Microheterogeneity in Dye-Doped Silicate and Polymer Films

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Received: June 10, 1998; In Final Form: July 22, 1998

Single molecule confocal microspectroscopic methods are used to characterize individual molecular-scale environments in silicate thin films for the first time. Rhodamine dyes doped into the materials at nanomolar levels are used as probes of the physicochemical environment in which each molecule is entrapped. The results are compared to those obtained from dye-doped organic polymer films. Static fluorescence spectra and time-dependent fluorescence signals recorded for a large number of single molecules show the silicate materials to be highly inhomogeneous in comparison to the polymer films. Histograms of the fluorescence maxima for encapsulated Rhodamine B show a full width at half-maximum of 13.3 nm for the silicate host framework and 6.7 nm for the polymer film. The integrated fluorescence signal from single molecules, recorded with millisecond time resolution, under continuous illumination conditions, is also sensitive to the local environment. The time-dependent signal traces show dramatic intensity fluctuations for some molecules and none for others. The fluctuations occur most frequently for the silicate-entrapped dye. In the present work, the signal fluctuations are proposed to result from time-dependent variations in the molecular environment, which in turn cause changes in the excitation and emission characteristics of the molecules. The photophysical phenomena behind these fluctuations include quantum yield variations, intersystem crossing to a long-lived dark state, and, to a lesser extent, spectral diffusion. All such effects are highly dependent upon the physicochemical properties of the molecular-scale environment, as shown by comparison to results obtained for the polymer samples. The results are used as further evidence for the heterogeneous nature of entrapment in silicate host structures. The dynamic nature of many of the molecular-scale environments in these materials is demonstrated as well.

I. Introduction

Sol−gel technology provides a convenient tool for the fabrication of inorganic and inorganic−organic hybrid materials via the hydrolysis and condensation of metal alkoxides.[1] The ease with which these materials can be prepared, modified, and processed in conjunction with their high optical quality, photochemical and electrochemical stability, and good mechanical and chemical stability has made them an attractive alternative to conventional organic polymers for various optical applications, composite material fabrication, and chemical sensor development.[2−7] While these porous solids are routinely used as stable host matrices for advanced applications, they are chemically complex. The detailed synthetic, aging, and drying conditions used in their preparation determine their average chemical and physical properties. These properties also vary dramatically within individual samples, on a local scale. Such heterogeneity results from variations in pore size, shape, surface composition, and local solvent composition, leading to the formation of a wide range of chemically- and physically distinct molecular-scale environments.[8] These spatially varying attributes control local material and dopant stability, as well as dopant and solvent mass transport. A better understanding of the types of environments found in these materials is therefore of both fundamental and technological interest.

Previous bulk spectroscopic and chemical measurements have provided valuable information on the average chemical environment found in these materials.[3,5,8−19] However, very few sites actually possess the average properties determined by such means. Indeed, a few recent studies have noted the presence of several microdomains in the silicate host structure.[8,13,15−21] The presence of these environments was deduced from bulk spectroscopic studies of dye-doped silicate materials in which broadened spectral transitions were observed in static spectroscopic experiments[19] and complex time-dependent fluorescence signals were recorded in time-resolved studies.[13,15−18,20,21] However, the data obtained still represent average information about large numbers of environments. While these environments may be similar, they are not necessarily identical. Therefore, new methodologies need to be established to accurately probe the microenvironments of these complex materials at the molecular level.

The goal of understanding the inhomogeneous distribution of sites present in solid-state and condensed-phase chemical systems is not new. Spectroscopists have developed a number of tools that allow for such site-selective measurements to be performed.[22] Although significant progress has been made using these methods, many are experimentally and theoretically difficult and are instrumentally intensive. Recently, with the realization that single molecules can be readily detected by optical spectroscopic means,[23−33] a new class of site-selective experiments has become available. It is now possible to dope fluorescent probe molecules into a sample at very low concentration, isolate an individual molecule either spectrally[23,34,35] or spatially,[24−26,28−31,34,36] and use its observed spectroscopic properties as a direct probe of the local, molecular-scale environment in which it resides. By spectroscopically charac-

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S1089-5647(98)02574-7 CCC: $15.00
Published on Web 08/20/1998

terizing a large number of single molecules, a detailed picture of the range of local environments present is obtained. To date, however, very limited investigations have been directed along these lines.

In this work, single-molecule fluorescence spectroscopy is used for the first time to characterize the static and dynamic local properties of individual molecular-scale environments in silicate films prepared by the sol−gel process. For comparison purposes, similar experiments are performed on dyes doped into nonpolar organic polymer films. Several different rhodamine dyes are employed and are doped separately into the hosts at nanomolar concentrations. The individual molecules are then located and probed by confocal microspectroscopic methods. Fluorescence spectra of the individual immobilized dye molecules are obtained, providing information about the properties of the static (i.e., time-averaged) local chemical environments and overall sample heterogeneity. Likewise, time-dependent fluorescence spectral and intensity data are used to characterize dynamic variations in the local sample properties. Interpretations of these results are developed and presented in conjunction with the conclusions of previously published single-molecule studies.[26,28,30−33,37−39] These earlier studies have primarily addressed the photophysical origins of phenomena similar to those observed here. In the current work, the value of utilizing single-molecule spectroscopy to characterize material heterogeneity is demonstrated. These results show that the silicate host structures are highly heterogeneous, particularly relative to the polymer films.

II. Experimental Section

Equipment. The confocal microscope employed in these studies was of the sample-scanning variety. The sample stage was obtained from Queensgate instruments. This stage incorporates closed-loop X,Y feedback circuitry, providing ±3 nm accuracy in the stage position. The stage was mounted on a Nikon inverted microscope (model TE-300). Epi-illumination of the sample and detection of the resulting fluorescence were accomplished through its side port. The 514.5 nm line from an argon ion laser was employed as the excitation source. A fused-silica window was used as the beam splitter, directing ≈200 nW of light into the microscope. This light was focused through a 200 μm pinhole placed in the primary image plane of the microscope. In most experiments, a polarization scrambler was placed between the beam splitter and pinhole. A 100 X, 1.30 numerical aperture objective (Nikon) was employed to form a nearly diffraction-limited focus of 300 nm diameter on the sample. This same objective collected the resulting fluorescence. The fluorescence was imaged onto the 200 μm pinhole and transmitted back through the beam splitter. A holographic notch filter (Kaiser Optical) and two 530 nm longpass filters were used to remove residual excitation light. Detection of the fluorescence was accomplished with a single-photon-counting avalanche photodiode (EG&G).

The microscope was operated in three different imaging/spectroscopy modes. (i) Fluorescence images were recorded by raster scanning the sample in X and Y over a distance of 5 μm. The pixel size and dwell time in the images were 50 nm and 40 ms, respectively. (ii) In other experiments, the time-dependent fluorescence signal from individual molecules was recorded. The sample was held fixed such that the molecule being studied was positioned within the focused spot of the excitation light. The fluorescence was recorded as a function of time with 40 ms resolution. (iii) Finally, single-molecule fluorescence spectra were recorded by imaging the fluorescence

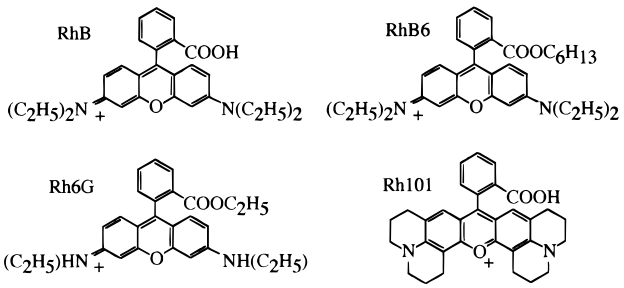

Figure 1. Molecular structures of the rhodamine dyes employed.

onto the entrance slit of a spectrograph (Acton Research) and detecting it with a liquid N_2-cooled CCD detector (Princeton Instruments). The spectra were typically integrated for 10 s. In studies of spectral diffusion phenomena,[37] integration times of 0.5−1.0 s were employed.

Procedures. Rhodamine B (RhB), rhodamine B hexyl ester (RhB6), rhodamine 101 (Rh101), and rhodamine 6G (Rh6G) dyes were obtained from Aldrich and Molecular Probes and were used as received. The structures of these dyes are shown in Figure 1. For studies of RhB in poly(butyl methacrylate) (PBMA, Aldrich), the dye (1 nM in methanol) was either spin-cast on top of a precast polymer film (from toluene solution) or a dye/polymer mixture (1 nM dye in chloroform) was spin-cast directly onto the substrate. The resulting films were allowed to dry, yielding thin polymer films containing well-dispersed dye (see below). Microscope cover glasses (Gold Seal, Fisher) were employed as substrates. The cover glasses were cleaned by washing with spectrograde methanol prior to use and contained negligible levels of fluorescent impurities.

For single-molecule studies in silicate films, a silica sol was prepared by the acid-catalyzed hydrolysis and condensation of tetramethoxysilane (TMOS, Aldrich, 98%). In a typical preparation, 1.6 mL of TMOS was combined with 2.4 mL of methanol and 1.2 mL of deionized water, followed by 0.3 mL of 0.1 M HCl. The final mole ratio of TMOS:H_2O:MeOH:HCl was 1:8:7:0.003. After ≈12 h, dye was doped into the sol so that its final concentration in solution was 1 nM. A ≈100 μL aliquot of the dye-doped sol was then spin-cast (room temperature, ≈7000 rpm) onto a clean cover glass and the resultant thin film dried overnight (relative humidity = 25−35%, ambient temperature) prior to use. The film thickness was ≈240 ± 30 nm, as measured via surface profilometry (Alpha Step 500, Tencor Instruments).

III. Results

Figure 2 shows a representative fluorescence image of a silicate film doped with RhB. (Dye-doped polymer film images are similar in appearance.) The brightness and vertical scale on this image depict the fluorescence intensity detected for each image pixel. The bright spots result from *single-molecule* fluorescence. Proof of this conclusion is drawn from a number of experimental observables.[25] (i) The fluorescence count rates measured for each spot are consistent with that expected for single-molecule emission at the excitation rate employed. (ii) The areal density of fluorescent spots observed is in agreement with the expected density of single molecules in a 240 nm thick film prepared from a sol of 1 nM dye concentration. (iii) Photochemical bleaching of each fluorescent spot is observed to occur in a discrete jump to the background level in time-dependent traces (40 ms resolution) of the fluorescence signal (see below). (iv) Fluorescence spectra recorded for each spot (see Figure 3) provide proof that the signal comes from isolated

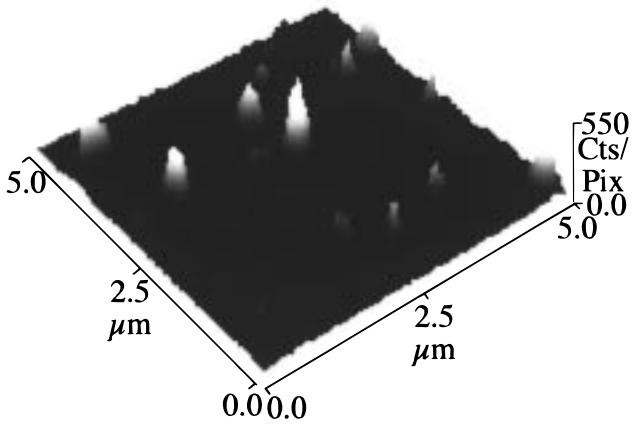

Figure 2. Fluorescence image of a silicate film produced by gelation of a sol containing rhodamine B dye at a concentration of 1 nM. The brightness and vertical intensity scales correspond to the fluorescence signal intensity observed for each pixel. The bright spots depict fluorescence from single dye molecules. Typical fluorescence signals are ≈200 counts/pixel where the signal from each pixel is integrated for 40 ms. The background signal is ≈20 counts/pixel.

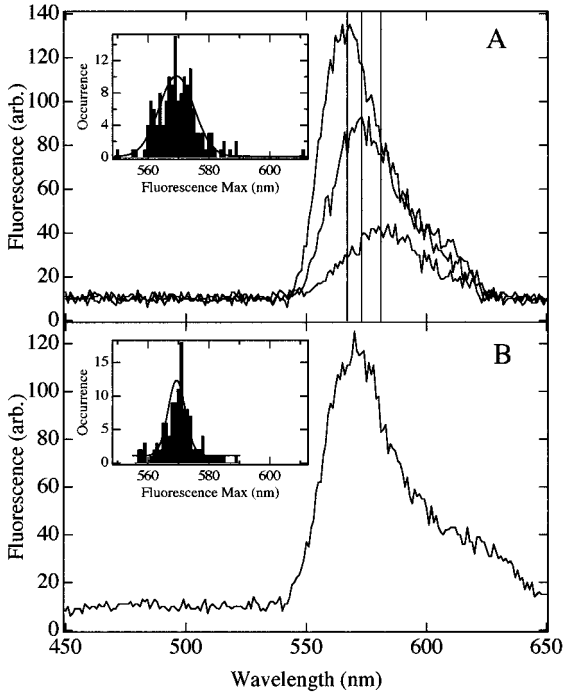

Figure 3. Representative fluorescence spectra for single rhodamine B molecules entrapped in (A) a silicate film and (B) a poly(butyl methacrylate) film. The vertical lines in (A) were appended to more clearly show the differences between the fluorescence maxima. The spectra were recorded by integration of the signal for 10 s. All three silicate spectra were recorded in the same film. The insets in (A) and (B) show histograms of the wavelength of maximum emission for large numbers of single molecules in different films.

(monomeric) single molecules, rather than aggregates, which also are not expected to form at such low concentrations.[14]

The fluorescence images obtained enable individual molecules to be located. Information on the local environment surrounding each molecule is best obtained by recording their individual fluorescence spectra. Spectra recorded for single RhB molecules in a single silicate film are shown in Figure 3A. Significant variations in the static (time-averaged) spectra are observed. In contrast, smaller variations are found for molecules trapped within the polymer host. A representative spectrum is shown in Figure 3B. The insets in Figure 3 present a better view of the differences. Histograms of the emission maxima measured by curve fitting the fluorescence spectra for numerous single molecules are shown. The approximate widths for the inhomogeneous distributions in silicate and polymer were determined by fitting these histograms to Gaussian profiles. The full-width at half-maximum (fwhm) for the silicate data is 13.3 nm, while that for the polymer is 6.7 nm, proving that there is a much broader distribution of molecular-scale environments in the silicate films than in the polymer. However, the peaks for both distributions occur at nearly the same wavelength (569 nm).

It is possible that the presence of protonated and deprotonated forms of RhB in the silicate materials contribute significantly to the increased width in the spectral distribution. The acid and base forms of RhB emit at 576 and 565 nm in water, respectively.[40] To determine the extent to which acid−base chemistry contributes, the experiments were repeated using rhodamine 6G (Rh6G), which does not have a carboxylic acid group. The spectral distribution for Rh6G was observed to have a similar width (18.3 nm, centered at 546 nm) to that observed for RhB. Thus, the presence of the two forms of RhB is not a prominent contributor to the distribution width, which primarily represents a measure of inhomogeneity in the silicate material itself. Note that the lactone form of RhB is only very weakly fluorescent and is not likely to be observed in these experiments.[41]

While the fluorescence spectra recorded above provide information on the static properties of each environment, the integrated fluorescence signal, recorded as a function of time, provides valuable information on dynamic variations in the environmental properties. Figure 4 shows representative time-dependent fluorescence traces (40 ms resolution) for individual RhB molecules encapsulated in silicate and polymer films. As noted above, individual molecules in both materials undergo irreversible photochemical destruction of the chromophore, as evidenced by the discrete, permanent drop in fluorescence to the background level near the end of each trace. In addition, some molecules show dramatic, reversible variations in their fluorescence signal levels (called "switching" here) under conditions of continuous illumination. These variations are most dramatic for RhB in the silicate film and less dramatic for the polymer samples (see Figure 4).

A more quantitative view of the relative number of molecules that switch in the silicate and polymeric films is obtained through statistical analysis of data recorded for numerous single molecules. Figure 5 shows histograms of the number of molecules exhibiting a given average switching rate. These plots were prepared by counting the number of fluorescence intensity jumps observed for each molecule and dividing by the total time of observation. Only jumps greater than 2 standard deviations above or below the mean signal level (local) were counted, thus excluding most (>95%) of the fluctuations due to shot noise. As is readily apparent from Figure 5, dramatically more fluctuations are observed for RhB entrapped in the silicate host, compared to the case of the polymer films. These results indicate that the molecular-scale environments in the silicate are much more dynamic than in the polymer.

For both samples, the characteristics of the time-dependent signal were observed to vary dramatically between individual molecules as well. Again, the greatest number of fluctuations were observed in the silicate materials. The signal characteristics can be categorized into three different classes. First, and perhaps most frequently, the fluorescence signal was observed to repeatedly jump between large nonzero values and the background level (Figure 4A). As in the case of irreversible

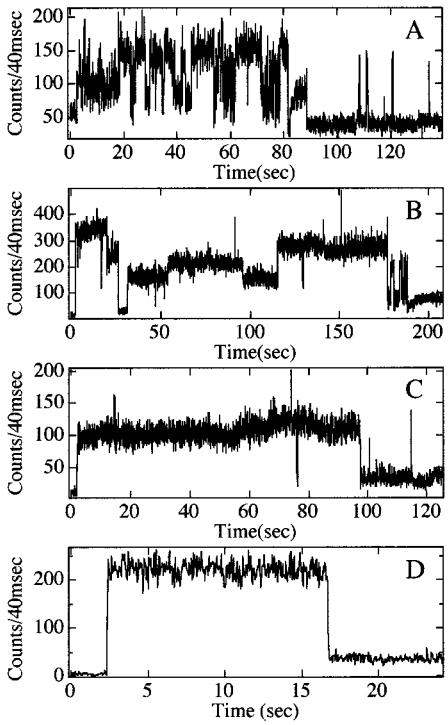

Figure 4. Representative time-dependent fluorescence signal traces showing the range of behavior observed for single rhodmine B molecules entrapped in the silicate matrix. Three general classes of behavior are observed: (A) large, "reversible", discrete jumps between the background level and nonzero signals; (B) discrete jumps between various nonzero signal levels; and (C) slow variations or approximately stable fluorescence signals. The variations shown by these data indicate that the molecules are entrapped in environments with different physical and chemical properties. (D) A representative time-dependent fluorescence signal trace for RhB in a poly(butyl methacrylate) film. The fluorescence signal is relatively stable in most cases for RhB in polymer. At the end of the trace, a discrete jump to the background level occurs due to permanent photodestruction of the chromophore.

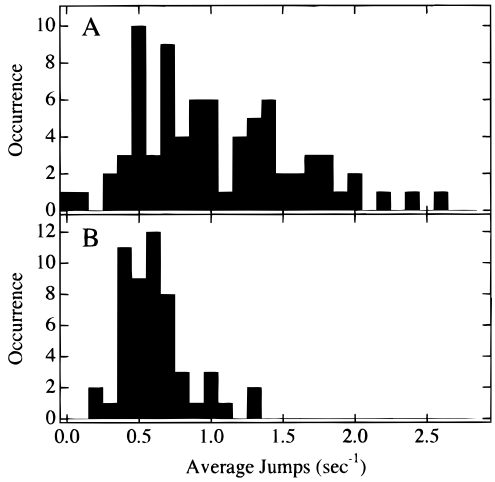

Figure 5. Histograms showing the extent of single-molecule fluorescence fluctuations observed in (A) silicate and (B) polymer films. Plotted are the number of molecules observed to exhibit a given average switching rate. The number of fluorescence "jumps" is counted and divided by the obervation time for each molecule. All jumps to new signal levels differing from the average signal by more than 2 standard deviations are counted, regardless of magnitude.

photochemistry, these transitions appeared as discrete jumps (shown here with 40 ms time resolution). Second, on a less frequent basis, discrete jumps between various nonzero signal levels also occurred (Figure 4B). Increasing the time resolution to 5 ms did not dramatically increase the number of jumps observed in either case.[28] Third, for a few molecules, the fluorescence signal was observed to remain approximately constant (except for shot noise) or to show slow variations (on a seconds or longer time scale) (Figure 4C). Such long-term fluctuations are not counted as "switching" in Figure 5 but are treated like stable signals. The dramatic differences observed between individual molecules within the same $5 \times 5\ \mu m^2$ area of the silicate film further points to the heterogeneity of the silicate.

To help determine the origins of these intensity fluctuations, experiments were performed using several different dyes. The acidic and basic forms of RhB have different fluorescence quantum yields.[40] The contributions of RhB acid−base chemistry were investigated by substituting the hexyl ester of rhodamine B (RhB6) and rhodamine 6G (Rh6G) for RhB. Neither RhB6 nor Rh6G has carboxylic acid groups (see Figure 1), and therefore do not have the same pH dependence as RhB. The time-dependent emission characteristics of these molecules could not be distinguished from that of RhB, indicating that such chemistry is not an important cause of the fluctuations.

Time-dependent variations in the quantum yield of emission for RhB may also lead to the observed fluctuations. The fluorescence quantum yield for RhB has been shown to vary with solvent.[42−44] The rate of nonradiative decay for RhB is believed to be greater in environments allowing pendant amine group motions.[44] To address this issue, rhodamine 101 (Rh101) was employed as the probe. As shown in Figure 1, Rh101 is structurally similar to RhB but has its amine groups rigidly bonded to the ring structure; hence, its fluorescence quantum yield is stated to be unity (in solution).[45,46] It is then expected that much less switching should be observed for this molecule. The time-dependent fluorescence signal from Rh101 in the silicate host was found to behave in a manner similar to RhB (i.e., switching occurs). However, a statistical analysis of the time-dependent data from a large number of molecules suggests that the fluctuations occur less frequently with Rh101.

Molecular rotational motion may also cause the fluctuations as the molecular transition dipole rotates with respect to the incident polarization vector.[31,32] However, fluorescence traces recorded with and without a polarization scrambler placed in the path of the excitation light showed no discernible differences. The scrambler renders the excitation light unpolarized in the plane of the sample and effectively removes any variations due to molecular rotation in this plane. It remains a possibility that the molecular dipoles may rotate out of the plane of the sample. Contributions from such motion cannot readily be discounted, but since rotational motion in the plane of the sample is an insignificant contributor, rotation out of the plane may be excluded as a possibility as well.

The contribution of translational (i.e., Brownian) motion of the molecules was examined by acquiring several sequential images of an identical sample area. Figure 6 shows two images recorded ≈10 min apart. If fast, large-amplitude motions were occurring, little correlation between images would be observed. In the case of much slower motion, it is possible that the individual molecular trajectories could be followed.[30,31,38] Qualitative inspection of the images indicates that no observable motion is occurring for the vast majority of molecules. For a more quantitative analysis, the fluorescent spots recorded for individual molecules were fit with Gaussian profiles to determine their positions to within 30 nm in each image.[30,38] None of the molecules selected for analysis were observed to move

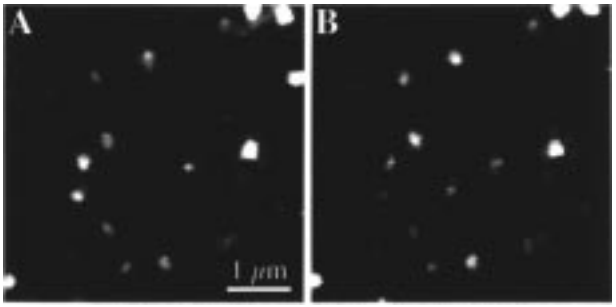

Figure 6. Sequential fluorescence images of the same area in a silicate film. Approximately 10 min elapsed between images. The images show that the single molecules do not move a distance greater than ≈30 nm on this time scale.

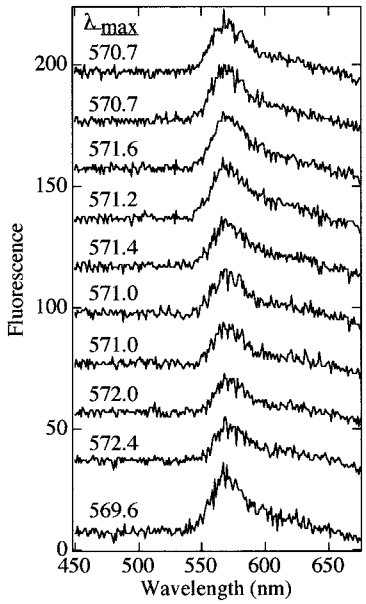

Figure 7. Time-dependent fluorescence spectra recorded for RhB in a silicate film. The spectra were recorded at 1 s intervals. The fluorescence maxima, determined by curve fitting the spectra, are shown as well. The data demonstrate that spectral diffusion is not a major contributor to the signal fluctuations observed in Figure 4.

within the precision of the measurement. Since a molecule must move a distance greater than ≈300 nm in much less than 40 ms to cause a significant change in the fluorescence signal, it is concluded that the majority of molecules in these films are trapped in "fixed" locations.

Spectral diffusion[33,37] resulting from time-dependent variations in the properties of the environment surrounding the molecule may also contribute to the observed fluctuations. A series of fluorescence spectra were acquired as a function of time for single RhB dye molecules to investigate this possibility. Representative data are shown in Figure 7. The spectra were recorded with 0.5−1 s time resolution. In all experiments of this type, the fluorescence maxima observed did not shift by more than ≈3 nm (peak-to-peak), indicating that spectral diffusion is only a minor cause of the observed signal fluctuations.

IV. Discussion

The observation and interpretation of the emission characteristics for individual molecules entrapped within a matrix represents a valuable means for obtaining detailed information about the local environment in which each molecule resides. Taken together, the results for a large number of molecules provide valuable information on the microheterogeneity of complex materials. The variations observed between single-molecule spectra provide new insights into the physicochemical properties of the host structure. Time-dependent fluctuations in the single-molecule emission signal provide additional data and yield a means for observation of molecular-scale environmental dynamics. Important knowledge about how each environment differs from all others, and how these environments vary in time, is obtained. Such single-molecule studies are likely to yield a better understanding of the material at the microscopic level.

The results presented above provide important new information on the properties of individual molecular-scale environments present within silicate films. The static single-molecule spectra recorded for the silicate films (shown in Figure 3) indicate that the silicate materials are highly inhomogeneous. The degree of inhomogeneity in the silicate is gauged here by comparison of these results with those obtained for the polymeric materials (also shown in Figure 3). It is readily apparent from the much (≈2 times) broader distribution of emission maxima that the silicate materials incorporate many more chemically distinct sites than are found in the polymer. As noted above, the broad distribution observed in the silicate is not simply due to the presence of the acid and base forms of RhB.

The chemically different sites found in the silicate materials arise during sol−gel formation due to spatial inhomogeneities that develop in the physical properties and rate of reactivity for the sol as polymerization proceeds. The local rates of hydrolysis and condensation may vary dramatically during this process. Such effects lead to the formation of pores of different sizes, surface properties, surface polarity, solvent content, and interconnectivity. Variations in the local concentration of surface hydroxide and alkoxide groups, entrapped or surface adsorbed solvent, and the sizes of interstitial cavities will strongly influence the photophysical properties of the individual dye molecules via subtle differences in hydrogen bonding, surface adsorption, and molecular rotation, for example.[8] The presence of all such sites in the silicate samples is manifested in the broad distribution of molecular spectra observed. As shown previously, RhB absorption and fluorescence spectra are sensitive to the polarity of the local environment.[42] These studies showed that RhB spectra recorded in water are red-shifted by 5−15 nm from those recorded in butanol (for example). It may therefore be concluded that the least polar sites lead to RhB emission near the blue edge of the distribution and the most polar cause emission to the red. It is proposed here that the sites on the blue edge are pores with significant numbers of methoxy groups remaining on the surface. The sites furthest to the red are pores with primarily residual hydroxy-terminated surfaces. These pores may also contain significant amounts of surface-adsorbed or interstitial water. Because of its higher volatility, it is expected that most of the methanol formed during hydrolysis of the precursor species has evaporated prior to study. The numerous sites between these extremes simply represents a nearly continuous variation in pore properties found in these materials. Conclusive assignment of the observed spectra to specific chemical phenomena requires detailed studies of their dependence on pore size and size distribution, silicate surface properties, and solvent content in films with carefully controlled properties.

The fact that dramatic fluctuations are observed in the integrated, time-dependent fluorescence from RhB in the silicate films, while few fluctuations are observed for RhB in the polymer film, indicates that this behavior is also highly

dependent on local environment. Such measurements may also be employed as probes of the molecular-scale environmental properties. The fact that dramatic variations in the switching behavior for single molecules within small regions of the silicate films are observed, again, indicates that the silicate films are highly heterogeneous, particularly compared to the polymeric material.

Similar fluctuations have been observed in the past in other systems.[26,33,36,39] The primary goal of these previous studies was elucidation of the mechanism by which such fluctuations occur. Their causes were attributed variously to molecular motion,[32] spectral diffusion,[33,37] energy transfer to nonfluorescent sites,[36] and the formation of long-lived "dark" states.[28,39] As discussed above, it is unlikely that the switching observed here is due to translational or rotational molecular motion. It is equally unlikely that the fluctuations occur from rapid interconversion of RhB between the acid and base forms.

The time-dependent fluctuations observed are believed to result primarily from time-dependent variations in the local environment. The fact that rapid, large-amplitude signal fluctuations are observed for some molecules in the silicate while others show a constant signal level (neglecting shot noise) indicates that some environments are more dynamic while others are totally static. Such differences point to variations in the physical (i.e., morphological) and chemical makeup of the region of entrapment for each molecule. In a recent review, Dunn and Zink have described the location of dopant molecules in the silicate host structure in terms of four "very general" areas: solvated pore interior, interfacial region, pore wall, and constrained region.[8] When the photophysical characteristics of individual dye molecules are closely examined, these individual microenvironments can clearly be observed.

In the present work, the molecules exhibiting constant levels of fluorescence are likely entrapped in completely enclosed or constrained pores. In such pores, solvent and other solutes (i.e., dissolved gases) as well as the dye itself cannot migrate in and out of the pore. Hence, the environment remains essentially constant in time. Such molecules may also be adsorbed to the surface of the pore. Finally, they may simply be entrapped in dry (no solvent present) environments. The molecules exhibiting rapid fluctuations are of course in much more open environments and likely reside in large pores or pores with large numbers of channels to surrounding pores. In such a system, solvent molecules may migrate relatively freely. However, because of the small size of the pores, a relatively small number of solvent molecules are present at any one time. Therefore, migration of just a few chemically different solvent molecules in or out of the pore will lead to a dramatic change in the environment. Such changes in the solvent composition may induce adsorption or desorption of the probe from the pore surface. Future studies will address such issues.

Variations in the local environmental properties lead to changes in the photophysical characteristics of the probe molecule, which in turn are manifested as changes in the fluorescence signal level. As noted above, the time-dependent changes in signal for some molecules may result from spectral diffusion, changes in the fluorescence quantum yield, or crossing to a long-lived dark state (likely a triplet state). Fluctuations caused by spectral diffusion result from variations in the excitation efficiency which are caused by shifts in the absorption spectrum due to changes in the local environmental properties. Such effects are particularly pronounced in solvatochromic dyes like RhB and have been observed previously to occur on time scales similar to the fluctuations observed here (>0.1 s).[33] However, only slight time-dependent variations in the position of the fluorescence maxima are observed (see Figure 7), indicating that in this system spectral diffusion is not likely to be important. However, it may contribute to the smaller discrete jumps observed and the slow signal variations.

A more likely explanation for the signal fluctuations is that the fluorescence quantum yield is changing in time. Again, photophysical changes brought about by solvation/desolvation and surface adsorption/desorption phenomena may contribute. Such environmental variations lead to changes in the rate of nonradiative decay of the excited state. Although significant controversy exists as to the exact mechanism for nonradiative decay in RhB, it is now generally accepted that it occurs by pyramidilization and rotation of the pendant amine groups (see Figure 1).[44] Such motion occurs on a very fast time scale ($\approx 10^{-14}$ s) and is not directly observed here. However, the extent to which amine group motion occurs is highly solvent dependent, as indicated in numerous studies of the solvent-dependent fluorescence quantum yield for RhB.[42−44] The acid and base forms of RhB are reported to have quantum yields of 0.24 and 0.31 in water, respectively.[44] In ethanol, the quantum yields have been measured to be 0.53 and 0.70, respectively.[44] It is hypothesized that time-dependent variations in the solvation shell surrounding a molecule will lead to dramatic time-dependent variations in the quantum yield and, hence, the dramatic fluctuations observed. Indeed, as evidenced by the data presented in Figures 4 and 5, RhB entrapped in the more polar silicate spends significantly more time in weakly fluorescent or nonfluorescent states than it does when entrapped in the nonpolar polymer. Hence, the RhB molecules have a lower (time-averaged) quantum yield in the silicate, consistent with the previously published solvent dependence.[44] Additional confirmation that quantum yield variations contribute to the fluctuations observed is obtained from the studies of Rh101 in the silicate materials.[46] Again, this molecule is believed to have a quantum yield near one because amine group motions are restricted.[46] The fluorescence signal fluctuations are still observed for this molecule but are somewhat less prevalent than for RhB. Experiments in which the level of hydration in the film is varied may yield valuable proof of the above hypothesis; these experiments are presently underway.

The sensitivity of the intersystem crossing rate to the local environment is also a likely cause of the differences in switching rates observed for different molecules. The discrete jumps to the background level that are observed to occur in the silicate glass most definitely result from the formation of a "dark" (nonfluorescent) state, which may be a triplet state. If such a dark state is long-lived (i.e., >40 ms), the signal level will drop to the background level until the molecule returns to the ground state. Again, this process has been used in the past to explain the switching behavior observed in different samples.[28,39] Here, the time scale between jumps (seconds) suggests a triplet yield in the $10^{-5}-10^{-6}$ range. Previous measurements of this parameter, however, place it at $\approx 10^{-3}$ for RhB.[47] This discrepancy is attributable to the sensitivity of intersystem crossing rates to the surrounding environment. Similar dye molecules exhibit solvent-dependent intersystem crossing rates because of the close proximity and different solvent dependencies of $n-\pi^*$ and $\pi-\pi^*$ transitions.[48,49] The energies of these states are influenced differently by different solvent environments, leading to large differences in the intersystem crossing yield for different solvents.[49] This could also explain the distinct molecule-to-molecule differences in the observed switching behavior. The RhB molecules entrapped in the more polar

environments therefore may show greater switching due to a greater intersystem crossing rate, while those in less polar environments show less switching. Alternatively, entrapped O_2 may play a role. However, experiments performed in N_2 and O_2 environments showed no distinguishable differences in switching behavior.

The triplet lifetime for RhB is also sensitive to local environment and has been measured to be 1.6 μs in aqueous and alcohol solutions,[47] a value too short to observe here. The fact that long-lived dark states are observed may be indicative of the environmental dependence of this phenomena. It is well-known that triplet lifetimes (and phosphorescence quantum yields) are dramatically lengthened when a molecule is trapped within a solid matrix or similar environment. Therefore, RhB molecules showing long-lived (seconds) dark states are believed to reside in small, enclosed pores, while those showing short-lived dark states ($\approx$40 ms) exist in more open pores.

V. Conclusions

In summary, we have presented the results of single-molecule studies of sol−gel-derived silicate films and organic polymer films. It was shown that the static and time-resolved photophysical properties of the single molecules employed are sensitive to the local, molecular-scale environment in which they are entrapped. The studies revealed the silicate materials to be highly inhomogeneous in comparison to the polymer with many chemically and physically distinct environments present. In the future, it is likely that detailed single-molecule studies will lead to a better, more quantitative understanding of the molecular-scale environments present in these and other materials. It may be possible to directly probe properties such as local matrix polarity, pore size and interconnectivity, solvent incorporation, and solvent composition by these methods. Time-dependent variations in the local environment may also be probed. Such studies are extremely important from a materials perspective because they allow for the entire distribution of individual sites found within such materials to be directly probed. While previous studies have identified different classes of sites within these materials, the present work represents the first studies of individual molecular-scale sites. The results will be useful to those developing silicate materials for device applications because they clearly demonstrate the high degree of inhomogeneity found in silicate thin films. These effects must be taken into account when developing devices from such materials because they may lead to dramatic variations in the mechanical and chemical stability of these materials, as well as the chemical reactivity of guest molecules doped within.

Acknowledgment. D.A.H. and A.M.B. acknowledge the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this work. Also acknowledged are the NSF CAREER Award Program and Kansas State University (startup funds). M.M.C. and H.W. acknowledge the Office of Naval Research for its support of this work.

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