decays convoluted with the instrument response function, IRF. The lifetimes assumed to be the same across the whole dataset while the pre-exponential factors are left free. The estimated pre-exponential factors/amplitudes represent the estimated spectra, with a lifetime given by the inverse of the rate constant of the exponential decay. The first type of global analysis that was used to analyze presented data utilizes a model of number of parallel decaying components. In this case, the estimated amplitudes at each wavelength are termed decay-associated spectra (DAS). A positive amplitude means a fluorescence decay, whereas a negative amplitude means a rise of fluorescence. A DAS which is positive in one wavelength region and negative in another can be indicative of an excited-state population transfer with the lifetime being associated with that DAS. It is important to note that the DAS do not necessarily represent the spectra of pure physical (chemical) species but rather a mixture of states with similar lifetimes, however in case of parallel decaying species DAS will represent true spectra. A second, more advanced form of the global analysis here uses a kinetic compartmental model. In this case each microscopic rate constant represents the transfer of population of one (excited-state) species into another or the decay to the ground state. The estimated spectra resulting from such an analysis are termed "species associated spectra" (SAS). In our analysis, a maximum of 3 compartments were applied at the same time, and the results of the target analysis were always cross validated with a result from the global analysis, utilizing individual datasets as well as the analysis of multiple datasets at the same time.

3.8. Average excited-state lifetime calculation

The data were fitted globally in terms of the multiexponential model. The data were analyzed by the iterative reconvolution method using the instrument's FAST software that utilizes the Levenberg–Marquardt algorithm to minimize χ^2 . The contribution of each time constant to the steady-state intensity is given by

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

where the sum in the denominator is over all the decay times and amplitudes.

The average lifetime at a given wavelength is then given by

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

Acknowledgements

All authors thank the research program in United Arab Emirates University for financial grant numbers ECEER-1-2017.

Associated content

Supporting information paragraph

Global and target analysis of TRPL data at different pH values; TRPL of TLM in water (pH2–4); TRPL of TLM in MeOH and D₂O; TRPL of TLM in water (pH4–6); TRPL of TLM in water at (pH6–8) and (pH8–10); TRPL of TLM in water (pH10–12); TRPL of TLM in water (pH6–8) and (pH8–10); TRPL of TLM in water (pH10–12); Global analysis of TRPL data in the solid state; Average excited-state lifetime in the solid state; UV–vis absorption and FTIR spectra for TLM–Hg²⁺ complex; References; Appendix I: Standard deviation calculations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.saa.2019.117189>.

References

- [1] D. Klemm, F. Kramer, S. Moritz, T. Lindström, M. Ankerfors, D. Gray, A. Dorris, Nanocelluloses: a new family of nature-based materials, *Angew. Chem. Int. Ed.* 50 (2011) 5438–5466.
- [2] J. Wu, N. Wang, Y. Zhao, L. Jiang, Electrospinning of multilevel structured functional micro-/nanofibers and their applications, *J. Mater. Chem. A* 1 (2013) 7290–7305.
- [3] E. Omollo, C. Zhang, J.I. Mwasiagi, S. Ncube, Electrospinning cellulose acetate nanofibers and a study of their possible use in high-efficiency filtration, *J. Ind. Text.* 45 (2016) 716–729.
- [4] L.T.H. Nguyen, S. Chen, N.K. Elumalai, M.P. Prabhakaran, Y. Zong, C. Vijila, S.I. Allakhverdiev, S. Ramakrishna, Biological, chemical, and electronic applications of nanofibers, *Macromol. Mater. Eng.* 298 (2013) 822–867.
- [5] M. Wang, G. Meng, Q. Huang, Y. Qian, Electrospun 1,4-DHAQ-doped cellulose nanofiber films for reusable fluorescence detection of trace Cu²⁺ and further for Cr³⁺, *Environ. Sci. Technol.* 46 (2012) 367–373.
- [6] X. Sun, Y. Liu, G. Shaw, A. Carrier, S. Dey, J. Zhao, Fundamental study of electrospun pyrene–polyethersulfone nanofibers using mixed solvents for sensitive and selective explosives detection in aqueous solution, *ACS Appl. Mat. Int.* 7 (2015) 13189–13197.
- [7] B.W. Davis, N. Niamnont, R. Dillon, C.J. Bardeen, M. Sukwattanasinitt, Q. Cheng, FRET detection of proteins using fluorescently doped electrospun nanofibers and pattern recognition, *Langmuir* 27 (2011) 6401–6408.
- [8] B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, 2002.
- [9] Y.E. Greish, M.A. Meetani, E.A. Al Matroushi, B.A. Shamsi, Effects of thermal and chemical treatments on the structural stability of cellulose acetate nanofibers, *Carbohydr. Polym.* 82 (2010) 569–577.
- [10] N.S. Moyon, P.M. Gashnga, S. Phukan, S. Mitra, Specific solvent effect on lumazine photophysics: a combined fluorescence and intrinsic reaction coordinate analysis, *Chem. Phys.* 421 (2013) 22–31.
- [11] J.F. Ireland, P.A. Wyatt, Acid-base properties of electronically excited states of organic molecules, *Adv. Phys. Org. Chem.* 12 (1976) 131–221.
- [12] L.M. Tolbert, K.M. Solntsev, Excited-state proton transfer: from constrained systems to “super” photoacids to superfast proton transfer, *Acc. Chem. Res.* 35 (2002) 19–27.
- [13] N. Agmon, Elementary steps in excited-state proton transfer, *J. Phys. Chem. A* 109 (2005) 13–35.
- [14] O.F. Mohammed, D. Pines, J. Dreyer, E. Pines, E.T. Nibbering, Sequential proton transfer through water bridges in acid-base reactions, *Science* 310 (2005) 83–86.
- [15] S. Mondal, K. Sahu, P. Sen, D. Roy, S. Ghosh, K. Bhattacharyya, Excited state proton transfer of pyranine in a γ -cyclodextrin cavity, *Chem. Phys. Lett.* 412 (2005) 228–234.
- [16] T. Förster, Die pH-Abhängigkeit Der Fluoreszenz Von Naphthalinderivaten, *Z. Elektrochem. Angew. Phys. Chem.* 54 (1950) 531–535.
- [17] I. Presiado, Y. Erez, R. Gepshtein, D. Huppert, Excited-state intermolecular proton transfer of lumazine, *J. Phys. Chem. C* 114 (2010) 3634–3640.
- [18] R. Simkovitch, D. Huppert, Photoprotolytic processes of lumazine, *J. Phys. Chem. B* 121 (2017) 129–142.
- [19] R. Simkovitch, D. Huppert, Excited-state proton transfer of weak photoacids adsorbed on biomaterials: 8-Hydroxy-1,3,6-pyrenetrisulfonate on chitin and cellulose, *J. Phys. Chem. A* 119 (2015) 1973–1982.
- [20] R. Simkovitch, D. Huppert, Excited-state proton transfer of weak photoacids adsorbed on biomaterials: proton transfer on starch, *J. Phys. Chem. B* 119 (2015) 9795–9804.
- [21] N. Amdursky, R. Simkovitch, D. Huppert, Excited-state proton transfer of photoacids adsorbed on biomaterials, *J. Phys. Chem. B* 118 (2014) 13859–13869.
- [22] K. Ohkawa, Nanofibers of cellulose and its derivatives fabricated using direct electrospinning, *Molecules* 20 (2015) 9139–9154.
- [23] B. Fischer, S.J.N. Burgmayer, Models for the pyranopterin-containing molybdenum and tungsten cofactors, in: A. Sigel, H. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 2002, pp. 265–305.
- [24] N. Saleh, A.-M. Rawashdeh, Y. Yousef, Y. Al-Soud, Structural characterization of new Cd²⁺ fluorescent sensor based on lumazine ligand: AM1 and Ab initio studies, *Spectrochim. Acta, Part A* 68 (2007) 728–733.
- [25] N. Saleh, J. Graham, A. Afaneh, Y. Al-Soud, G. Schreckenbach, F. Esmadi, Peridine-based fluorescent pH sensors designed for physiological applications, *J. Photochem. Photobiol. A* 247 (2012) 63–73.
- [26] N. Saleh, Luminescent sensor for Cd²⁺, Hg²⁺ and Ag⁺ in water based on a sulphur-containing receptor: quantitative binding-softness relationship, *Luminesc* 24 (2009) 30–34.
- [27] P. Mahato, S. Saha, P. Das, H. Agarwalla, A. Das, An overview of the recent developments on Hg²⁺ recognition, *RSC Adv.* 4 (2014) 36140–36174.
- [28] X. Wang, C. Drew, S.-H. Lee, K. Senecal, J. Kumar, L. Samuelson, Electrospun nanofibrous membranes for highly sensitive optical sensors, *Nano Lett* 2 (2002) 111273–111275.
- [29] M. Kaur, J. Cho, D. Choi, A phenothiazine-based “naked-eye” fluorescent probe for the dual detection of Hg²⁺ and Cu²⁺: application as a solid-state sensor, *Dyes Pigments* 125 (2016) 1–7.
- [30] A. Afaneh, G. Schreckenbach, Conformation/tautomerization effect on the pK_a values of lumazine and 6-thienyllumazine, *J. Phys. Org. Chem.* 27 (2014) 690–700.
- [31] J. Snellenburg, S. Liptonok, R. Seger, K. Mullen, I. Stokkum, Glotaran: a java-based graphical user interface for the R package TIMP, *J. Stat. Softw.* 49 (2012) 1–23.
- [32] A. Afaneh, G. Schreckenbach, Fluorescence enhancement/quenching based on metal orbital control: computational studies of a 6-thienyllumazine-based mercury sensor, *J. Phys. Chem. A* 119 (2015) 8106–8116.
- [33] D. Dai, Z. Li, J. Yang, C. Wang, J.-R. Wu, Y. Wang, D. Zhang, Y.-W. Yang, Supramolecular assembly-induced emission enhancement for efficient mercury(II) detection and removal, *J. Am. Chem. Soc.* 141 (2019) 4756–4763.
- [34] S. Tang, X. Zhou, S. Zhang, X. Li, T. Yang, W. Hu, J. Jiang, Y. Luo, Metal-free boron nitride nanoribbon catalysts for electrochemical CO₂ reduction: combining high activity and selectivity, *ACS Appl. Mat. Int.* 11 (2019) 906–915.
- [35] X.-Y. Lou, N. Song, Y.-W. Yang, Fluorescence resonance energy transfer systems in supramolecular macrocyclic chemistry, *Molecules* 22 (2017) 1640.
- [36] E.S. Childress, C.A. Roberts, D.Y. Sherwood, C.L.M. LeGuyader, E.J. Harbron, Ratiometric fluorescence detection of mercury ions in water by conjugated polymer nanoparticles, *Anal. Chem.* 84 (2012) 1235–1239.