



Surface plasmon coupled phosphorescence (SPCP)

Michael J.R. Previte^a, Kadir Aslan^a, Yongxia Zhang^a, Chris D. Geddes^{a,b,*}

^a *Institute of Fluorescence, Laboratory for Advanced Medical Plasmonics, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 West Lombard St., Baltimore, MD 21201, United States*

^b *Center for Fluorescence Spectroscopy (CFS), Medical Biotechnology Center, University of Maryland School of Medicine, 725 West Lombard St., Baltimore, MD 21201, United States*

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Abstract

We report the first observation of surface plasmon coupled phosphorescence (SPCP) for PtOEP (2,3,7,8,12,17,18-octaethyl-21H,23H-porphyrin platinum II) immobilized in polyvinyl chloride films on continuous gold films of 47 nm thickness. We confirm the observation of SPCP by comparing the spectra and lifetimes for plasmon coupled phosphorescence with the free space isotropic phosphorescence emission. Due to the directional emission inherent to plasmon coupled phosphorescence, we believe that SPCP can facilitate the study of phosphorescence in biological applications, such as protein dynamics and alkaline phosphatase studies, whose signal intensities are inherently weak and nearly always isotropic.

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1. Introduction

The study of surface plasmon coupling of radiative molecules to metallic surfaces dates back to the beginning of the 20th century with theoretical work by Zenneck and Sommerfeld among others [1,2]. Further work by Fano described that the negative dielectric constant of the metal is responsible for the binding of the surface EM waves to the metal–air interface at optical frequencies [3]. The existence of surface plasmon excitations at metal surfaces was demonstrated theoretically by Ritchie in 1957 and later experimentally observed by Damon and Eschbach [4,5]. Burnstein et al. provides an extensive review that summarizes the early work in surface polaritons and details the theoretical origin of the propagation of electromagnetic modes at interfaces [6].

Since the propagating electromagnetic fields for surface plasmons at interfaces propagate with a preferred direction and polarization, it was soon realized that the emission of

fluorescent species close to metal surfaces would lead to directional and polarized emission [7–10]. Consequently, the inherent isotropic fluorescence emission from a population of randomly oriented fluorophores above surfaces would directionally emit by coupling to the surface plasmons of the metal surfaces [8,10,11]. As a result, it became possible to achieve better detectability of fluorophores at the metal interfaces and facilitate the detection of surface bound biomolecules [11]. Since its introduction, surface plasmon fluorescence spectroscopy, or surface plasmon coupled emission (SPCE), as it is alternatively known, has become an active area of research for applications in the measurement of biomolecular interactions on/at surfaces [12–15].

Changes to radiative decay rates of emitting dye molecules in the vicinity of metal substrates had been an active area of research that preceded the study of plasmon resonance [16]. Decreased fluorescent lifetimes and enhanced intensities that resulted from fluorescence emission coupled with metal surfaces was first realized with the early observations that fluorescent lifetimes are dramatically affected by the excited state molecules and the metal surface [16–20]. Due to the metal–chromophore interactions, the fluorophores exhibit increased brightness, decreased lifetimes,

* Corresponding author. Address: Institute of Fluorescence, Laboratory for Advanced Medical Plasmonics, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 West Lombard St., Baltimore, MD 21201, United States. Fax: +1 4107064600.

E-mail address: geddes@umbi.umd.edu (C.D. Geddes).

which indicates a substantial increase in system (metal–fluorophore) radiative decay rates and increased photostability [21–26]. More recently, enhanced phosphorescence emission and increased decay rates were reported for Pt/porphyrin complexes in polymer films near nanotextured silver surfaces. In addition, metal-enhanced phosphorescence for Rose Bengal, a halogenated fluorescein derivative that phosphoresces at low temperature, on silver island films (a non-continuous surface) was also reported in our most recent report of metal-enhanced phosphorescence (MEP) [27]. In each of these reports, the phosphorescence lifetimes of the ensemble average of molecules in close proximity to the metal surfaces decreases significantly.

In this manuscript, we subsequently report the first observation of surface plasmon coupled phosphorescence (SPCP) of PtOEP (2,3,7,8,12,17,18-octaethyl-21H,23H-porphyrin platinum II) immobilized in polyvinylcarbonate (PVC) films on continuous gold films of 47 nm thickness. We confirm the observation of SPCP by comparing the spectra and lifetimes for plasmon coupled phosphorescence with the free space isotropic phosphorescence emission. Like surface plasmon coupled fluorescence in polymer films on Au substrates, we show that SPCP is highly angularly dependent and preferentially p-polarized for PtPOEP complexes in thin and thick PtPOEP/PVC samples [28,29]. However, unlike previously reported results for SPCE in polymer films, we observed that the SPCP angular distribution broadens with increasing PVC film thickness [28,29]. While SPCE reports either p- or s-polarized emission for different thicknesses at discrete detection angles, we observe *both* p- and s-polarized phosphorescence (SPCP) at a single range of angles and found that the p:s polarization ratios decreased with increasing PVC film thickness. Polarization results for SPCP of PtOEP are partially explained with 4-phase reflectivity calculations for p- and s-polarized emission from PtOEP in a thin polymer film on a thick Au film substrate [30]. Our findings strongly suggest that like fluorescence, phosphorescence signals can also be collected in a directional manner greatly enhancing the sensitivity of detection.

2. Theory

The theory of surface plasmon coupled emission has its origins in the theory of surface plasmon resonance (SPR), which is covered in detail elsewhere [6–8,11]. Briefly, the coupling of light emission from a chromophore to metal surfaces depends on the matching of the wavevector of the incident light (k) with the wavevector of the surface plasmons (k_{sp}). The wavevector of the incident light is given by

$$k_p = \frac{2\pi}{\lambda} = \frac{n_p \omega}{c} = n_p k_o \quad (1)$$

where n_p is the refractive index of the prism, λ is the wavelength, ω is the frequency in radians/s, and k_o is the wavevector in free space. The wavevector (k_{sp}) for the surface plasmon is given by

$$k_{sp} = k_o \left(\frac{\epsilon_m \epsilon_s}{\epsilon_m + \epsilon_s} \right)^{1/2} \quad (2)$$

where ϵ_m and ϵ_s are the real parts of the dielectric constants of the metal ($\epsilon_m = \epsilon_r + i\epsilon_i$) and the sample ($\epsilon_s = \epsilon_r + i\epsilon_i$) above the metal film (Fig. 1, left) respectively. The surface plasmon phenomenon persists when the X-axis component of the emissive light from the chromophore (incident light) equals the wavevector of the surface plasmon. If the wavevector of the incident light on the prism is given by $k_p = k_o n_p$, the x component or projection of the wavevector along the interface is $k_x = k_o n_p \sin \theta_i$, where θ_i is the incidence angle in the prism measured from the normal to the metal surface. It follows that the conditions for SPR excitation are met when

$$k_{sp} = k_x = k_o n_p \sin \theta_{sp} \quad (3)$$

where θ_{sp} is the surface plasmon coupling angle. From this equation, it follows that the reflectivity and transmissivity of incident light at the metal/dielectric interface are determined by θ_{sp} .

The reflectivity at the surface below the θ_{sp} is very high due to the presence of an evaporated metal layer that acts

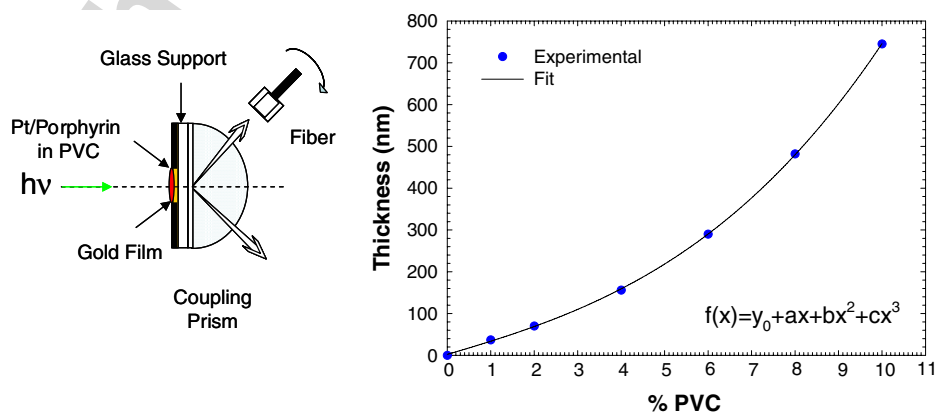


Fig. 1. SPCP experimental set-up (left). Experimental data points adopted from [29], fit with equation $f(x)$ (right). PVC film thicknesses estimated from percentage of PVC and fitting function $f(x)$.

as a mirror and reflects most of the transmitted light [11]. At just above θ_{sp} , the metal surface acts as a resonator for incident light from the emitting dye molecule and gives rise to the resonant excitation of a surface plasmon, which reradiates according to the dispersion curve for surface plasmons. The surface plasmon dispersion curves are predicted with Fresnel calculations, which take into account the interactions of light impinging upon media with multiple phases [31].

3. Methods and materials

The reverse Kretschmann configuration is implemented for all free space and SPCP measurements such that the sample is excited from the air or sample side and the angle of incidence was normal to the sample surface (Fig. 1, left) [32]. The SPCP (prism side or back plane) for the PtOEP/PVC mixtures was collected over a range of detection angles at 2° increments to determine the angular distribution of SPCP, and free space phosphorescence emission was measured at 175° and 190° (excitation side or front plane). Polarization measurements for the samples of Pt/porphyrin in PVC films were performed by placing a polarizer in the path of the SPCP emission and rotating it 90° to collect both s- and p-polarization. Au films were deposited on silanized glass slides by vapor deposition with an EMF Corp. (Ithaca, NY) instrument. Samples of platinum porphyrin (PtOEP) complexes were dissolved in toluene and mixed with 10% PVC stock solutions. Final PVC solutions were mixed at 1%, 2.5%, and 5% PVC in toluene with a final concentration of 0.48 mM PtOEP. Forty microliters of the PVC mixtures were then spin coated on the Au/glass substrates. Previously published data by Gryczynski et al. that describes the relationship between polymer percent composition and film thickness was fit to a cubic polynomial function, which was subsequently used to estimate PVC film thicknesses from PVC percentages used for these experiments (Fig. 1, right) [29]. Lifetime measurements for free space emission and SPCP from PtOEP samples were measured using a sub-microsecond Xenon flashlamp operating at a frequency of 300 Hz (IBH, Model 5000XeF) as the excitation source, a cooled high speed PMT detector head for photon counting (PMC-100, Becker-Hickl), and a gated photon counter/multiscalar card (MSA-300, Becker-Hickl). Lifetime analysis fitting was performed using FluoFit software provided by Picoquant GmbH.

Lifetime data was fit using a multiexponential model

$$I(t) = \int_{-\infty}^t \text{IRF}(t') \sum_{i=1}^n \alpha_i e^{-\frac{t-t'}{\tau_i}} dt' \quad (4)$$

where $\text{IRF}(t')$ is the measured instrument response, τ_i is the lifetime of component i and α_i is its amplitude where $\sum_i \alpha_i = 1.0$. The contribution of each component to the steady-state intensity is given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \quad (5)$$

the mean decay time is given by

$$\bar{\tau} = \sum_i f_i \tau_i \quad (6)$$

and the amplitude weighted lifetime is given by

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \quad (7)$$

4. Results and discussion

Figs. 2a–c show the normalized free space and surface plasmon coupled phosphorescence (SPCP) for 1%, 2.5% and 5% PVC film thicknesses on Au films. These results show no noticeable spectral shift in the SPCP versus the free space emission for different polymer film thicknesses. Typically, spectral shifts in chromophore emission are ascribed to the changes in the electronic distribution of the energies of electronic transitions [33]. Since we do not observe any noticeable spectral shifts for the coupled phosphorescence with respect to free space emission, we concluded that the reported observations of SPCP are not due to changes in photophysical properties. This is consistent with reports of SPCE [28,29].

In addition to comparing the spectral features of SPCP and free space phosphorescence, we also compared the lifetimes of SPCP and free space emission on glass and gold for Pt/porphyrin complexes in 1% and 5% PVC films (Table 1) to further qualify our observation of plasmon coupled phosphorescence. The results in Table 1 demonstrate that the free space phosphorescence lifetimes for Pt/porphyrin complexes in 5% PVC films on glass (72.3 μs) and gold (80.3 μs) are longer than those for the complexes in 1% PVC films (glass, 47.7 μs ; Au, 51.5 μs). Since oxygen can diffuse more quickly in thinner PVC films, it is likely that the observed decrease in lifetimes in the thinner films is due to increased oxygen quenching of phosphorescence. We believe that the slight decrease of the free space phosphorescence lifetimes on glass versus Au surfaces could also be explained by oxygen quenching. Due to the differences in the wettability of the substrates to the hydrophobic PVC films, it is possible that the less hydrophobic glass surfaces will be more wettable with respect to the slightly polar PVC films. As a result, the PVC films on the glass surfaces may be slightly thinner than those on gold, which may account for the slight decrease in the free space phosphorescence lifetimes on the glass substrates.

For SPCP emission, the lifetime components for Pt/porphyrin complexes in 5% PVC films were 59.3 μs and 24.7 μs , which yield a mean lifetime of 51.6 μs and an amplitude weighted lifetime of 45.2 μs . Conversely, the lifetime of the complexes in 1% PVC films was fit to a single exponential model and found to be 52.9 μs . For the SPCP lifetime in 5% PVC, we are not quite certain as to the origin of the faster lifetime component (21.7 μs). We hypothesize that it may be a result of a more complex plasmon coupled

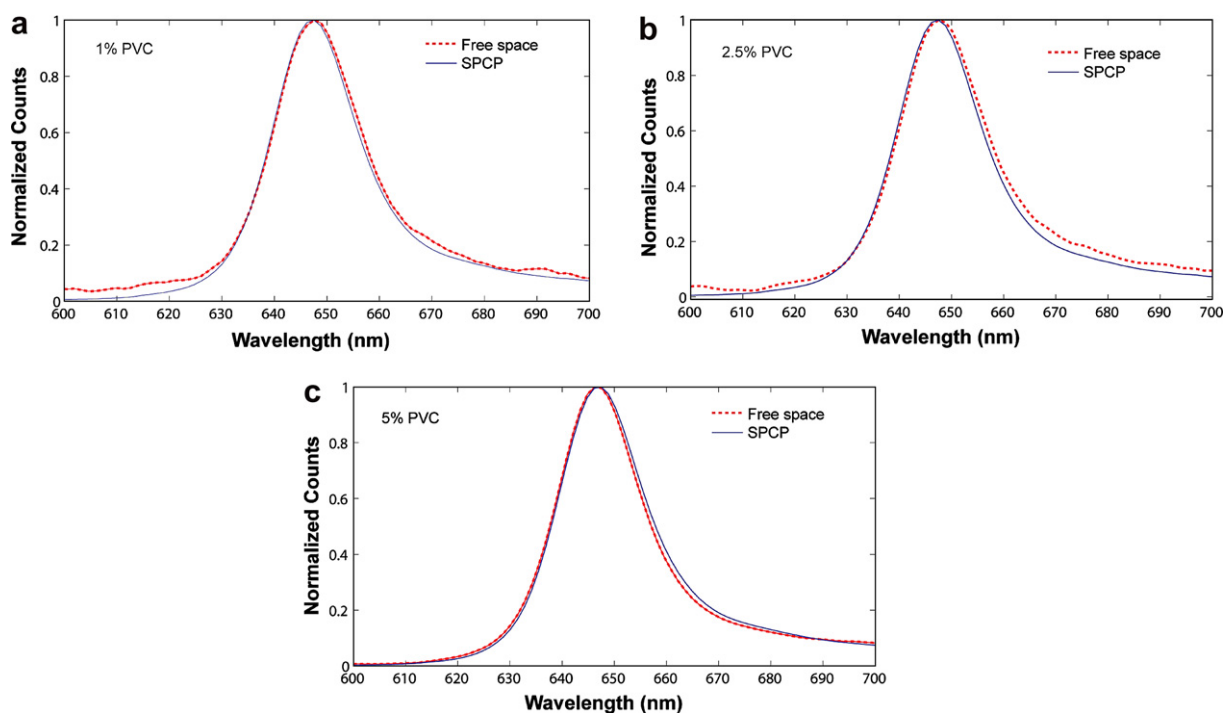


Fig. 2. Normalized spectra for free space and SPCP emission for 0.48 mM PtOEP in: (a) 1%; (b) 2.5%; (c) 5% PVC films deposited on 47 nm thick gold films.

Table 1
Lifetime data for SPCP and free space emission of the PtOEP complex in PVC films

Sample:	A_1	$\tau_1(\mu\text{s})$	A_2	$\tau_2(\mu\text{s})$	$\bar{\tau}$	$\langle\tau\rangle$	χ^2
Glass Free 5% PVC (1 exp)	1.00	73.3	–	–	–	–	.996
Glass Free 1% PVC (1 exp)	1.00	47.0	–	–	–	–	.959
Au – Free 5% PVC (1 exp)	1.00	80.2	–	–	–	–	.975
Au – Free 1% PVC (1 exp)	1.00	51.5	–	–	–	–	1.002
Au – SPCP 5% PVC (2 exp)	0.59	59.3	0.41	24.7	51.6	45.2	1.003
Au – SPCP 1% PVC (1 exp)	1.00	52.9	–	–	–	–	.934

phenomenon (optical waveguide modes), which we have yet to further explore [34]. In 1% PVC the SPCP lifetime for the PtOEP was slightly longer than that measured in the free space. In order to insure that these slightly longer observed SPCP lifetimes were not a result of experimental error, multiple measurements were performed and confirmed that the slightly longer SPCP lifetime could be reproduced (data not shown). In addition, photostability measurements for SPCP and free space phosphorescence further support that these slightly different lifetimes are meaningful. As shown in Fig. 3, the SPCP is slightly more photostable than free space phosphorescence, which is corroborative evidence for the observed longer SPCP lifetimes. Although we do not have a working explanation of this result, it is consistent with previously published data that reports the lifetimes for surface plasmon coupled fluorescence are slightly longer than those measured for free space emission [28,29].

Fig. 4, left shows the SPCP detected at different measurement angles. These results show that the angular distribution of the coupled phosphorescence emission

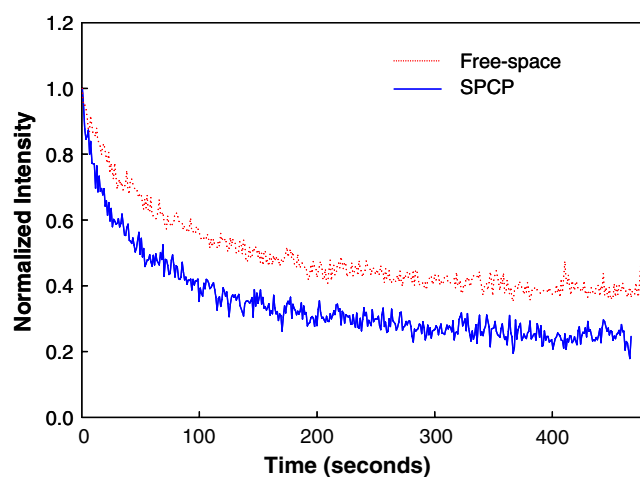


Fig. 3. SPCP and free space photostability curves for 0.48 mM PtOEP in 1% PVC on 45 nm thick Au films.

through the back of the film increases with increasing film thicknesses (Fig. 5a–c). On the one hand, SPCP measurements for a thin polymer film of about 90 nm have an

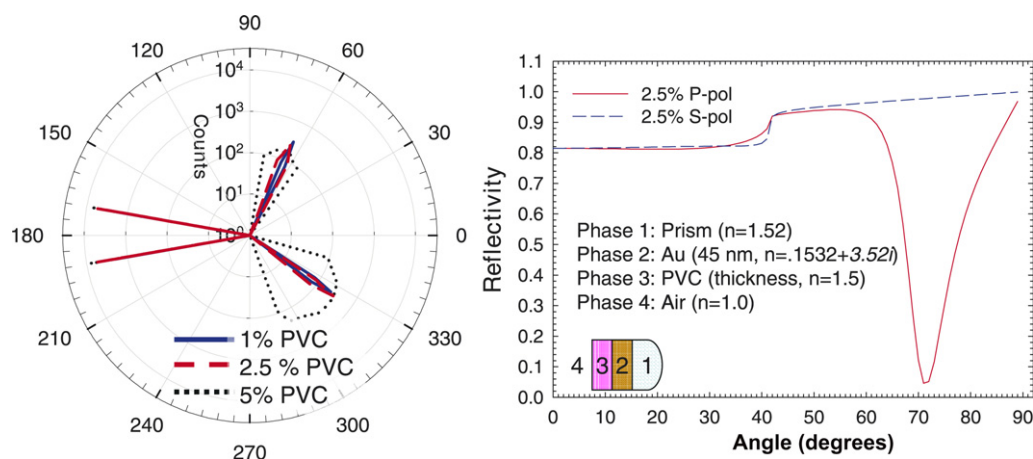


Fig. 4. Angular distribution of emission from PtOEP/PVC films on Au substrates (left). Calculated reflectivity curves for PVC samples using 4-phase Fresnel calculation [30] (right).

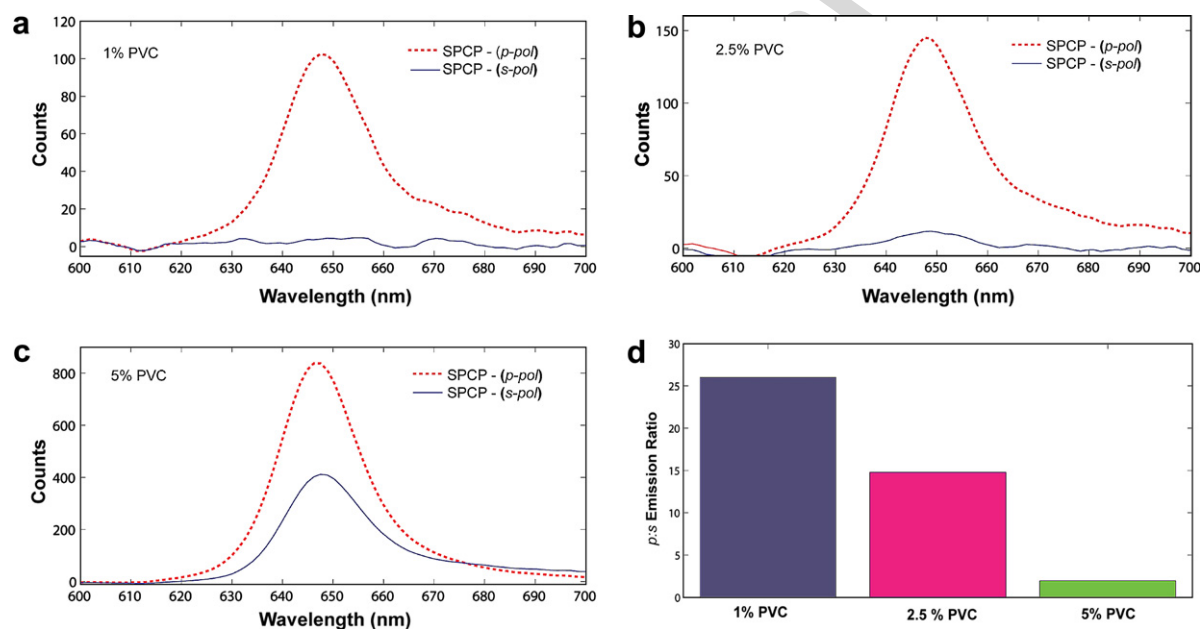


Fig. 5. SPCP p- and s-polarized emission for 0.48 mM PtOEP in: (a) 1% ; (b) 2.5% ; and (c) 5% PVC films deposited on 47 nm thick gold films; (d) p:s polarization emission ratios for PtOEP/PVC films.

angular distribution that is relatively consistent with the angle dependent reflectivity calculations for a 4-phase configuration: air, a thin polymer film, gold, and glass (Fig. 4, right). On the other hand, the angular emission distributions for the thicker film and the 37 nm film are not consistent with reflectivity calculations. In addition, we found that the p:s polarization ratios increased with decreasing film thicknesses (Fig. 5d), which is also inconsistent with reflectivity calculations (data not shown). While previously published results report *only* s- or p-polarized emission at discrete SPCE angles, we do not observe angular emission with definitive s- or p-polarization [28,29]. On the contrary, we measure a ratio of the polarization of SPCP over a range of angles for the different thicknesses, such that

the p:s polarization ratio decreases with increasing film thickness.

The theory of SPCE precludes the plasmon coupling of s-polarized emission for thin films, which is consistent with the results for the Pt/porphyrin phosphorescence in 1% PVC films. For thicker multilayer dielectric slabs, both s- and p-polarized plasmon phosphorescence emission is supported by waveguide modes that exist for thicker dielectric films. Thus, the decreased s:p polarization ratio that we observe for the plasmon coupled phosphorescence emission in 5% PVC films is consistent with the reported coupling of s-polarized fluorescence emission through waveguide modes for thicker films [29,35]. Incongruently, it is reported that s-polarized coupled fluorescence emission is recorded

at discrete emission angles, but we measure similar p:s polarization ratios for each sample over the range of SPCP angles that were measured at 2° increments. Recently, we reported a similar result that yielded p:s polarization ratios over a range of angles with surface plasmon coupled chemiluminescence (SPCC). It may be possible that the increased decay times for phosphorescence and chemiluminescence as compared to nanosecond fluorescence decay times may play a role in the coupling of the radiating species to the surface plasmons, such that additional p- and s-polarized plasmons become allowed. Work is currently underway in this regard.

5. Conclusions

In conclusion, we have successfully demonstrated the first observation of surface plasmon coupled phosphorescence. We have observed that the spectral characteristics of SPCP are consistent with that observed for free space phosphorescence. In addition, we have observed that the lifetimes of SPCP are slightly longer than free space phosphorescence.

We have also observed a polarized angular distribution of SPCP in thin films that differs slightly from the previous reports of SPCE in polymer films, but is consistent with surface plasmon coupled chemiluminescence (SPCC). Although the thin film results for the polarized SPCP are consistent with reflectivity calculations, we observe a p:s polarization ratio for thicker films which contradicts reflectivity calculations that predict the existence of discrete polarized waveguide modes with different film polymer thickness [28,29]. Furthermore, we report an overall angular distribution of SPCP for thicker films and a narrower distribution for thinner films than those predicted for SPCE from reflectivity calculations [28,29]. However, our new results are consistent with a recent report of SPCC, which may suggest that slower decaying chromophores, such as phosphorescent and chemiluminescent species may have different allowed plasmon coupled modes. Due to the directional emission inherent to plasmon coupled phosphorescence, we believe that SPCP can facilitate the study of phosphorescence in biological applications, such as protein dynamics and alkaline phosphatase studies. Further work is currently underway in our laboratories to capitalize on the improved detectability of surface plasmon coupled phosphorescence signals, as demonstrated here.

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