

Flashy Silver Nanoparticles

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Temporal fluctuations in the Raman spectrum from molecules adsorbed to noble metal colloids (i.e., “blinking”) has been viewed as a single molecule signature. It is demonstrated here that silver colloids exhibit blinking that is independent of the nature of the adsorbate. Blinking was observed on silver colloids in the presence and absence of a SERS probe (e.g., R6G, BPE), sodium chloride, poly-L-lysine, an alkanethiol layer, and a liquid overlay (e.g., water, methanol). These observations suggest that the mechanism that gives rise to blinking is insensitive to the nature of the adsorbate and is likely to be a property of the nanoparticle. Consistent with this idea, blinking was observed from vapor-deposited silver films and silver powders with no added molecular probe. In addition, two distinct broad luminescence-like (continuum) features also exhibited blinking and spectral diffusion, with no apparent adsorbate dependence. Evidence that SERS and the underlying continuum are associated is provided by an observed correlation of “on” and “off” states for both processes.

Introduction

Noble metal nanoparticles are capable of enhancing the Raman scattering efficiency of nearby molecules by as much as 10^{14} .^{1,2} Although the mechanism that results in such enhancements remains incompletely understood, the effect has enabled single molecule detection using surface enhanced Raman scattering (SERS). Seemingly related to the SERS enhancement mechanism is the observation of the dramatic spectral and temporal fluctuations of Raman bands noted in single molecule SERS (smSERS).¹ As in single molecule fluorescence experiments, “blinking” is often attributed to Raman scattering from a single molecule.^{2–6} The observation in our laboratory, and others,⁷ of SERS blinking from silver colloids coated with hundreds of molecules motivated further study of the blinking mechanism, which, in turn, may provide insight into the SERS mechanism.

It has been noted since the late 1970s that a broad continuum background is commonly associated with SERS.^{8,9} Both Raman and the continuum have been described for smSERS as well, and Michaels and Brus reported a correlation of their respective peak intensities with time.¹⁰ Several authors have also reported “spurious” SERS, that is, Raman bands whose shifts do not correspond to the molecule under study.^{11–13} Silver colloids prepared by both the citrate and borohydride reduction procedure exhibit spurious SERS.¹³ Although the origin of these peaks is not clear, spurious SERS also exhibits blinking.

Here we report blinking of SERS and the continuum from silver colloids in the absence of a SERS probe. The most widely accepted hypothesis for blinking, which is based on adsorption/desorption cycles or diffusion of the adsorbate, is discussed in light of the insensitivity that blinking exhibits toward a variety of adsorbates. A blinking mechanism associated directly with particle properties appears to be more likely.

Experimental Section

To explore the blinking phenomenon in smSERS, we initially employed the conditions typically used to demonstrate smSERS.

The colloids were prepared according to the procedure first published by Lee and Meisel¹⁴ and adapted by Michaels et al.¹⁵ The particles were characterized by both transmission electron microscopy (TEM) and atomic force microscopy (AFM). The SERS probe was added to the colloid preparation by incubating 250 μL of the Ag colloid solution with 5 μL of 1×10^{-10} M Rhodamine 6G (R6G) in methanol and 10 μL of 20 mM NaCl for 2–3 h. A 5- μL aliquot of the mixture was deposited onto poly-L-lysine-coated glass slides using the procedure described by Emory and Nie.¹⁶ AFM images of the resulting slides indicated that the majority of the particles were single colloids or associated colloid clusters with a wide range of distances between particles, from zero (in a cluster) to several microns, depending on the quality and uniformity of the poly-L-lysine layer.

An air-cooled argon ion laser (Ion Laser Technology, 5500A) was used for excitation at 514.5 and 488.0 nm and a helium–neon laser (Melles Griot, 05-LHP-991) was used for 632.8 nm excitation. Light from the argon ion laser was passed through a pre-monochromator (Optometrics TGF-302) to remove spurious plasma lines. A narrow pass optical filter (Omega Optical) was used to remove spurious lines from the helium–neon laser. The beam was then expanded using a $10\times$ beam expander (Melles Griot), and an aperture (Melles Griot) was used to block stray light exiting the expander. The beam passed through a nonpolarizing, broadband beam splitter (Newport, 50/50) where the discarded light fell incident on a beam stop. An aluminum-coated mirror (Newport) directed the beam downward to a microscope objective (Leica, infinite achromat, 0.66 NA) that focused the light onto the sample. The microscope objective was mounted on a Z-translation stage (Newport, submicron resolution), which allowed focusing of the excitation light. Samples were manually scanned below the objective on an X–Y translation stage (Newport, submicron resolution).

Light was collected from the sample using the same objective and separated from the light path by the beam splitter. An aluminum-coated mirror (Newport) directed the beam toward two Raman notch filters (Kaiser Optical Systems), which removed the excitation wavelength. A monochromator matching f/4 lens (Newport, gradient index) was installed along the optical

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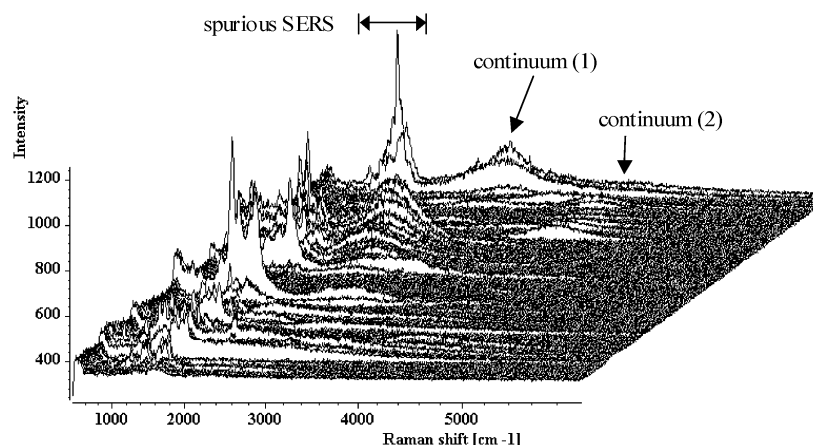


Figure 1. Silver colloids with no added SERS probe (Control 2 in **Table 1**). 514.5 nm excitation with 1 s integration (time increases away from the *x*-axis).

TABLE 1: Summary of Conditions under Which Silver Colloids “Blink”

	λ_{ex}	citrate (synthesis)	NaCl (incubation)	poly-L-lysine (immobilization)
R6G (~0.003 mlc/particle)	514 nm	present	present	present
R6G (~30 mlc/particle)	514 nm	present	present	present
BPE (~0.3 mlc/particle)	514 nm	present	present	present
BPE (~30 mlc/particle)	514 nm	present	present	present
control 1 (no probe added)	514 nm	present	present	present
control 2 (untreated colloid)	488 nm	present	not present	not present
control 3 (silicon ^a substrate)	514 nm	present	not present	not present
control 4 (bulk water overlayer)	514 nm	present	not present	present
control 5 (bulk methanol overlayer)	514 nm	present	not present	present
control 6 (decanethiol monolayer)	514 nm	not present	not present	not present
	632 nm			

^a Silicon (100), p-doped, 0.03 $\Omega \cdot \text{cm}$.

path of a 0.3 m monochromator (Acton Research Corp., 150 μm slit, 300 or 1800 grooves/mm) to collect spectra on a liquid nitrogen cooled, charge coupled device (CCD) (Roper Scientific, 400 \times 1340 pixels). The CCD was controlled with a Princeton Instruments controller (ST-133) and data was acquired using Winspec software (Princeton Instruments). The CCD was typically binned over 20 pixels in the 400 pixel direction (*Y*-axis) to increase the signal-to-noise ratio. Calibration of the CCD was performed by collecting a Raman spectrum of polystyrene and fitting the pixel data to known peak Raman shifts using a 2nd-order polynomial.

The laser power was measured with a power meter (Scientech, 372) and the spot size was estimated from a Raman image of silicon using 514-nm excitation. The dimensions of the Raman image were calibrated using an objective micrometer and line scan analysis was performed through the center of the spot. Points were found where the intensity was a factor 1/e less than the center, maximum intensity, and these points defined the edge of the spot. A 1/e spot diameter of 14 μm was measured. Using the measured laser power, optics transmission characteristics, and the measured spot size, the average excitation intensity was found to be $\sim 10^{22}$ photons/(s cm^2) at the surface, consistent with that reported by Nie and co-workers.¹

Results

Colloids with calculated^{10,16} single molecule coverage (e.g., ~ 0.003 R6G mlc/particle and ~ 0.3 BPE mlc/particle) exhibited blinking of the SERS peaks as well as blinking of the continuum, as reported by Michaels and Brus.¹⁰ However, in addition to smSERS spectra similar to those reported in the literature, spurious SERS was observed in every case. As shown in Figure 1, spurious SERS is characterized by a variable number of peaks, with seemingly random shift frequencies and fluctuating relative intensities.

Consistent with the observations by Käll and co-workers,⁷ blinking was also observed when the colloids were incubated with much higher concentrations of SERS probe molecule. As shown in Table 1, colloids incubated with relatively high concentrations of both resonantly and nonresonantly enhanced probe molecules exhibited SERS and continuum blinking. Of course, this observation does not rule out single molecule detection because the dominant signal could originate from localized areas of high enhancement, or “hot spots”.

Control Studies. Several control studies were conducted in the absence of an added SERS probe in an attempt to determine whether the blinking phenomenon is more closely associated with the adsorbate or with the nanoparticle. When the same chemistry is used in preparation and immobilization of the

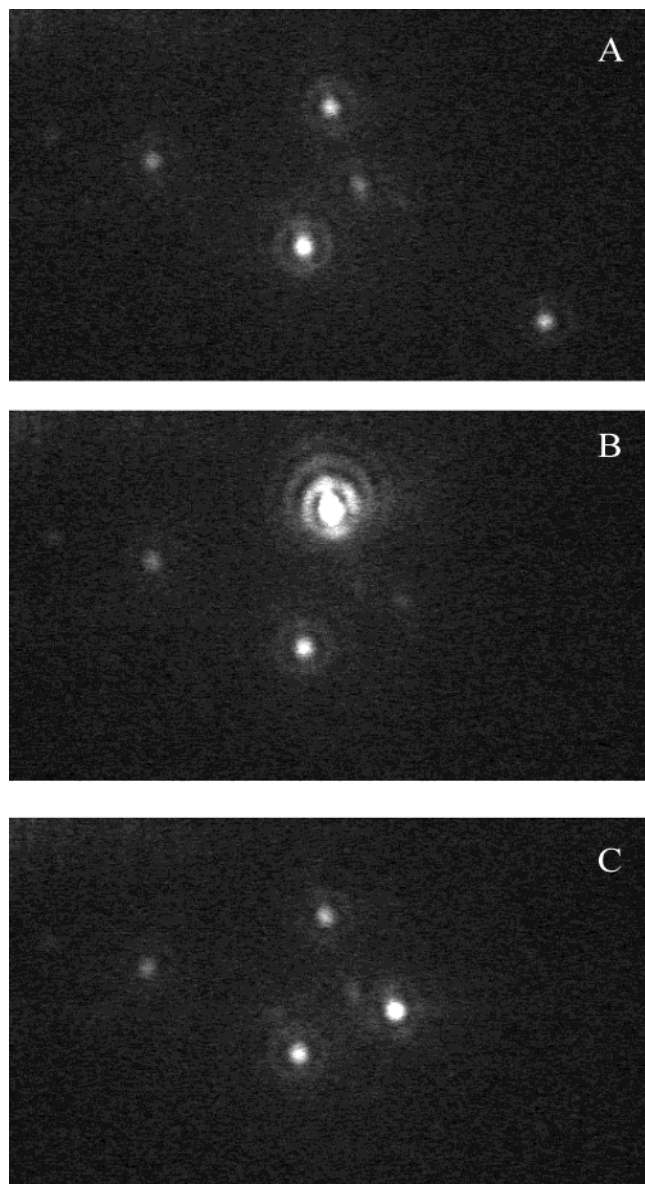


Figure 2. Optical images of Ag colloids dispersed on clean silicon. The colloids were deposited from the reaction mixture with no NaCl added, no SERS probe added and no poly-L-lysine on the surface. The image size along the x -axis is $\sim 16 \mu\text{m}$. Two Raman notch filters were used to remove the Rayleigh scattering and the CCD integration time was 1 s with 514.5 nm excitation. **A** is the image obtained at 30 s, **B** at 127 s, and **C** at 137 s. Note the diffraction pattern around the most intense spots.

colloids, but no SERS probe is added (Control 1 in Table 1), the colloids exhibit spurious SERS bands and two distinct continua that blink in the manner shown in Figure 1. The spurious SERS bands were not assignable to the reference Raman spectra of citrate, silver nitrate, or poly-L-lysine. To test the role of NaCl, we conducted the same study but eliminated the NaCl incubation step (Control 2 in Table 1). Despite the lack of incubation with NaCl, the colloids exhibited blinking of both spurious SERS and the continua. The influence of the nature of the substrate on blinking behavior was investigated by eliminating the poly-L-lysine coating on fused silica (Control 2) as well as by employing clean silicon as the substrate (Control 3 in Table 1). In both cases, the colloids blinked. Blinking of silver colloids on clean silicon is shown in Figure 2. In an attempt to control the liquid overlayer more quantitatively, the colloids were examined on fused silica under bulk water and

methanol (Controls 4 and 5 in Table 1). In both cases, the colloids exhibited blinking in spurious SERS and the continua.

Blinking appeared to be relatively insensitive to optimum excitation of the surface plasmon for colloids prepared without added adsorbates (Table 1, Control 2). A single colloid (cluster) examined with 514.5 nm excitation continued to blink when the excitation wavelength was changed to 488 nm. In the experiments described above (Table 1, Controls 3, 4, and 5), variation in the index of refraction, which is known to lead to a shift in surface plasmon resonance,¹⁷ caused no noticeable difference in the blinking behavior. The same was true with a drastic change in the substrate refractive index (i.e., silicon vs fused silica).

The origin of spurious peaks is unclear and the random nature of the peak frequencies greatly complicates their assignment. Because the colloids were prepared by the citrate reduction process, citrate and citrate oxidation products likely coat the colloids and may be a source of spurious SERS peaks.¹⁸ To displace the citrate and provide a controlled surface layer, the colloids were coated with 1-decanethiol using self-assembly conditions (Table 1, Control 6). Self-assembled monolayers of 1-decanethiol have a strong free energy of adsorption (-27 kcal/mol)¹⁹ and have been shown to displace most organic adsorbates from silver SERS substrates.²⁰ Surprisingly, even with an apparent monolayer of a strongly adsorbed species the spectrum blinked. In this case, the observed spectrum contained Raman bands clearly identifiable as decanethiol during the first 10 seconds of excitation. However, with time, the decanethiol bands decayed, and “spurious” bands grew in. At long excitation times, typical graphitic bands grew in, indicating that photodecomposition takes place at the surface.

Vapor Deposited Silver Films. Our hypothesis that the phenomenon is a property of the nanoparticles rather than the adsorbate was tested by investigating the behavior of vapor-deposited silver films, in which there were no added chemical species. On the basis of TEM and AFM images of vapor-deposited silver films, the average particle diameter was $\sim 25 \text{ nm}$, with only a few nanometers separation between particles. Figure 3 shows a series of images collected as a function of time from 4 nm of vapor-deposited silver on fused silica. Clearly, particles within the film exhibit independent blinking. These “hot spots” on the film may explain the recent observation by Aroca and co-workers of single molecule SERS on vapor-deposited films using high spatial resolution.²¹ Raman spectra from the vapor-deposited films are the same as the background spectrum that is often attributed to a “carbonaceous” contamination layer on the surface.²⁰ Perhaps even more surprising was the observation of blinking from silver powder taken directly from the container (Alrich). These silver particles have a large nominal diameter ($5\text{--}8 \mu\text{m}$), but characterization by TEM also shows many smaller particles.

Spectral Diffusion of the Continuum. While spectral diffusion has been reported for SERS bands from molecules adsorbed on noble metal colloids, we are not aware of reports of such behavior for the long wavelength continuum (continuum 2 in Figure 1). In nearly all of the dozens of data sets acquired for this study, the continuum was observed to drift. An example of spectral diffusion for a continuum is shown in Figure 4a. Note that there are two distinct continua. In this case, as in most, the continuum observed near 3000 cm^{-1} remains fixed, while the longer wavelength continuum shifts drastically as a function of time. In this example, the higher wavenumber continuum shifts

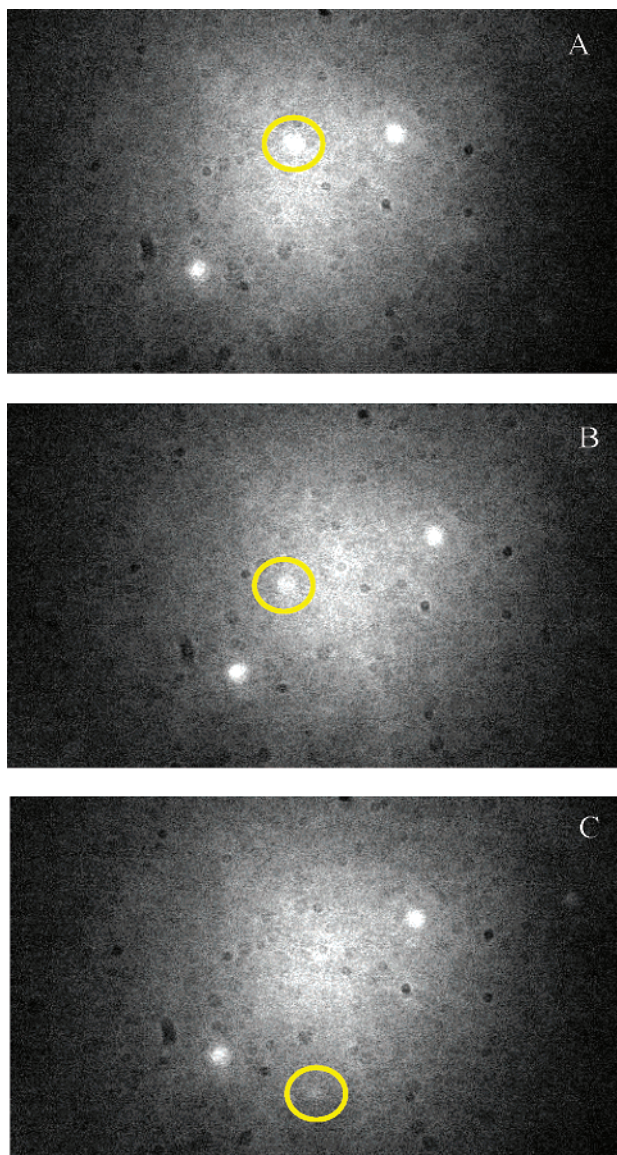


Figure 3. Optical images of a vapor-deposited Ag film (4 nm) thickness. The image size along the x -axis is $\sim 16 \mu\text{m}$. Two Raman notch filters were used to remove the Rayleigh scattering and the CCD integration time was 1 s with 488 nm excitation. **A** is the image acquired at 22 s, **B** at 35 s, and **C** at 49 s. Yellow circles are employed to aid the reader in identifying points that fluctuate.

by nearly 4000 cm^{-1} in just over one minute. In an attempt to quantify the shift and the apparent intensity changes in the higher wavenumber continuum, the spectra were fit (Grams/AI) with two Gaussians and a background. The results of the fit are shown in Figure 4b. It is important to note that fits to the data at times where the continuum is near the Rayleigh line were “manual,” because a quantitative evaluation of the notch filter profile was not possible in the absence of prior knowledge of the low frequency background. Thus, the peak height and width shown represent only one possible fit to the data. However, the fit was made in such a way as to account for an underlying continuum that, at times 68–70 s in the data set, contributes intensity on the blue side of the Rayleigh line. Enhanced signal on the “anti-Stokes” side of the Rayleigh is surprising, especially considering that the intensity reaches a maximum 1 s after the Stokes side reaches its maximum (i.e., the intensity contribution does not appear to be exactly correlated but rather it exhibits a behavior consistent with a continuum maximum that is shifting blue).

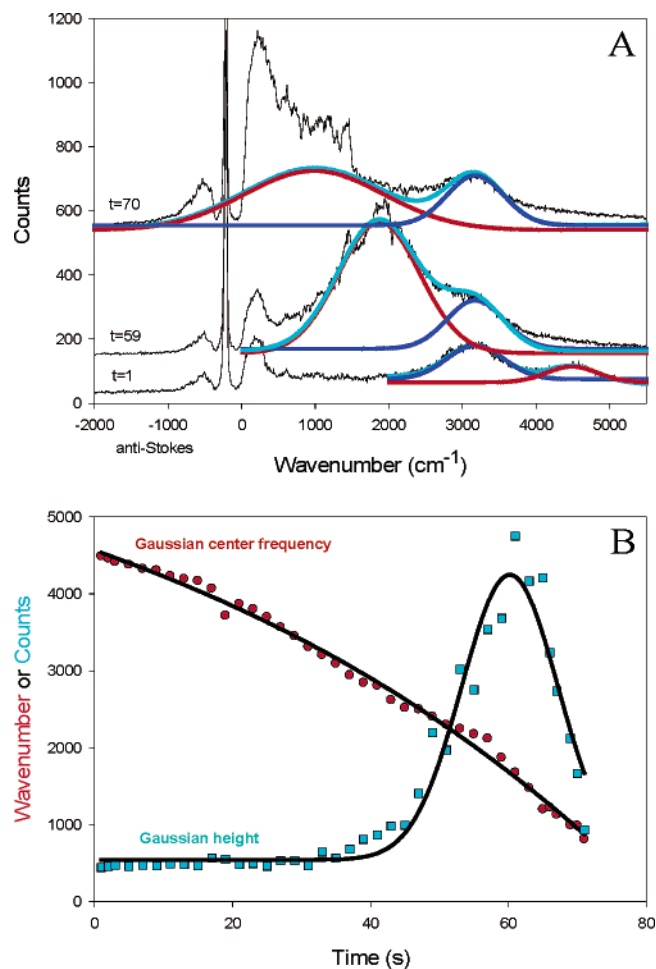


Figure 4. The spectra shown in **A** are from Ag colloids incubated with NaCl and R6G at a calculated coverage of tens of molecules per particle. As noted, the spectra correspond to 1, 59, and 70 s excitation (514.5 nm). The peak fit in red corresponds to a continuum that shifts and increases in magnitude with time. **B** contains a plot of the center frequency and height of the Gaussian fit. Note in **A** that the spurious SERS is not observed until the continuum appears to be “in resonance.”

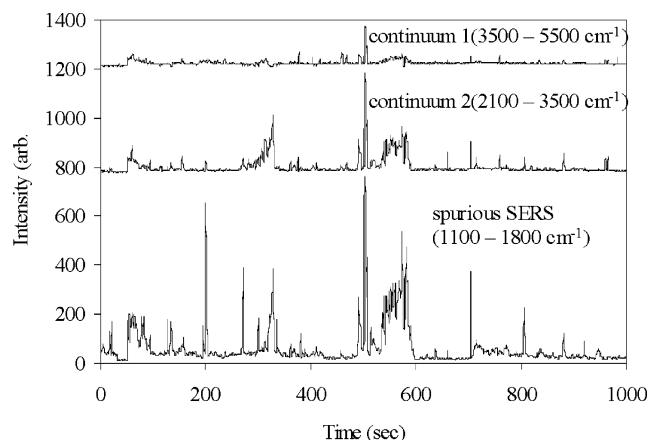


Figure 5. Time profile of maximum amplitude in each of the specified frequency ranges. These data were extracted from the data set shown in Figure 1 by measuring the maximum peak or band intensity within the specified region.

Time Correlation. To address whether the Raman bands and the continuum likely originate from the same fundamental source, the time correlation in the “on” and “off” states of each of the bands was examined. Figure 5 contains a time plot of

the most intense peak within three distinct wavenumber regions, corresponding to SERS and two continua. It is clear that while there is little correlation in the absolute intensities, the processes generally appear to turn on and off in a correlated fashion. We are in the process of analyzing a much larger dataset using a more sophisticated two-dimensional correlation algorithm.

Discussion

While several theories for SERS blinking have been postulated, blinking is commonly believed to arise from the adsorbate as it undergoes either rapid adsorption/desorption cycles¹⁵ or diffuses in and out of high enhancement “hot spots” on the particle surface.³ On the basis of the work conducted in this study, it does not appear that many of the molecules associated with typical smSERS experiments, including a strongly enhanced Raman probe, are required for the colloids to exhibit blinking. Moreover, the blinking appears to be independent of the adsorbate, even in the case of strongly adsorbed species such as citrate and 1-decanethiol. If blinking were due to adsorbate desorption or diffusion, one would not expect to observe blinking in the presence of such a strongly adsorbed molecule as 1-decanethiol, which has a ΔG_{ads} of -27 kcal/mol¹⁹ and a diffusion coefficient on the order of $10\text{--}12$ cm²/s.²² Of course, it is possible that photodegradation of the decanethiol leads to weakly adsorbed species within high field areas around the particle and that these molecules may undergo rapid adsorption/desorption.

A common blinking mechanism for the continuum and at least spurious SERS seems likely, given that blinking of these processes show strong temporal correlation. The optical signals appeared to bleach together (data not shown), further suggesting that the continuum and SERS enhancement mechanism are intrinsically linked. In addition, the continuum and SERS intensities have been reported to exhibit near identical laser power dependencies.¹⁰ Another example of the inter-relatedness of the processes is apparent in Figure 4. Note that the SERS region of the spectrum does not become “active” until continuum 2 shifts into that region. This implies a “resonance” condition for the two processes. The transient nature of that continuum with respect to the Rayleigh line also suggests that the process is not Raman scattering and is more likely to be luminescence.

Consistent with concepts presented by others, we hypothesize that e/h pairs in the silver colloids link SERS and the process that gives rise to the continuum. Further, we postulate that the continuum observed at longer wavelengths is due to radiative recombination of e/h pairs in the metal. Burstein and co-workers were the first to point out that e/h pairs may play an important role in both SERS and the continuum.^{23,24} Furtak and Reyes²⁵ summarized Burstein's four proposed mechanisms as (A) coulomb interaction, (B) inelastic electron scattering, (C) negative ion resonance, and (D) electron–hole polaron. Furtak and Reyes interpreted mechanism A as “... the excitation of the e/h pairs near the metal surface is transferred to (and back from) electronic excitations in the molecule, creating a molecular vibration in the interim”.²⁵ Mechanism B is essentially the “Frohlich” mechanism, in which the electron (or hole) is inelastically scattered from the vibrating charge on the adsorbate. The e/h then radiatively recombines. Mechanism C involves the localization of an electron (or hole) on the adsorbate. The de-excitation process leaves the molecule in an excited vibrational state. In mechanism D, an e/h pair polaron is formed by an admixture of molecular vibrations and a single (e/h) neutral particle. This mechanism could be viewed as a “driven” process, in which the radiative recombination of the e/h pair occurs

essentially simultaneously with excitation and deposition of one vibrational quantum into the adsorbed molecule. Recently, Brus and co-workers postulated that Raman scattering occurs from a resonant state formed between a ballistic e/h pair and the excited state of the adsorbate.^{10,15} Otto has proposed, and provided evidence for, electronic Raman scattering as a mechanism that may yield a broad continuum associated with SERS.²⁶

Recent research in the area of photoinduced luminescence from noble metal nanoparticles has demonstrated that radiative recombination of e/h pairs must occur.^{27,28} In an exploration of the single and multiphoton emission spectra from both smooth and rough films of Cu, Ag, and Au, Shen and co-workers attributed the luminescence to field-enhanced radiative recombination of e/h pairs.²⁹ They describe the system as a three step process, photoexcitation of an e/h pair, partial nonradiative relaxation of the excited electrons and holes followed by radiative recombination. Khriachtchev et al. report that the red photoluminescence from Au island films is strongly dependent on the film thickness and substrate roughness,³⁰ similar to the trends observed for SERS on thin films.²⁸ In El-Sayed's work with Au nanorods, noble metal luminescence was attributed to field-enhanced radiative recombination of excited sp-electrons with holes in the d-band.³¹

A simple two-state kinetic model suggests that a number of e/h pairs may exist in the metal during irradiation.³² These e/h pairs, regardless of whether they behave as excitons (e.g., a weakly bound e/h pair or Wannier-Mott-like exciton), could interact with the enhanced field, as pointed out by Boyd et al.²⁹ and Mohamed et al.,³¹ with incident photons, with Raman shifted photons, or as proposed by several researchers, the adsorbed molecule. It is anticipated that further investigation of the relationship between the optical behavior of SERS and the continuum will lead to a greater understanding of the mechanism(s) by which both processes occur.

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