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C₆₀ as an Active Smart Spacer Material on Silver Thin Film Substrates for Enhanced Surface Plasmon Coupled Emission

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Abstract

In this study, we present the use of C₆₀ as an active spacer material on a silver (Ag) based surface plasmon coupled emission (SPCE) platform. In addition to its primary role of protecting the Ag thin film from oxidation, the incorporation of C₆₀ facilitated the achievement of 30-fold enhancement in the emission intensity of rhodamine b (RhB) fluorophore. The high signal yield was attributed to the unique π - π interactions between C₆₀ thin films and RhB, which enabled efficient transfer of energy of RhB emission to Ag plasmon modes. Furthermore, minor variations in the C₆₀ film thickness yielded large changes in the enhancement and angularity properties of the SPCE signal, which can be exploited for sensing applications. Finally, the low-cost fabrication process of the Ag-C₆₀ thin film stacks render C₆₀ based SPCE substrates ideal, for the economic and simplistic detection of analytes.

Introduction

There is a critical need to significantly improve the ability of existing diagnostic devices, to sensitively and selectively identify disease markers. Addressing this need would provide high-quality sensing at low-cost, in resource-limited settings (RLS). As a result, the development of low-cost point-of-care technologies, to diagnose pathogens and infection is a major subject of current research in healthcare and medicine. Although fluorescence based sensors are often used for the detection of chemical and biological agents¹, they are not sensitive enough to detect analytes at very low concentrations. This is due to the fact that the emitted light is isotropic, and only a fraction of it reaches the detector resulting in a low signal collection efficiency of <1%.² Nevertheless, Lakowicz *et al.* have overcome this

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fundamental limitation of signal collection from isotropic free space emission, without the need for expensive or cumbersome optics. They showed that the emission from excited fluorophores, in the vicinity of thin noble metal films such as Ag or Au (separated from the fluorophore/analyte by a passivating layer), can couple to the surface plasmon modes of the metal leading to a highly directional, *p*-polarized and wavelength resolved emission known as surface plasmon coupled emission or SPCE. The high directionality of SPCE, leads to high signal collection efficiency (>50%).²⁻⁴ Although both Au and Ag thin films can be used, Ag thin film substrates have been used extensively for SPR based detection due to their greater sensitivity.⁵ Ag has also been the substrate of choice in many SPCE platforms due to economic viability and its unique property of absorbing high amount of incident energy at sharper plasmon angles compared to Au.^{2, 3, 6, 7} Theoretically, SPCE can support impressive emission signal enhancements (up to the factor of 10^3 fold), but only 3–10 fold signal enhancement, with respect to the isotropic free space emission, has been achieved in conventional SPCE studies.^{2, 8} Some of the issues limiting this potential have been identified and addressed to a certain extent by several groups. However, the enhancements achieved were meager and extraneous to the SPCE platform that involved expensive and cumbersome optical configurations.⁹⁻¹¹

Recently, we showed that graphene as an “active” spacer layer, yielded 40-fold amplification of the emission intensity of rhodamine B (RhB) fluorophore, via π - π interactions between graphene and RhB¹²; (see supporting information). Here, the term “active” refers to the innate ability of the spacer to contribute towards the generation of the coupled emission signal, unlike SiO₂ which has no actual role in the process. We also showed that the SPCE signal increases with an increase in the in-plane crystallinity (L_a) of graphene (inferred from micro-Raman spectroscopy).¹² Although graphene is an attractive spacer layer for SPCE, its synthesis with high L_a and subsequent transfer of a continuous film to an SPCE platform is a cumbersome task. Therefore, we investigated the feasibility of directly coating SPCE platforms with other nanocarbon allotropes (viz., C₆₀) and tested its performance as a spacer material. The low sublimation temperature of C₆₀ facilitates the fabrication of scalable and economically viable SPCE sensors, unlike the conventional substrates where coating of SiO₂ is cumbersome and expensive. Unlike graphene, uniform C₆₀ layers with precisely determined thickness, can be deposited on Ag films via physical vapor deposition. C₆₀ is an interesting material with the following unique properties: (i) robust chemical and mechanical nature, (ii) very efficient charge transfer processes that can be leveraged in sensing applications^{13, 14} (iii) excellent propagation of surface plasmon modes¹⁵ which is crucial for SPCE, and (iv) radially directed π -orbitals of C₆₀¹⁶ that can promote π - π interactions with fluorophore molecules as was recently reported.¹³ As detailed below, in addition to the enhancement of the SPCE signal by 30 times using C₆₀ layers, we also show that SPCE experiments along with some numerical models, (discussed later in the manuscript), could be used to obtain linear refractive index of spacer layers.

Experimental Section

Fabrication of Silver Substrates by Physical Vapor Deposition

The Ag substrates were prepared by depositing silver (~50 nm thickness) onto Pyrex microscope slides (75×25 mm). A home-built physical vapor deposition system ($\sim 10^{-5}$ Torr) was used, where the Ag film thickness was determined by a quartz-crystal, film thickness monitor.

Spacer Layer Synthesis

About 50 mg of C₆₀ powder (purchased from Sigma-Aldrich; 99.5% purity, CAS number 99685-96-8) was loaded into a W evaporation boat and sublimed onto the 50 nm thick Ag films (described in the preceding paragraph) at $\sim 10^{-5}$ Torr. C₆₀ films of desired thicknesses (2, 4, 8, 12 nm) were prepared using an in-built shutter, (placed over the W boat), and regulated by the quartz-crystal film thickness monitor.

Preparation of RhB-PVA Solution and Coating the Ag-C₆₀ Substrates

An aqueous solution of RhB in a 1% polyvinyl alcohol (PVA) matrix was prepared and spin coated @ 3000 rpm onto the four Ag-C₆₀ thin film stacks to achieve an overcoat of 30 ± 3 nm.^{2, 3}

SPCE Measurements

A schematic depiction of the experimental setup for SPCE measurements, used in this study, of RhB-PVA on Ag thin films can be found in Ref. 12. The four RhB-PVA coated, Ag-C₆₀ (2, 4, 8, 12 nm) thin film stacks were first affixed to a BK7 hemi-cylindrical prism using glycerol as the index matching fluid ($n = 1.47$). Next, the slide affixed to the prism was positioned on a calibrated 360° rotary stage and the fluorescence measurements were performed in the Reverse Kretschmann (RK) configuration.^{2, 3} The incident excitation source was a *p*-polarized, 532 nm c.w. laser. The isotropic free space emission was recorded on the frontal side of the substrate and the SPCE emission arising from the distal side of the prism was recorded through a 550 nm long wave pass filter. The fluorescence (free space) and the SPCE signals were recorded using the USB4000 fiber optic spectrometer, interfaced to the spectrasuite software (Ocean Optics®). Three aspects of SPCE namely; enhancement, polarization and angularity of the coupled emission signal were determined.

Surface Plasmon Resonance (SPR) Based Reflectivity Simulations

Computer simulations based on the SPR theory were performed using the TFCalc© software (Software Spectra Inc., Portland, OR) to predict the angle of minimum reflectivity (θ_{sp}); which can be correlated to the experimentally determined angle at which maximum directional emission intensity was observed. The various parameters of the fabricated thin film stacks such as; the thicknesses of Ag (50 nm), C₆₀ spacer layers (2, 4, 8, and 12 nm) and RhB-PVA coat (30 nm) were entered as input parameters in the software. The refractive indices for Ag (n , k values from 300–1000 nm)¹⁷, C₆₀ ($n = 2.1$),⁵ and PVA ($n = 1.50$)² were also entered into the software to generate the minimum reflectivity plots. A summary of all the results from various studies is shown in Table 1.

Results and Discussion

Enhancement

The signal enhancement is defined as the ratio of the intensity of the SPCE signal to that of the isotropic free space fluorescence signal. From our studies, the recorded enhancement, for each of the four Ag-C₆₀ stacks is depicted in Figure 1(a) and a 30 fold enhancement is inferred in the case of the Ag+2 nm C₆₀ stack. Although the remaining thin film stacks exhibited a <30 fold enhancement, (see table 1), it is noteworthy that their enhancement is superior to the 3–5 fold signal enhancement reported for traditional 5 nm SiO₂ spacer under similar experimental conditions.² The observed trend of decreasing signal enhancement with increasing thickness of the C₆₀ spacer is consistent with the very small amplitude (~2 nm) of surface plasmon resonance mode of Ag. An increase in the thickness of the C₆₀ spacer naturally leads to an inefficient coupling between the RhB emission and the plasmonic modes of Ag.

Polarization

The *p* and *s* polarized emission intensities of the SPCE signal, arising from the four Ag-C₆₀ thin film stacks were recorded by placing a sheet polarizer between the detector and the thin film stack. The percentage *p*-polarization of the SPCE signal for the four Ag-C₆₀ stacks was found to be 96 %, 96 %, 94 % and 92 %, respectively, represented in Figure 1(b) and (Table 1), in coherence with the nature of emission in SPCE. These findings are also consistent with the fact that the Ag-C₆₀ interface supports the propagation of surface plasmon polaritons.¹⁵ More importantly, in the case of free space, percentage of *p*- and *s*-polarization was equal (data not shown) indicating that the presence of C₆₀ increases *p*-polarization through π - π stacking akin to graphene. Graphene consists of a honeycomb lattice of carbon atoms with pure *sp*² hybridization unlike C₆₀ where in *p_z* orbitals partially participate in bonding resulting *sp^x* hybridization (2<*x*<3). Such partial availability of *p_z* orbitals reduces the π - π interactions in C₆₀ leading to a comparatively lower enhancement.

Directionality of Coupled Emission

The directional nature of the SPCE emission as observed in our studies is attributed to the fluorescence-plasmon coupling which occurs only at specific angles (θ_{SP}), where the reflectivity of the metal is at a minimum. The observed angles θ_{SP} for the four Ag-C₆₀ (2, 4, 8 and 12 nm) stacks are at 50°, 52°, 55° and 60° respectively, Figure 2(a–d). This is consistent with the predicted angles (dips in the reflectivity data) in Figure 2(e).

It is well established that an increase in the thickness/refractive index leads to a red-shift in the angle of minimum reflectivity,¹⁰ which was observed both experimentally and predicted theoretically (Figure 2). From the present work, it is also interesting to note the correlation between: (i) an increase of thickness of the arms in the radial plot, Figures 2(a–d), and (ii) the FWHM of the minimum reflectivity curves, as a function of increasing thickness of C₆₀; and also (iii) the negligible change in the intensity of the plasmon dips, (Figure 2(e)). The negligible change in the extent of the dip stems from the presence of a greater number of absorbing species (C₆₀) in the thin film architecture, while the increase in its FWHM is due to the alteration in the local refractive index¹⁰ of Ag in the presence of C₆₀.

The high degree of enhancement and also of the *p*-polarized SPCE signal is a direct evidence for C₆₀ as a good “active” spacer material in SPCE platforms. The observed SPCE signal augmentation is attributed to the interaction of radially projecting π -orbitals of C₆₀ with the aromatic rings of RhB. In our previous studies, we demonstrated that the enhanced SPCE signal arises from the π - π interactions between the honeycomb lattice of graphene and the aromatic rings of RhB.¹² To validate the same in this study, we performed SPCE measurements on two *non-silvered* glass slides; one coated with C₆₀ and another without; which served as a control. We spin coated RhB-PVA solutions onto the two glass slides (thickness ~ 30 nm) and monitored the polarization intensity of emission in both cases. It is noteworthy that the RhB emission (@ ~580 nm) which arose from the C₆₀ coated glass slides was predominantly *p*-polarized, in contrast to the emission from the bare glass slides that exhibited fairly isotropic *p/s* polarization intensities; (see supporting information). These results suggest that the C₆₀ spacer to some extent enables orientation of a large number of fluorophores (RhB) in a manner that generates significant *p*-polarized emission necessary for plasmon propagation and efficient coupling at the silver surface.

In addition to orienting the fluorophores in a suitable manner; it is important to note that C₆₀ facilitates efficient transfer of energy from the excited state of RhB to Ag plasmon modes. This stems from the fact that the energy levels of C₆₀ enable photo-induced charge transfer from an excited dye.^{18, 19} The mechanism of photo-induced charge transfer occurs on the picosecond time scale, which is 10³ times faster than the conventional fluorescence decay rate. This results in channeling of greater amount of energy in a given time. C₆₀ also allows multiple electron transfer reactions to occur from multiple fluorophores, which has been reported in reference to charge transfer from rhodamine. Consequently, a single fullerene molecule has been shown to accept up to 6 electrons at a time.¹⁸ Based on these factors, in our studies, a single C₆₀ molecule would enable the ultra-fast transfer of energy from multiple rhodamine b molecules at any given time. Considering that there are several C₆₀ molecules interacting with several RhB molecules in our thin film architecture; the rate of energy transfer and the amount of energy transferred from RhB to Ag, is expected to be rapid and exponential. In this manner C₆₀ facilitates the efficient and effective transfer of energy from RhB to Ag resulting in the generation of SPCE signal with enhanced intensity, as observed in our studies. A schematic illustrating the mechanism of SPCE signal generation from Ag-C₆₀ thin film substrates is depicted in Figure 3.

As described earlier, the 2 nm thick C₆₀ spacer supported the most efficient generation of SPCE signal. However, to gauge the potential use of Ag-C₆₀ substrates in sensing applications, it is important to understand the mechanism of detection that occurs on an SPCE sensing platform. Typically, the surface of the SPCE platform is functionalized with ligands for sensing target analyte molecules that may be present in its vicinity. Since SPR/SPCE are surface phenomena, binding of analyte at the surface leads to a local change in film thickness and/or refractive index of the thin film. The angularity of the SPCE signal is highly dependent on minute variations in thickness/refractive index of the different layers that constitute the thin film stacks and hence the angular variation associated with binding of analytes is a much better indicator for sensing. From Figures 2(a–d) and the simulated values of θ_{SP} in Figure 2(e), we deduced θ_{SP} (variation of surface plasmon angle) as a

function of C_{60} film thickness in the four thin film stacks, and compared the data to the system where C_{60} was replaced by SiO_2 . In both cases the standard spacer thickness of 2 and 5 nm was used. The minimum reflectivity plots displaying θ_{SP} for these systems are shown in Figure 4.

We can observe that for a 3 nm increase in thickness, the angular variation determined was $\theta_{SP1} = 0.8$ degrees (SiO_2 spacer) and $\theta_{SP2} = 2$ degrees (C_{60} spacer). This simulation provided the first indication that the C_{60} spacer layer was relatively more sensitive to variations in thickness than the conventional SiO_2 spacer layer. It is important to note that these studies on angular variation with thickness are of relevance because target analytes such as proteins, bacteria, DNA, etc. typically form layers that extend up to few tens of nanometers from the sensing platform. We were hence motivated to study the variation in angularity and SPCE pattern, for higher thicknesses of C_{60} in comparison to the SiO_2 spacer, which is depicted in Figures 5(a,b).

We have already shown in Figure 2(e) that the change in the extent of the dip in reflectivity is negligible for C_{60} spacers whose thickness range from 2–12 nm. However, the minimum reflectivity curve disappears completely when the C_{60} thickness exceeds 20 nm, (Figure 5(a)), which is in sharp contrast to that exhibited by SiO_2 where the curve disappears above a thickness of 150 nm, Figure 5(b). The results depicted in Figures 4 and 5(a,b) collectively suggest that C_{60} films serve as superiorly sensitive spacer layers, (film thickness <20 nm and greater θ_{SP2}), when compared to the traditional SiO_2 spacer layers.

In relation to these studies, Ekgasit et al.¹⁰ have shown that a linear dependence exists between θ_{SP} and thickness of thin films. Based on this, we plotted the variation of θ_{SP} with film thickness of C_{60} and SiO_2 , depicted in Figure 5(c). While the TFCalc. data showed a clear linear dependence between θ_{SP} and film thickness for SiO_2 , the dependence for C_{60} was relatively non-linear. Importantly, the experimental data of C_{60} was in strong coherence to theoretical prediction. Approximating the plots in Figure 5(c) as linear plots (which is valid for small d), the slope 'S' was determined to be ~1.32 and ~0.32, for C_{60} and SiO_2 respectively. The slope for each plot can be directly correlated to sensitivity, where the greater S-value of C_{60} (1.32) indicates a larger change in θ_{SP} as a function of increasing d , and thus implying greater sensitivity. In this manner, from all the studies performed in this work, C_{60} proved as an optimal spacer material for sensitive detection schemes.

During the course of our numerical calculations, we have also found a very interesting relationship between the refractive index (n) of a material, and the thickness-angularity data that can be obtained from SPCE experimental measurements. A linear dependence between thickness and angularity was observed for materials of low ' n ' (1–1.5), values shown in Figure 5(d). The dependence however became increasingly exponential for materials with $n > 1.5$ and increasing film thickness. The same was observed experimentally for C_{60} as was shown in Figure 5(c). We generated theoretical plots for thickness versus angularity as a function of different values of ' n ' (1.33–3.4), including that of C_{60} ($n = 2.1$), which was in strong correlation to the experimentally observed data; represented in Figure 5(d). This exercise elucidated that it is possible to discern the approximate refractive index ' n ' of a spacer layer in a thin film stack, from its experimentally obtained thickness-angularity data.

Hence, the SPCE measurements may also be used as a facile technique to obtain the refractive index of thin films. For instance, any thin film (e.g., bacterial protein films or emerging two-dimensional materials such as MoS₂, BN) may be used in place of the spacer layer, and the observed/numerical angular emission values could be used, (as described in Figure 5(d)), to obtain values of refractive index.

Conclusions

In this study we established C₆₀ as an effective “active” spacer material in an SPCE substrate, which led to 30 fold signal enhancement of fluorescence emission, compared to the isotropic emission. This high enhancement stems from the orientation of RhB molecules at the surface of the C₆₀ thin film through π - π interactions, and the unique ultra-fast photo-induced electron transfer mechanism associated with fullerene molecules. Importantly, the synthesis and fabrication of C₆₀ thin films is simplistic and economical when compared to conventionally used SiO₂ spacer. Furthermore, based on experimental evidence and reflectivity calculations, minor changes in the thickness of C₆₀ films were found to influence large variations in the angular and polarization modes of SPCE patterns; thereby enabling efficient signal detection. The feasibility of determining a material’s refractive index from its experimentally determined thickness-angularity SPCE data was also demonstrated. In this manner, we have substantiated C₆₀ as a smart spacer material that can be potentially employed on sensing platforms promising high sensitivity, towards detection of analytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) The isotropic free space emission intensity and SPCE intensities for the four Ag-C₆₀ (2–12 nm) thin film stacks, plotted as function of wavelength. (b) Polarized emission intensity plot displaying *p*-polarized *I*(*P*) versus *s*-polarized *I*(*S*) emission intensities of the SPCE signal, from Ag-C₆₀ (2–12 nm) thin film stacks.

Figure 2.

Radial plots displaying the angle of directional emission (θ_{SP}) observed for the four Ag-C₆₀ stacks: (a) @50° (2 nm C₆₀), (b) @52° (4 nm C₆₀), (c) @55° (8 nm C₆₀) and (d) @60° (12 nm C₆₀). (e) Simulated reflectivity plots showing the angle of minimum reflectivity (which corresponds to θ_{SP}) for the four Ag-C₆₀ stacks.

Figure 3.

A schematic (not to scale) depicting the mechanism of SPCE signal generation on Ag-C₆₀ substrate, affixed to a hemi-cylindrical prism. Multiple RhB molecules are oriented on C₆₀ via π - π interactions. RhB molecules emit ~580 nm (depicted by curved orange arrows) when excited at 532 nm. C₆₀ spacer layer in our experiments facilitates multiple electron transfer reactions from RhB to itself via ultra-fast photo-induced transfer of energy. The energy of RhB emission couples to the surface plasmon modes of the Ag substrate, resulting in radiative plasmons or the directional SPCE signal at an angle θ_{sp} that travels through the prism.

Figure 4.

A simulated reflectivity response for SiO₂ and C₆₀ spacer layers as a function of increasing spacer layer thickness. The angular spread θ_{SP2} in the case of C₆₀ is greater as compared to θ_{SP1} for SiO₂.

Figure 5.

Minimum reflectivity plots showing the variations in the emission angles as a function of spacer thickness for (a) C₆₀ and (b) SiO₂; (c) Plot depicting the linear dependence of $\Delta\theta_{SP}$ with thickness d of the spacer material (C₆₀/SiO₂) showing a strong correlation between experimental and predicted values for C₆₀ and (d) Plot depicting the Thickness-Angularity relationship for different values of refractive index 'n' of a material.

Table 1SPCE parameters for the Ag-C₆₀ thin film stacks used in this study

Slide Type	Enhancement	<i>p</i> -Polarization (%)	Predicted Angularity (deg.)	Observed Angularity (deg.)
Ag 50 nm + 2 nm C ₆₀	30	96	50.3	50
Ag 50 nm + 4 nm C ₆₀	25	96	51.6	52
Ag 50 nm + 8 nm C ₆₀	23	94	54.7	55
Ag 50 nm + 12 nm C ₆₀	14	92	59	60