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THE CHARACTERIZATION OF XANTHENE DYES ON A GLASS SUBSTRATE FROM DIFFERENT COATING TECHNIQUES

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THE CHARACTERIZATION OF XANTHENE DYES ON A
GLASS SUBSTRATE FROM DIFFERENT COATING
TECHNIQUES

BY
ELSA ORTEGA

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY

UNIVERSITY OF RHODE ISLAND

2019

DOCTOR OF PHILOSOPHY DISSERTATION
OF
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2019

ABSTRACT

Chapter 1 of this dissertation is an investigation of rhodamine 560 (Rh560), a cationic dye similar to the well-studied rhodamine 6G (Rh6G). The spectral properties of Rh560 and Rh6G have similar changes as a function of thickness. At low surface coverage the spectra indicate monomers, at 1-2 monolayers dimers dominate, and thicker films show larger aggregates. The difference between Rh6G and Rh560 is that the transition from monomer to dimer occurs at different thickness, ~ 1.2 nm for Rh6G and ~ 0.5 nm for Rh560. This difference is accounted for by the molecular size. Chapters 2 and 3 describe photophysical investigations of the zwitterionic dyes, Sulforhodamine 640 (SRh640, Chapter 2) and sulforhodamine B (SRhB, chapter 3). The charge appears to have little effect as the thin film behavior in the zwitterions. As with Rh6G and Rh560, the films change from monomer to dimer to aggregate. The monomer to dimer transition occurs at ~ 1 nm for SRh640 and at ~ 1.5 nm for SRhB, consistent with their molecular sizes.

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PREFACE

The following dissertation is presented in a manuscript format. The dissertation is presented into three chapters. The first chapter is titled “Investigating the Structures of Rhodamine 560 in Thin Films”. The second chapter is titled “Investigating the Structures of Sulforhodamine 640 in Thin Films”. The third chapter is entitled “Investigating the Structures of Sulforhodamine B in Thin Films”.

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CHAPTER 1

Investigating the Structures of Rhodamine 560 in Thin Films

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The following will be planned to be publish in *Langmuir* and is presented here in manuscript format.

Abstract

Xanthene dyes are well known with respect to their photophysical properties and their applications. However, they are well known in solution not so much in thin films. The cationic fluorophore Rhodamine 560 in thin films is studied. Rhodamine 560 of varying concentrations was either dip coated or spin coated onto glass substrates. The coating technique and the thickness of the Rhodamine 560 is a key factor for interpreting both the absorption and emission spectra. The transition from monomers to dimers is at the thickness of ~ 0.5 nm. By controlling the aggregation of the fluorescent dye we gained an understanding of its photophysical properties to improve our fluorescence-based sensor efficiently, especially where the optimum sensitivity can be obtained.

Introduction:

Fluorescence-based sensors have been reported and efforts are continuing to improve method in enhancing the fluorescent signal. A three layer structure that consists of a glass substrate, a polymer, and a fluorophore has been reported and shown as a sensor in detection of analytes.¹ Results show that with a little amount of fluorophore present in the sensor, the signal response was strong. Five xanthene dyes, rhodamine 6G (Rh6G), rhodamine 560 (Rh560), sulforhodamine B (SRhB), rhodamine 640 (Rh640), and fluorescein 548 (Fl548), on different substrates, either poly(methyl methacrylate), PMMA, or polyvinylidene difluoride, PVDF, were measured in absorption and emission. These xanthene dyes have a common chemical structure. Rhodamine 6G and rhodamine 560 are cationic dyes, sulforhodamine B and

rhodamine 640 are zwitteronic dyes, and fluorescein 640 is an anionic dye. Each showed a different response to the analytes in the emission spectra, either an enhancement or quenching. The interface between the polymer and the fluorophore plays a role in the changes of emission.¹

Over the years, there has been an interest in the properties of fluorescence dyes such as xanthene dyes, especially rhodamine 6G. Rhodamine 6G is a well-known cationic fluorophore used as a fluorescent tracer due to its high fluorescence quantum yield.²⁻⁴ In high concentrations, rhodamine 6G has been known to form aggregates, which can significantly affect the photophysical properties.⁵⁻⁸ Exciton theory has been used to predict the splitting of the two excited states based on dipole-dipole interaction in the dimer that causes spectral changes. For dimers there are two extremes labeled H- and J- dimers. An H-dimer has a sandwich structure and is identified by a blue shift in the absorption spectrum with respect to the monomer. Spectra of H-dimers are typically found at lower wavelength and are non-emissive. On the other hand, a J-dimer is a head-to-tail dimer and shows a red shift in the absorption spectrum with respect to a monomer and are emissive.⁹⁻¹⁰

Results show that aggregation of the dye molecules can be controlled depending on certain preparation conditions.¹¹⁻¹³ For rhodamine 6G the aggregation is dependent on the thickness of the thin film. As the thickness of the thin film decreases we see that the emission is higher and have shorter wavelength maxima whereas when the thin films increase in thickness we see a quench in emission and longer wavelength maxima. The structure of the dye and thickness of the thin films can be detected from the absorption spectra.² By controlling the aggregation of the fluorescent

dye we gained an understanding of its photophysical properties to improve our sensors efficiency. In this paper we are investigating the spectroscopic details of a xanthene dye similar to rhodamine 6G, rhodamine 560 also known as rhodamine 110. Similar to rhodamine 6G, rhodamine 560 is a cationic dye in the Xanthene family.¹⁴⁻¹⁷

Scheme 1. Structures of Rhodamine 560 and Rhodamine 6G.

Experimental Methods:

Pre-cleaned Borosilicate glass microscope slides were used from Scientific Products. These glass slides were cut into a certain length and width, 3.75 cm by 1.70

cm. These slides were washed twice in a Branson 3510 sonicator. The first wash contains 95% ethanol and was sonicated for 15 minutes. It was then followed by a second wash of distilled water for another 15 minutes. Then the slides were dried by using nitrogen gas.

Rhodamine 560 was purchased from Exciton. A series of diluted Rh560 solutions were made in 95% ethanol from different concentrations ranging from 1.0×10^{-2} M to 1.0×10^{-7} M. Each clean glass slide was coated with rhodamine 560 both by a spin coater (Laurell Technologies Spincoater) and by a dip coater (MTI Corp. HWTL-01 desktop dip coater). For the spin coater each substrate was covered with 50 microliters of solution and spun at 1200 RPM. For the dip coater, each substrate was removed from solution at a pull rate of ~ 60 mm/min.

Spectra of the absorbance were measured using a Perkin Elmer Lambda 1050 spectrometer. The spectra were collected using an integration time ranging from 0.20 s to 10.0 s, depending on the concentration and from a wavelength of 300-800 nm. The fluorescence spectrum was measured using a Horiba Fluorolog-3 Fluorimeter. For each sample steady state fluorescence measurements were measured at an incidence angle of 60 degrees relative to the excitation beam with a slit width of 2 nm. The excitation light source was used at a wavelength of 450 nm. The emission wavelength ranged from 460 to 800 nm. Excitation spectra were also measured. The excitation range was done in four scans, one from 400 nm to 510 nm and the second one is from 525 nm to 700 nm using a detection wavelength of 520 nm. The third one is from 400 nm to 610 nm and the fourth one is from 625 nm to 700 nm using the a detection wavelength of 620 nm. A Filmetrics microscope was used to determine the

thicknesses. Lifetime measurements were made using a Horiba Fluorohub time-correlated single photon counting (TCSPC) system. A Horiba NanoLED N-460 pulsed diode laser was used as the light source with a wavelength of 464 nm, a repetition rate of 1.0 MHz, and 160 ps pulse duration time with a power output of about 7 pJ/pulse.

Results and Discussion

The glass substrates were deposited by either dip coating or spin coating in a series of concentration solutions of Rh560. The two different coating techniques were both set at a certain speed (1200 RPM) and at a certain pulling rate (~60 mm/min). The dip coated samples were measured twice: immediately after the dip coat with both sides of the substrate and again after one side was polished clean. All absorption spectra of the two-side coated samples were twice the intensity of the sample with the one-side cleaned. This indicates the uniformity of the coating. Henceforth all reference to dip coated samples are single sided. As shown in Figure 1 there is a trend from the two coating techniques that shows similar spectral properties. As expected for the casting and in solution form, the absorbance increase as concentration increases. However it does change between solution and thin films. In solutions, at lower concentrations, the absorption maximum is at 500 nm with a shoulder at 469 nm. As the concentration increases we noticed there is no shift and we can see no changes in the line shape throughout the concentrations. This suggests no aggregation occurs in solution.

However, thin films show different results from solutions. In spin coating we noticed at lowest concentration the maximum peak is at ~503 nm with a shoulder at

~468 nm. As the concentration increases the line shape broadens and there is a slight shift in the main peak to ~510 nm and the shoulder to ~479 nm. This broadened peak suggests aggregation is occurring. Similar spectral behaviors occurred in dip coating. At the lowest concentration the maximum wavelength is at ~503 nm with a shoulder of ~468 nm. As the concentration increases the lineshape broadens and there is slight shift of the main peak to ~503 nm and the shoulder to ~468 nm. Between the two coating methods, the absorbance from spin coating is slightly greater than dip coating at the same concentration. With both spin coated and dip coated samples being broader at low and high energies this would imply an oblique dimer forms on the glass substrate.

Figure 2 shows that as the concentration increases the absorbance increases as expected. Spin-coated samples show a linear increase of absorbance with respect to concentration. However the dip-coated samples are non-linear, especially at lower thickness. This implies that dip coating is more reliable for thicker films than thin films. A reason for this effect could be caused by the coating process on the glass substrate, which it is known as the withdrawal phenomenon. This refers back to the model of Landau and Levich¹⁸, where the model can explain the relationship from the thickness of the sample and the withdrawal speeds on certain parameters such as viscosities, surface tension, and the evaporation rate of the solution.¹⁹⁻²⁰

Figure 1 Absorption spectra of a series of different concentration of Rh560 in A.) solutions, in B.) Spin coating and C.) Dip coating.

Figure 2. Top is spin coating on different film thicknesses over either absorbance (left) or concentration (right). Bottom is dip coating on different thicknesses over either absorbance (left) or concentration (right).

Figure 3. Normalized Absorbance spectra of various concentration of Rh560 in A.) solutions, B.) Spin coating and C.) Dip coating.

Normalized absorbance spectra were used to see the difference in their line shape. The solution spectra show that throughout the concentrations all have the same narrow absorption peak with a high energy shoulder. However, for both coating methods the line shape of the spectra are broadened. From the two different coating

techniques it can be seen there is a trend where at low concentrations the peak starts out with a narrow peak with the broad shoulder and as the concentration increases the peak shifts towards lower energy at ~ 500 nm with a prominent broad shoulder at ~ 470 nm. This peak has both broad low and high energies shoulders, which implies the presence of oblique dimers in thicker films, similar to what was found in Rh6G.² The spin coated samples show broader peaks than dip coated samples at high concentrations, which indicates there are more molecules on the surface when spin cast than dip cast.

Spin Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	475	15	0.007154	494	28	0.028300	516	15	0.019695
5.0×10^{-4} M	473	10	0.002764	493	27	0.018393	513	14	0.010459
1.0×10^{-4} M	474	10	0.000987	485	30	0.001703	505	14	0.003630
5.0×10^{-5} M	478	14	0.001191	484	37	0.000678	505	12	0.003487
1.0×10^{-5} M	485	21	0.000455	505	11	0.000735	506	52	0.000058
Dip Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	474	8	0.002201	494	26	0.02513	513	13	0.016398
5.0×10^{-4} M	475	8	0.001653	493	27	0.012258	511	13	0.009306
1.0×10^{-4} M	474	10	0.000987	485	30	0.001703	505	14	0.003630
5.0×10^{-5} M	478	14	0.001191	484	37	0.000678	505	12	0.003487
1.0×10^{-5} M	477	17	0.000199	505	13	0.000546	506	7	0.000060

Table 1: Deconvolution from the absorbance spectra from both spin-coated and dip-coated samples as shown for Peak position ($\lambda_{\max}$) and FWHM (Γ). A Gaussian function was used to describe each peak.

Deconvolutions of the absorbance spectra from both coating methods are done and all were fitted to three peaks. Both coating methods have similar results. The peak at ~ 494 nm is assigned to a monomer. The pair of peaks at ~ 474 and ~ 511 nm are

assigned to an oblique exciton dimer. As shown in the absorption spectra the increasing thickness affects a change in intensity, but it does not require any additional peaks to fit.

Figure 4. Fluorescence spectra of Rh560 as a function of various concentrations from A.) Spin coating and B.) Dip coating.

These samples were measured to see their fluorescence intensity. As shown in Figure 4, in rhodamine 560 thin films as concentration changes intensity changes. As the concentration decreases the intensity reaches a maximum. Then it continues to decrease as the line shape get narrowed. For dip-coated samples its highest intensity is at a concentration of 5.0×10^{-5} M. For spin-coated samples the highest intensity is found at lower concentration, 5.0×10^{-6} M.

Figure 5. Normalized Fluorescence spectra of different serial concentration of Rh560 for A.) Spin coating and B.) Dip coating.

From the normalized fluorescence spectra, as shown in figure 5, the trend in line shapes can be seen. Both coating techniques have similar line shape. Both having a maximum wavelength of $\sim 530\text{-}534$ nm with a shoulder at $\sim 600\text{-}610$ nm at high concentration that then shifts to $\sim 520\text{-}526$ nm with the shoulder disappearing at low concentration. This suggests that at lower concentrations monomers dominate in these thin film samples and at high concentration aggregates form. This can also correlate to the thickness and how it affects with fluorescence intensity.

From figure 6 it shows a trend that as the highest concentration fluorescence intensity is lowest. As the concentration decreases the fluorescence intensity increases dramatically. This implies we can control our aggregation based on what concentration we use.

Figure 6. These two graphs show the average fluorescence intensity from the different concentrations from the two different coating methods A.) Spin coating and B.) Dip coating.

It is evident in figure 7, which shows the intensity as a function of film thickness, both coating methods show a similar trend. As the thickness increases the fluorescence intensity reaches at maximum then drops at films that are more than 0.5 nm thick. This implies that we are seeing a transition from primarily monomers to primarily dimers. In previous work, it is shown that the transition for Rh6G is around ~1 nm. This would mean Rh560 is more susceptible in aggregation than it is with Rh6G.

Figure 7. Fluorescence Intensity as a function of thickness from A.) Spin coating and B.) Dip coating.

Spin Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	525	16	5313	550	22	4390	609	55	2959
5.0×10^{-4} M	524	14	11981	544	19	12213	588	41	11273
1.0×10^{-4} M	527	14	47613	549	20	38301	585	35	17302
5.0×10^{-5} M	522	12	48475	541	18	27066	570	29	10668
1.0×10^{-5} M	524	13	41978	542	18	19624	569	27	6514
Dip Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	525	14	8896	546	17	8796	595	42	8184
5.0×10^{-4} M	527	14	19139	550	21	11073	590	39	7125
1.0×10^{-4} M	527	13	10137	548	19	66846	583	34	21615
5.0×10^{-5} M	526	13	83067	545	18	50301	575	29	16028
1.0×10^{-5} M	520	12	45711	536	16	33830	563	27	12189

Table 2. Deconvolution from the fluorescence spectra from both spin-coated and dip-coated samples as shown for Peak position (λ_{max}) and FWHM (Γ). A Gaussian function was used to describe each peak.

The fluorescence spectra from both coating techniques were deconvoluted and the results are shown in Table 2. Both coating methods required only three peaks to fit the spectra at ~ 525 nm, ~ 548 nm, and ~ 590 nm. The monomer was assigned at the peak of ~ 525 nm. The oblique exciton dimer is assigned to the peak of ~ 548 nm. Also, at higher concentration larger aggregation is assigned to ~ 590 nm that shifts to lower energy as the aggregates grow.

As shown in figure 8 thickness of the thin films have no effect on the line shape of the excitation spectra detected at 520 nm. However, when detecting at 620 nm maxima wavelength maxima shift to lower energy and the line shape broadens. These results demonstrate that when detecting at the monomer wavelength, ~ 520 nm, all of the absorbed light is emitted from monomer. When detected at 620 nm, both

monomers and aggregates contribute to the emission that is, some of the energy in the excited monomer is transferred to aggregates.

Figure 8. Normalized Excitation spectra from two different techniques Dip coating (**Top**) and Spin coating (**Bottom**). This was detected at 520nm (**left side**) and 620nm (**right side**).

Figure 9 and 10 shows several normalized absorbance, emission, and excitation spectra for the thin films. For both coating techniques, at low concentration the excitation and absorbance spectra overlap, independent of the detection wavelength. This supports the assignment that thin films are primarily monomers. The same features are observed for dip-coated sample at intermediate concentrations. However, the spin-coated sample shows that the excitation spectra are slightly

narrower than the absorbance. This behavior is accentuated in the thickest sample for both coating techniques. This implies that only absorption into monomers leads to emission. Although the oblique dimers absorb some light, these excited states do not contribute significantly to the emission.

Figure 9. Normalized absorption, emission, excitation spectra (the detection wavelength of 520 nm (Left) and 620 nm (Right)) from Dip coating at different concentrations.

Figure 10. Normalized absorption, emission, excitation spectra (detection of wavelength of 520 nm (Left) and 620 nm (Right)) from spin coating at different concentrations.

Figure 11. Fluorescence Lifetime decays of various concentrations of Rh560 for A.) Spin coating and B.) Dip coating. IRF =instrument response function.

	Spin Coating		Dip Coating	
Concentration (M)	τ_1 (ns)	χ^2	τ_1 (ns)	χ^2
1.0×10^{-5} M	2.41	1.58	1.51	1.32
1.0×10^{-6} M	2.65	1.76	2.48	1.42

Table 3. Lifetime decays fits for spin coating and dip coating.

Figure 11 shows the excited state decays for both coating methods. The decays are collected at 525 nm. The black curve is the instrumental response function and other colors represent various concentration of Rh560. Table 3 show the measured lifetimes of the samples. At low concentration the decay curves are best fitted using two exponential functions. Decays less than 1 ns are not considered as they arise from scattering. The longer decays are about 2.5 ns which is consistent for all

concentrations. These values are close to the lifetime of Rh560 in solution, previous reported, 4 ns.²¹

Conclusion

	Thin films	Thin Films	Thick Film	Thick Films
	Rhodamine 6G	Rhodamine 560	Rhodamine 6G	Rhodamine 560
λ_{max} Abs. (nm)	526	503	540	510
Shoulder (nm)	485	451	503	479
Emission max (nm)	562	529	601	527
Shoulder (nm)	Narrow peak no shoulder	Narrow peak no shoulder	Broad peak no shoulder	616

Table 4. Summary of spectral behaviors for Rhodamine 6G and Rhodamine 560.

Overall the spectral behavior is similar from the two different coating methods. This implies that the samples are not dependent on the coating methods. This would imply controlling concentration instead of using different coating methods affect the spectral behavior and the molecules orientation. Molecule orientation is a key importance when dealing with photophysical properties. Table 4 shows that both Rh560 and Rh6G have similar spectral behavior with small differences in both absorbance and emission. In thick films of Rh560 the peak is found at a higher energy than Rh6G. There is a significant shift for Rh6G than Rh560 from the transition thick to thin thickness. Also, in the emission there is noticeable difference at higher

thickness where Rh6G has a broad peak while Rh560 has a broad peak with shoulder which implies aggregation is much influential in Rh560 than Rh6G. For Rh560, the transition from monomers to aggregates is at the thickness of 0.5 nm. However, Rh6 G the transition is found to be at around 1.0 nm. This indicates the Rh560 is very easily susceptible to aggregation than Rh6G. This shows us further understanding of Rh560 in thin films whereas in fluorescence it can help indicate which sample is aggregating or is more like monomer like structure and how useful this information in the process of sensors.

NOTES

The authors declare no competing financial interest.

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CHAPTER 2

Investigating the Structures of Sulforhodamine 640 in Thin Films

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The following will be planned to be publish in *Langmuir* and is presented here in manuscript format.

Abstract

There have not been many studies of the photophysical properties of xanthene dyes in solid thin films. From our previous work we have investigated cationic dyes such as Rhodamine 560 and Rhodamine 6G. We found that their spectral behavior is affected by how thick the thin films are. By controlling their aggregation, we gained an understanding of their photophysical properties. In this work we have studied on a different xanthene dye, Sulforhodamine 640. Sulforhodamine 640 is an anionic dye which is different from Rhodamine 560, or Rhodamine 6G which is a cationic dye. Sulforhodamine 640 was cast by spin coating or dip coating onto glass substrates using various concentrations. The spectral behavior is dependent on the thickness of the thin films. In the submonolayer region monomers dominate while in thicker films aggregation is more important. This is demonstrated in the emission spectra, where significant quenching begins at 1 nm thickness. For films greater than 1 nm thick, weak emission at long wavelengths dominates.

Introduction

Xanthene dyes are important dyes having high quantum yield. One of the best studied xanthenes is Rhodamine 6G, a cationic fluorophore that is used in extensive applications for fluorescence sensing.¹⁻⁶ These applications can be extended to detect explosives.⁵⁻⁷ Rhodamine 6G is a great candidate for this application based on its optical properties such as high quantum yield. When working in solid thin films, this fluorescent dye aggregates depending on the preparation settings. The forms of aggregation are commonly one of three types of dimers: H-dimers, J-dimers, or oblique dimers. The Exciton theory explains the spectroscopy of molecular

interaction. An H-dimer has a face to face arrangement causing a blue shift with of the absorbance maximum respect to the monomer. These H-dimers are non-emissive. J-dimers are oriented in a head to tail arrangement and are emissive. This arrangement is causing a red shift with of the peak maximum respect to a monomer. Oblique dimers are oriented in an angle between two monomer transition moments. This arrangement results in both a lower and higher energy peaks with respect to the monomer.⁸⁻¹¹ Layering the neighboring dye molecules in certain geometry onto substrates can alter their optical properties.

While Rhodamine 6G has been reported frequently,¹²⁻¹⁸ other family members of the Xanthene dyes have not had their spectral properties investigated in thin films. In chapter one, we discussed the spectral properties of Rhodamine 560, another cationic fluorescent dye similar to Rhodamine 6G. Results show very similar spectral behavior of Rh6G and Rh560 in thin films. Controlling the concentration can prevent or induce aggregation. Higher concentration tends to form aggregates and at low concentration monomer like structure is layered across the glass substrates. In this work, we will investigate sulforhodamine 640. Sulforhodamine 640 is an anionic dye in the Xanthene family unlike Rhodamine 6G and Rhodamine 560, which are a cationic dye.

Scheme 1. Structure of Sulforhodamine 640

Experimental Methods

Pre-cleaned Borosilicate glass microscope slides were used from Scientific Products. These glass slides were cut into a certain length and width, 3.75cm by 1.70 cm. These slides were washed twice in a Branson 3510 sonicator. The first wash contains 95% ethanol and was sonicated for 15 minutes. It was then followed by a second wash of distilled water for another 15 minutes. Then the slides were dried by using nitrogen gas.

Sulforhodamine 640 was purchased from Exciton. A series of diluted SRh640 solutions were made in 95% ethanol from different concentrations ranging from 1.0×10^{-2} M to 1.0×10^{-7} M. Each clean glass slide was coated with Sulforhodamine 640 both by a spin coater (Laurell Technologies Spincoater) and by a dip coater (MTI Corp. HWTL-01 desktop dip coater). For the spin coater each thin film was covered with 50 microliters and spun at 1200 RPM. For the dip coater, each thin film was removed from solution of a pull rate of ~ 60 mm/min.

Spectra of the absorbance were measured using a Perkin Elmer Lambda 1050 spectrometer. The spectra were collected using an integration time ranging from 0.20 s to 10.0 s, depending on the concentration and from a wavelength of 300-700 nm. The fluorescence spectrum was measured using a Horiba Fluorolog-3 Fluorimeter. For each sample steady state fluorescence measurements were measured at an incidence angle of 60 degrees relative to the excitation beam with a slit width of 2 nm. The excitation light source was used at a wavelength of 540 nm. The emission wavelength ranged from 550 nm to 800 nm. The Excitation spectra were also measured. The excitation ranged in two scans, one from 400 nm to 590 nm and the second one is from 605 nm to 700 nm using a detection wavelength of 600 nm. A Filmetrics microscope was used to determine the thicknesses. Lifetime measurements were made using a Horiba Fluorohub time-correlated single photon counting (TCSPC) system. A Horiba NanoLED N-460 pulsed diode laser was used as the light source with a wavelength of 464 nm, a repetition rate of 1.0 MHz, and 160 ps pulse duration time with a power output of about 7 pJ/pulse.

Results and Discussion

In this study, Sulforhodamine 640 thin films were produced by dip coating and spin coating onto glass substrates in a range of different concentrations from 1.0×10^{-3} M to 1.0×10^{-7} M. The two different coating techniques were both set at a certain speed (1200 RPM) and at a certain pulling rate (~ 60 mm/min). The dip coated samples were measured twice: immediately after the dip coat with both sides of the substrate and again one side was polished clean. All absorption spectra of the two-side coated

samples were twice the intensity of the sample with the one-side cleaned. This indicates the uniformity of the coating. Henceforth all references are to dip coated samples are single sided. Figure 1 shows the absorbance spectra of SRh640 on thin films as a function of the concentration of SRh640 used in solutions, dip coating and spin coating. As expected for the casting and in solution form, the absorbance increases as the concentration increases. In solutions, at the lowest concentration the absorption maximum is at 578 nm with a shoulder at 538 nm. As the concentration increases, we noticed there is no shift wavelength maxima and we can see no changes in the line shape. This suggests no aggregation occurs in solution. However, thin films show different results from solutions. In spin coating we noticed at the lowest concentration the maximum peak is at 576 nm with a broad shoulder 519 nm. As the concentration increases the line shape broadens and there is a gradual shift of the main peak to 590 nm and the shoulder to 549 nm. This broadened peak suggests aggregation of the dye molecules. Similar spectral behavior occurred in dip coating. At lowest concentration the maximum wavelength is at 581 nm with a broad shoulder at 524 nm. As the concentration increases the line shape broadens and there is a gradual shift of the main peak to 586 nm and the shoulder to 546 nm. Between the two coating techniques, the absorbance from spin coating is four times greater than from dip coating at the same concentration. This is consistent with the spin coated sample being is broader at both high and low energies onto the glass substrate for a given concentration.

Figure 2 shows the normalized absorbance of the two casting methods and in solution form. The solution spectrum shows that the same narrow absorption peak

with a higher energy shoulder. There are no shifts. However, when the fluorescent dye is cast either by dip coating or spin coating the line shape of the spectra broadened. For both coating methods, the absorbance spectra maximum shifts to lower energy as the film thickness increases. The high energy shoulder also increases. These observations imply the presence of oblique dimers in the thicker films, similar to what was found in Rh6G.¹⁶

Figure 1. Absorption spectra of a series of different concentration of SRh640 in A.) Spin coating, in B.) Dip coating and C.) Solutions.

Figure 2. Normalized Absorbance spectra of various concentrations of SRh640 for solutions spin and dip coating of SRh640 for A.) Spin coating B.) Dip coating and C.) Solutions.

Figure 3. Absorption spectra as a function of concentration for both coating methods spin coating (top left) and dip coating (top right). Absorbance as a function of SRh640 film thickness for spin coating (top left) and dip coating (top right).

Spin Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	592	20	0.038562	546	16	0.030267	510	15	0.006221
5.0×10^{-4} M	596	19	0.021752	549	18	0.013774	510	14	0.002002
1.0×10^{-4} M	592	15	0.009462	552	22	0.003860	506	11	0.000326
5.0×10^{-5} M	587	14	0.004399	548	22	0.001681	510	5	0.000067
1.0×10^{-5} M	590	13	0.000770	550	26	0.000303	508	6	0.000211

Dip Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	581	17	0.009668	538	27	0.008376	492	12	0.000665
5.0×10^{-4} M	575	18	0.005300	531	23	0.004294	490	10	0.000592
1.0×10^{-4} M	573	17	0.001026	531	23	0.000840	492	10	0.000133
5.0×10^{-5} M	570	16	0.000510	527	19	0.000468	487	20	0.000074
1.0×10^{-5} M	584	14	0.000298	557	19	0.000364	520	16	0.000304

Table 1. Deconvolution from the absorbance spectra from both spin-coated and dip-coated samples as shown for Peak position (λ_{max}) and FWHM (Γ). A Gaussian function was used to describe each peak.

Deconvolution of the absorbance spectra for both coating techniques were done, and all the spectra were fit to three peaks and the results are given in Table 1. For spin coating, at low concentration where the thickness is in the submonolayer to monolayer range, there have two main peaks, ~ 590 nm with higher energy shoulder around ~ 550 nm and a hidden feature at 510 nm. The peak at ~ 550 nm is assigned as arising from a monomer while the peaks at ~ 590 nm and ~ 510 nm are assigned to an oblique exciton dimer. As the concentration increases no additional peaks are needed to fit the spectrum but the exciton peaks become more prominent. Dip coating shows similar behavior but with the peaks slightly shifted to ~ 530 nm for the monomer and ~ 590 nm and ~ 490 nm for the oblique exciton dimer.

Figure 4. Fluorescence spectra of SRh640 as a function of various concentrations for both A.) Spin coating and B.) Dip coating.

Figure 4 shows the emission spectra for sulforhodamine 640 thin films. Similar to the absorption spectra, there are significant changes in the emission spectra as a function of concentration. At higher concentrations the fluorescence intensity is low. This intensity increase as the concentration of the dye decreases where at lowest concentration 1.0×10^{-5} M the emission intensity is the highest. Shown in Figure 5, the normalized emission spectra demonstrate the line shape and spectral shift changes as the concentration changes. Two broad peaks grow at higher concentration but with low intensity. This would imply again aggregation play into the effect. As the concentration decreases the broad peak gets narrower and shifts towards the higher energy side with a lower energy shoulder. This behavior is the same for the emission

spectra for dip coated samples, where at higher concentration the intensity is lower. At 1.0×10^{-5} M the maximum intensity is reached. Lower fluorescence intensities are found at lower concentrations.

Figure 5. Normalized Fluorescence spectra of various concentrations of SRh640 for both A.) Spin coating and B.) Dip coating.

Figure 6 shows the emission intensity as a function of concentration and thickness. For both coating methods, at high concentrations the intensity is lowest. As the concentration decreases the intensity reaches maximum intensity at a concentration of 1.0×10^{-4} M for dip-coated and at a concentration of 1.0×10^{-5} M for spin-coated samples. When it is plotted as a function in thickness ~ 1.0 nm is where we see the maximum intensity.

Figure 6. Fluorescence spectra as a function of concentration for both coating methods Spin coating (left side) and Dip coating (right side). Fluorescence intensity as a function of SRh640 film thickness for both coating methods Spin coating (left side) and Dip coating (right side).

Spin Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	553	16	17502	596	20	6096	593	67	6919
5.0×10^{-4} M	540	150	113	591	18	9255	610	47	9258
1.0×10^{-4} M	548	20	10689	599	21	11612	629	44	9227
5.0×10^{-5} M	561	12	8206	589	18	10399	585	53	10098
1.0×10^{-5} M	538	9	2762	587	15	6934	572	54	10656
5.0×10^{-6} M	544	14	1676	587	17	20094	589	47	20414
1.0×10^{-6} M	538	10	3698	588	16	4474	562	56	7737
Dip Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	532	13	1365	605	22	12553	647	41	9872
5.0×10^{-4} M	532	13	2440	597	22	11353	635	42	8149
1.0×10^{-4} M	589	17	10797	608	54	7018	613	23	4432
5.0×10^{-5} M	570	75	4605	585	17	9735	612	28	3944
1.0×10^{-5} M	576	79	8571	592	17	29074	619	28	13106
5.0×10^{-6} M	532	84	9047	588	17	14559	616	27	5709
1.0×10^{-6} M	533	14	6610	588	16	7808	584	49	8982

Table 2. Deconvolution from the fluorescence spectra from both spin-coated and dip-coated samples as shown for Peak position ($\lambda_{\max}$) and FWHM (Γ). A Gaussian function was used to describe each peak.

Deconvolution of fluorescence spectra for both coating methods are done and most of the spectra were fit to three peaks as shown in Table 2. At lowest concentrations the monomer is assigned at ~ 530 nm and the dimer is assigned at ~ 580 nm. At higher concentrations, higher aggregates are forming and for that peak is assigned to ~ 600 nm.

Figure 7 shows the normalized spectra absorbance, emission, and excitation spectra for a few concentrations of sulforhodamine 640. In all cases, the absorbance and excitation spectra are not superimposed. At low concentration the low energy side of the absorbance and excitation match but on the high energy side the excitation spectra are less intense.

Figure 7. Normalized absorption, emission, and excitation at the detection wavelength of 600nm from spin coating (left) and dip coating (right) at different concentrations.

Figure 8. Fluorescence Lifetime decays of various concentrations of SRh640 for A.) Spin coating and B.) Dip coating. IRF = instrument response function.

	Spin Coating		Dip Coating	
Concentration (M)	τ_1 (ns)	χ^2	τ_1 (ns)	χ^2
1.0×10^{-3} M	4.48	1.16	4.19	1.23
1.0×10^{-5} M	4.22	1.20	4.98	1.24
1.0×10^{-6} M	3.99	1.20	4.87	1.95

Table 3. Lifetime decays fits for Spin coating and Dip coating.

Figure 8 shows the lifetimes from both coating techniques. The decays are collected at 550 nm. The black curve is the instrumental response function and the other colors represent at various concentration of SRh640. The decay curves are best fit using three exponential functions. Two short decay times are from scattering and are not considered. Table 3 shows the measured the lifetime of the samples. These values are consistent with the lifetime of monomeric SRh640, consistent with previous measurements.¹⁹⁻²⁰

Conclusion

SRh640 cast from two different methods do not have a significant change in their spectral properties. At lower concentration monomer-like structures are dominating. These structures absorb less but have maximum fluorescence intensity. When there are more neighboring dye molecules higher order aggregates can form. This caused the fluorescence to be reduced even though it has a higher absorbance. Therefore, the thickness on our samples affects the spectral behavior.

NOTES

The authors declare no competing financial interest.

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CHAPTER 3

Investigating the Structures of Sulforhodamine B in Thin Films

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The following will be planned to be publish in *Langmuir* and is presented here in manuscript format.

Abstract

Xanthene dyes are used in many different applications especially involving sensors. There have been studies on their photophysical properties in solution but not very much information about them in thin films. Our previous studies on the cationic dyes rhodamine 6G and rhodamine 560 showed that dimerization occurs when the films were greater than about one monolayer thick. Sulforhodamine B is a zwitterionic dye and we were interested in seeing how this might affect the thin film photophysics. Sulforhodamine B was cast as thin films from two different coating methods, dip coating or spin coating, using various concentrations of the dye. The coating method did not affect the spectral behavior but the concentration, related with thickness, was significant factor in our spectroscopic results. This helped further our understanding of its photophysical properties.

Introduction

There has been a significant amount of research in sensing.¹⁻⁶ There has been an interest in sensing explosives which can be a challenging task to achieve.^{4,7-8} In our research group we have been interested in sensing vapor gas explosives using fluorescence based sensors. Our sensor system is based on a three layer system, where we have a glass substrate coated with a transparent polymer, and then a fluorophore. Our results showed a great deal in efficiency with both the sensitivity and selectivity of gas phase explosives. However, the interfacial effects are not fully understood yet.⁹ Thus, it is important to look at the photophysics of the fluorophore in thin films.

Xanthene dyes are widely known. They have favorable photophysics such as high quantum yields and photostability. These have been used in many applications as

a lasing medium in dye lasers and fluorescent markers in biological studies.^{6,10-17} The most studied on is Rhodamine 6G. Rhodamine 6G is a cationic fluorescent dye. However, these xanthene dyes especially for rhodamine are known to aggregate, which can affect the photophysical properties.¹⁸⁻²¹ Exciton Theory can explain these molecule interactions. There are two main molecular interactions that can occur. One interaction is the H-dimer. An H- dimer is a face to face arrangement, a sandwich structure. These are non-emissive. They are identified when there is a blue shift in the absorption spectrum with respect to the monomer. The second interaction is the J-dimer. J-dimers are a head to tail arrangement and are emissive. These are identified when there is a red shift in the absorption spectrum with respect to a monomer. The third type is the oblique dimer. Oblique dimers are oriented in an angle between two monomer transition moments. This arrangement causes to have a lower and higher energy peaks with respect to the monomer²²⁻²⁵

Depending on the preparation conditions, aggregation of the dye molecules can be controlled. From our previous work showed that aggregation of rhodamine 6G is dependent on the thickness of the thin film. Emission is at its highest when the thin films are approximately one monolayer and when the films are thicker the emission intensity is quenched. Structures were identified using the absorption spectra.^{17,22-28} From Chapter 1, the results for rhodamine 560 showed similar results to rhodamine 6G. Though we were seeing aggregation occur regardless of which coating method was used, aggregation only occurs at higher concentration. At lower concentration we tend to see more monomer like structure in our thin films.

In this work we will investigate sulforhodamine B (SRhB) in thin films.

Sulforhodamine B is a zwitterionic dye in the xanthene family. Sulforhodamine B is different from rhodamine 6G due to its different functional groups where as sulforhodamine B has tertiary amines, a sulfate group and a sulfonic acid attached to a benzene ring. Rhodamine 6G has only two secondary amines, methyl groups, and an ester attached to the benzene ring.

Sulforhodamine B

Scheme 1. Structure of Sulforhodamine B.

Experimental Methods

Pre-cleaned Borosilicate glass microscope slides were used from Scientific Products. These glass slides were cut into a certain length and width, 3.75cm by 1.70 cm. These slides were washed twice in a Branson 3510 sonicator. The first wash contains 95% ethanol and was sonicated for 15 minutes. It was then followed by a second wash of distilled water for another 15 minutes. Then the slides were dried by using nitrogen gas.

Sulforhodamine B was purchased from Lambda Physics. A series of diluted SRhB solutions were made in 95% ethanol from different concentrations ranging from 1.0×10^{-3} M to 1.0×10^{-7} M. Each clean glass slide was coated with sulforhodamine B both by a spin coater (Laurell Technologies Spincoater) and by a dip coater (MTI Corp. HWTL-01 desktop dip coater). For the spin coater each thin film was covered with 50 microliters and spun at 1200 RPM. For the dip coater, each thin film was removed from solution of a pull rate of ~ 60 mm/min.

Spectra of the absorbance were measured using a Perkin Elmer Lambda 1050 spectrometer. The spectra were collected using an integration time ranging from 0.20 s to 10.0 s, depending on the concentration, and from a wavelength of 300-700 nm. The fluorescence spectrum was measured using a Horiba Fluorolog-3 Fluorimeter. For each sample steady state fluorescence measurements were measured at an incidence angle of 60 degrees relative to the excitation beam with a slit width of 2 nm. The excitation light source was used at a wavelength of 490 nm. The emission wavelength ranged from 500 nm to 800 nm. The excitation measurements were also measured. The excitation range in two scans, one from 400 nm to 590 nm and the second one is from 610 nm to 700 nm using a detection wavelength of 600 nm. A Filmetrics microscope was used to determine the thicknesses. Lifetime measurements were made using a Horiba Fluorohub time-correlated single photon counting (TCSPC) system. A Horiba NanoLED N-460 pulsed diode laser was used as the light source with a wavelength of 464 nm, a repetition rate of 1.0 MHz, and 160 ps pulse duration time with a power output of about 7 pJ/pulse.

Results and Discussion

Sulforhodamine B thin films were produced by two different coating methods, dip coating and spin coating onto glass substrates. Different ranges of concentrations, from 1.0×10^{-3} M to 1.0×10^{-7} M were deposited onto the glass substrates. Figure 1 shows the absorbance spectra of SRhB on thin films as a function of concentration in solution to the two coating methods spin coat and dip coat. As expected for both casting methods and in solutions form, as the concentration increases the absorbance increases. At lowest concentration in solution the absorption maxima is at 556 nm with a high energy shoulder at 520 nm. The thin films show different results from solutions. In spin coating at lowest concentration the maximum peak is at 583 nm with a shoulder at 539 nm. As the concentration increases the line shape broadens and there is a gradual shift on the main peak and shoulder. However, at high concentration the shoulder is more prominent. The dip-coated films show similar results.

Figure 1. Different SRhB concentrations, in A.) Dip coating, and in B.) Spin coating, and in C.) Solutions.

Figure 2. Normalized Absorbance spectra of various concentrations of SRhB for A.) Spin coating B.) Dip coating and C.) Solutions.

Figure 2 shows the normalized absorbance spectra. The solution spectrum shows that the line shape is independent of the concentration. However, the thin films show different results. When the fluorescent dye is cast either by dip coating or spin coating the line shape of the spectra is broader than in solutions. For spin-coated samples the maxima absorbance peak shifts to lower energy as the film thickness increases. And the high energy shoulder increases as the thickness increases. However, there is no gradual shift of the peak maximum for the dip-coated samples. There is a change in the line shape where it broadens, and the higher energy shoulder increases as the concentration increases. This implies the presence of oblique dimers in the thicker films, similar to what was found in Rh6G.¹⁷

Figure 3. Absorption spectra maximum as a function of concentration for A.) Spin coating and B.) Dip coating.

Figure 3 shows the absorbance maximum as a function of thickness for both coating methods. As expected for both coating methods the absorbance increases as the concentrations increases. Both dip-coated and spin-coated samples show a non-linear increase of absorbance with respect to concentration. When plotted as a function

of film thickness, Figure 4, the absorbance is nonlinear for both coating methods. This implies that dimerization occurs at the thicker region.

Figure 4. Absorbance as a function of SRhB film thickness for A.) Spin coating and B.) Dip coating. Concentration as a function of SRhB film thickness for C.) Spin coating and D.) Dip coating.

Spin Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	536	22	0.032428	491	13	0.002938	579	16	0.043456
5.0×10^{-4} M	535	22	0.017406	487	12	0.001326	575	15	0.027168
1.0×10^{-4} M	533	19	0.003212	489	10	0.000347	572	15	0.006377
5.0×10^{-5} M	532	18	0.001593	491	8	0.000283	570	15	0.003656
1.0×10^{-5} M	529	21	0.000420	469	9	0.000073	569	13	0.000829
Dip Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	537	24	0.020533	488	14	0.001635	572	14	0.028456
5.0×10^{-4} M	537	23	0.011327	488	13	0.000948	571	14	0.017736
1.0×10^{-4} M	537	22	0.002413	489	9	0.000102	571	13	0.005128
5.0×10^{-5} M	538	22	0.001164	493	9	0.000141	569	13	0.002739
1.0×10^{-5} M	543	24	0.000317	496	9	0.000051	569	12	0.000730

Table 1. Deconvolution parameter for absorbance spectra of spin coating and dip coating. Peak position ($\lambda_{\max}$) and FWHM (Γ). A Gaussian function was used to describe each peak.

Deconvolution of the absorbance spectra for both coating techniques were done, and all the spectra were fit to three peaks and the results are given in Table 1 for both coating methods. Both coating methods give similar results. The peak at ~ 535 nm is assigned to absorption from a monomer. The pair of peaks at ~ 490 nm and ~ 575 nm are assigned to an oblique exciton dimer.

Figure 5. Fluorescence spectra of SRhB as a function of various concentrations from A.) Spin coating and B.) Dip coating.

Figure 5 shows the emission spectra for sulforhodamine B thin films. Similar to the absorption spectra, there are significant changes in the emission spectra. Both coating methods have similar spectral behaviors. At higher concentrations the fluorescence intensity is lower. This intensity increases as the concentration of the dye decreases where at a concentration 1.0×10^{-4} M the emission intensity is the highest. The line shape is such that the lower energy peak dominates over the higher energy shoulder. As the concentration further decreases the change in the line shape occurs where the once dominate lower energy shoulder starts to dissipate and the higher energy starts to dominate. Shown in Figure 6, the normalized emission spectra demonstrate the line shape and spectral shift changes as the concentration changes. Two broader peaks grow at lowest concentration with low intensity and a lower energy shoulder. As the concentration increases, the two broad peaks shift gradually, and the higher energy feature starts to dissipate and the lower energy starts to dominate, reaching at a maximum intensity at a concentration of 1.0×10^{-4} M. At higher concentrations the lower energy peak is nearly gone, the higher energy shoulder slowly decreases, and a newer lower shoulder start to appear. This implies that aggregation is occurring. Similar behavior is observed for the emission spectrum for dip coating.

Figure 6. Normalized Fluorescence spectra of various concentrations of SRhB for A.) Spin coating and B.) Dip coating

Spin Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	540	15	7334	601	18	14715	605	56	8232
5.0×10^{-4} M	528	17	4106	597	15	17081	626	36	7159
1.0×10^{-4} M	525	18	1811	592	13	24275	618	32	7272
5.0×10^{-5} M	536	17	7549	590	13	37218	609	34	11613
1.0×10^{-5} M	523	18	2444	587	12	43118	612	28	10910
Dip Coating									
Concentration (M)	λ 1 (nm)	Γ 1 (nm)	Intensity	λ 2 (nm)	Γ 2 (nm)	Intensity	λ 3 (nm)	Γ 3 (nm)	Intensity
1.0×10^{-3} M	518	17	2532	592	13	34964	619	34	12886
5.0×10^{-4} M	519	17	2014	591	13	31470	617	34	11589
1.0×10^{-4} M	520	17	2718	586	13	150232	611	28	42599
5.0×10^{-5} M	516	25	2931	584	13	47186	599	33	16149
1.0×10^{-5} M	525	24	3037	582	13	24771	591	36	8777

Table 2. Deconvolution parameter for fluorescence spectra of spin coating and dip coating. Peak position ($\lambda_{\max}$) and FWHM (Γ). A Gaussian function was used to describe each peak.

Deconvolution of fluorescence spectra for both coating methods are done and most of the spectra were fit to three peaks as shown in Table 2. At lowest concentrations the monomer is assigned at ~ 520 nm and the excimer are assigned at ~ 592 nm. At higher concentrations, higher aggregates are forming and for that peak is assigned to ~ 605 nm. For dip coating there is a shift where at lowest concentrations

the monomer is assigned at ~ 520 nm and the excimer are assigned at ~ 592 nm. At higher concentrations, higher aggregates are forming and for that peak is assigned to ~ 620 nm.

Figure 7. Fluorescence spectra as a function of concentration for A.) Spin coating and B.) Dip coating.

Figure 8. Fluorescence Intensity as a function of SRhB film thickness for A.) Spin coating and B.) Dip coating.

Figure 9. Normalized absorption, emission, and excitation at the detection wavelength of 600 nm from spin coating (left) and dip coating (right) at different concentrations.

Shown in Figure 7, for both coating methods there are similar spectral behavior as a function of concentration. At high concentrations the intensity is lower. As the concentration decreases the intensity reaches maxima intensity at a concentration of 1.0×10^{-4} M for both dip-coated and spin-coated samples. When it is plotted as a

function in thickness, shown in figure 8, the thickness where the thin films are around ~ 1.4 - 1.5 nm is where we see the maximum intensity. The transition from monomers to aggregates, as defined by the intensities, occurs at about one monolayer thickness.

Figure 9 shows the normalized spectra absorbance, emission, and excitation spectra for a few concentrations of sulforhodamine B. At high concentrations the absorbance and excitation spectra are not superimposed. This implies that absorption into aggregates does not contribute to emission. At the concentration 1.0×10^{-4} M the absorbance and excitation spectra are superimposed. At lower concentration the absorbance and excitation spectra mostly do align with similar peaks matching only with the lower energy portion of the peak.

Figure 10. Fluorescence Lifetime decays of various concentrations of SRhB for A.) Spin coating and B.) Dip coating. IRF = instrument response function.

	Spin Coating		Dip Coating	
Concentration (M)	τ_1 (ns)	χ^2	τ_1 (ns)	χ^2
1.0×10^{-5} M	2.58	1.15	1.89	1.08
1.0×10^{-6} M	3.61	1.87	3.38	1.03

Table 3. Lifetime decays fits for spin coating and dip coating.

Figure 10 shows the lifetimes from both coating techniques. The decays are collected at 570 nm. The black curve is the instrumental response function and the other colors represent at various concentration of SRhB. Tables 3 show the measured lifetime of the samples. At high concentration the decay curves are best fitted using three exponential functions while at lower concentration it was used only two exponential functions. Decay times less than 1 ns are from scattering and are not considered. The longer decay is similar, about 3 ns, throughout the concentrations. These values are consistent with the lifetime of SRhB, consistent with previous measurements.²⁹⁻³⁰

Conclusion

Sulforhodamine B cast from two different methods does not show a significant difference in their spectral properties. At lower concentration we see monomer-like structures. These structures absorb less but have the maximum fluorescence intensity. When there more neighboring dye molecules higher order aggregates can form. This caused the fluorescence to be lessened even though it has a higher absorbance. Results show how much thickness can affect their spectral behavior which we have seen from previous work Rhodamine 6G, from chapter 1 Rhodamine 560 and chapter 2

Sulforhodamine 640. With different functional group, they still have similar conjugated structures where they would all share similar behaviors.

NOTES

The authors declare no competing financial interest.

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APPENDICES

Statement of the Problem

We have developed a fluorescence-based sensor using Xanthene derivatives. Due to their high quantum yield and strong signal in emissions Xanthene dyes are widely used. These dyes interact with analytes such as trinitrotoluene (TNT) and Trinitrobenzene (TNB) and have shown high sensitivity in detection from fluorescent emission.¹ We also have done intense investigation on Rhodamine 6G and its photophysics properties. This led to our further understanding of the aggregation of the dye molecules and how the effect of thin film thickness plays a key role in aggregation.²⁻⁵ Now we had extended our results to other xanthene dyes including Rhodamine 560, Sulforhodamine B and Sulforhodamine 640. We had compared the spectroscopic results from a widely used fluorophore such as Rhodamine 6G with other Xanthene dyes and investigate these fluorophores to optimize their photophysics properties and enhance our fluorescence-based sensor.

Justification for and Significance of the Study

Fluorescence-based sensors have been reported and are continuing to improve the methods in enhancing the fluorescent signal. A three layer structure which consists of a glass substrate, a polymer, and a fluorophore has been reported and shown as a sensor in detection of analytes. Results show that with a little amount of fluorophore present in their sensor, the signal.

response was strong. The interface between the polymer and the fluorophore plays a role in the changes of emission. However, there isn't information about what happens with fluorophores to the analytes. Five xanthene dyes, Rhodamine 6G, Rhodamine 560, Sulforhodamine B, and Fluorescein 548, on different substrates either PMMA or PVDF were measured in absorption and emission. These Xanthene dyes have a common chemical structure with little variance. Rhodamine 6G and Rh560 are cationic dyes, Sulforhodamine B and Rhodamine 640 are zwitterionic dyes, and Fluorescein 640 is an anionic dye. Each in different absorption maxima and emission maxima showed a different response to the analytes in the emission spectra either an enhancement or quenched.¹

Rhodamine 6G is a common cationic fluorophore used as a fluorescent tracer due to its high fluorescence quantum yield.¹ In high concentrations, Rhodamine 6G has been known to form aggregates and higher order aggregation which can significantly affect the photophysical properties.² Results show aggregation of the dye molecules can be controlled depending on certain preparation settings.⁶⁻⁸ For Rhodamine 6G the aggregation is dependent on the thickness of the thin film. As the thickness of thin films decreases we see that the emission is higher and have shorter wavelength maxima whereas when the thin films increases we see a quench in emission and longer wavelength maxima. The structure of the dye and thickness of the thin films can be found in the absorption spectra.² From controlling the aggregation of the fluorescent dye we gained an understanding of its photophysical properties to improve our sensors efficiently.

In this work we had expanded our investigation on the Xanthene dyes coated on glass substrates. The xanthene dyes we had studied are Rhodamine 6G, Rhodamine 560, Sulforhodamine B, and Sulforhodamine 640. Each xanthene dye is structurally different: the Rhodamine 6G and Rh560 are cationic dyes, Sulforhodamine B is zwitteronic dye and Sulforhodamine 640 is an anionic dye. We had investigated the absorption, emission, and excitation spectra of Rh6G, Rhodamine 560, Sulforhodamine B, and Sulforhodamine 640. ¹We will also investigate the preparation of the thin films from coating techniques spin cast and dip coating.⁹⁻¹² We will use our spectroscopic spectra as a function of thickness to determine the structure of these dyes which will give us understanding to improve our sensing methods.

Methodology or Procedures

The experiments were conducted by the start with a substrate preparation. Pre-cleaned Borosilicate glass microscope slides was used from Scientific Products. These glass slides were cut into a certain length and width. These slides were washed twice in a Branson 3510 sonicator. First wash contained only 95% ethanol and was sonicated for fifteen minutes. It was then followed by a second wash of distilled water for another fifteen minutes. Then the slides were dried by using a nitrogen gas tank. Rhodamine solutions was made from Rhodamine 6G purchase from Acros Organics with 99% purity, Rhodamine 560 purchase from Exciton, Sulforhodamine B purchase from Lambda Physics, and Sulforhodamine 640 purchase from Exciton. The solvent used for these different concentrations of Rhodamine 6G will be 95% ethanol. Each clean glass slide was coated with Rhodamine 6G both by a spin coater and by a dip coater. For the spin coater each thin film was submerged with 50 microliters and spun coat at

1200 RPM. For the dip coater, each thin film was submerged from a constant pulling rate $\sim 60\text{mm/min}$.

Each sample was conducted to absorption, emission, excitation, and thickness measurements. The instrumentation and tools that was used which were

- Glass cutter
- Borosilicate glass microscope slides
- Rhodamine 6G
- Rhodamine 560
- Sulforhodamine 640
- Sulforhodamine B
- 95% Ethanol
- Glassware
- Analytical balance
- Branson 3510 Sonicator
- Laurell Technologies Spincoater
- MTI Corp. HWTL-01 desktop dip coater
- PerkinElmer Lambda 1050 spectrometer

- Horiba Jobin Yvon Fluorolog Fluorimeter
- Filmetrics F-40 Thin Film Analyzer

Resources Required

All instrumentation and tools were used and found in Beaupre Room 485 and Room 495. For this study the chemicals were purchased through Acros Organics Exciton, Lambda Physics, and Exciton. Resources for peer reviewed journals include the American Chemical Society database, Web of Science database, SciFinder database, and was found through the University of Rhode Island library system for this study.

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