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Determination of Critical Micelle Concentration by Hyper-Rayleigh Scattering

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Abstract: The critical micelle concentration (CMC) of several surfactants that contain an NLO chromophore, either at the hydrocarbon tail, or at the hydrophilic headgroup, or even as a counterion, was determined by hyper-Rayleigh scattering (HRS). In all cases, the HRS signal exhibited a similar variation with surfactant concentration, wherein the CMC is inferred from a rather unprecedented drop in the signal intensity. This drop is attributed to the formation of small pre-micellar aggregates, whose concentrations become negligible above CMC. In addition, a probe molecule, which upon protonation yielded a species with significantly enhanced HRS intensity, was developed and its utility for the determination of the CMC of simple fatty acids was demonstrated.

Introduction

Critical micelle concentration (CMC) of surfactants is readily determined by a variety of simple techniques, wherein an appropriate property such as osmotic pressure, ionic conductance, scattered light intensity etc., is monitored as a function of concentration. A break in the plot of the chosen property versus concentration often signifies the formation of micellar aggregates.[1] Most of these measurements rely on the change in the property due to the formation of large aggregates of the amphiphile molecules (typically, about 50−100 molecules) above CMC. Any further addition of amphiphiles to the solution, it is expected, will not alter the concentration of free surfactants in solution but will only lead to an increase in the number of such micellar aggregates. Because the aggregates and free species contribute to different extents toward the measured property, an abrupt change of slope occurs at the CMC. Both the size and the shape of these surfactant aggregates are known to depend on a variety of factors such as the surfactant and salt concentrations, temperature, etc. However, only a few methods, such as dynamic light scattering and SAXS, have been utilized directly to probe such size and shape changes.

Hyper-Rayleigh scattering[2] (HRS) has been shown to be an effective tool to examine spatially correlated NLO chromophores, which can lead to either a reduction or enhancement of their hyperpolarizability, β.[3] Rigidly tethering the NLO chromophores onto a helical polymer backbone, such that the chromophores make a fixed angle ($\neq 90°$) with respect to the helical axis has been shown to dramatically enhance the β value.[4] On the other hand, linking them to the periphery of a dendrimer was shown to cause an effective lowering of β.[5] In the former case, there is a cumulative addition of the individual chromophoric dipoles leading to a large net dipole along the helical axis, which in turn results in a large coherent second harmonic response. Although in the latter, it is due to the near spherical symmetry attained by the higher generation dendrimers causing just the opposite effect on the β value. More recently, significant enhancement in β has been realized in dendritic wedges in which the NLO chromophores are incorporated between the branching junctions.[6] Other examples in which the spatial fixation of the relative orientation of the chromophores has been exploited to modulate the net β value, include those in which the chromophores are linked to a calixarene skeleton[7] or other rigid molecular frameworks.[8] The formation of supramolecular H-bonded assemblies in solution has also been studied using HRS.[9]

HRS is forbidden in a centrosymmetric structure, and for a perfect spherically symmetric arrangement of NLO chromophores, it is expected, that β would be zero in the electric dipole approximation. However, any deviation from the perfectly spherical arrangement of molecular dipoles would lead to second

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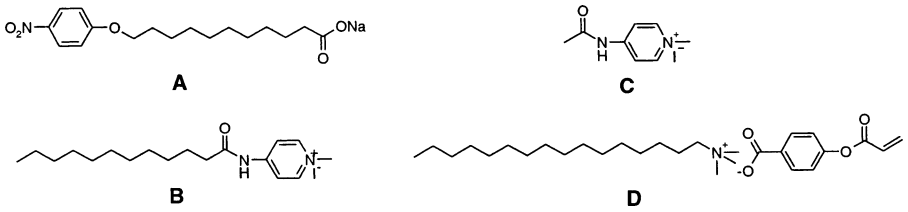
Scheme 1



harmonic scattering. Thus, detection of second harmonic scattered light could serve as a sensitive probe of structural organization. The application of this technique to micelle formation can add valuable insight into the structural properties of micelles. With this hypothesis, we decided to examine whether the formation of micellar aggregates is detectable by HRS in solution. Several surfactants that incorporate a NLO chromophore were designed, synthesized and their aggregation properties studied by HRS.

Results

The structures of different surfactants examined in this study are shown in Scheme 1.

Compound **A**, is an anionic amphiphile in which the *p*-nitrophenoxy-chromophore (a Donor−Acceptor NLO chromophore) is incorporated at the end of the hydrocarbon tail, while in **B** the NLO chromophore forms a part of the hydrophilic headgroup. Surfactant **A** was synthesized by coupling *p*-nitrophenol with 11-bromoundecenylic acid, ethyl ester followed by hydrolysis, while **B** was prepared by reacting lauroyl chloride with 4-aminopyridine followed by quarternization with methyl iodide. The model chromophore **C** was made similarly from 4-aminopyridine, using acetyl chloride instead of lauroyl chloride. Surfactant **D**, on the other hand, was synthesized by ion-exchange of the bromide counterion in cetyl trimethylammonium bromide (CTAB) with the appropriate benzoate.[10] The structures of all the surfactants were confirmed by their ^{1}H NMR spectra.

Hyper-Rayleigh Scattering. Hyper-Rayleigh scattering (HRS) experiment is based on a two-photon scattering process, in which the intensity of the second harmonic scattered light ($I_{2\omega}$), generated by focusing an intense laser beam (I_ω) on to an isotropic solution, is measured.[11] The intensity of the incoherently scattered second harmonic light depends on the different components of the molecular first hyperpolarizability tensor β. The intensity of the second harmonic signal in an HRS experiment is generally given as

$$I_{2\omega} = gN\langle\beta^2\rangle I^2_\omega$$

where N is the number density, $\langle\ \rangle$ indicates orientational averaging, and the factor 'g' clubs the scattering geometry and instrumental factors. For a system in which solute molecules are dissolved in a solvent

$$I_{2\omega}/I_\omega{}^2 = G[N_{\mathrm{sol}}\langle\beta^2\rangle_{\mathrm{sol}} + N_{\mathrm{solv}}\langle\beta^2\rangle_{\mathrm{solv}}]$$

Generally, for the measurement of first hyperpolarizability (β) of a molecule the external reference method[12] is used. In this method, the second harmonic signal from the sample and from a known reference compound (for example, para-nitroaniline) in the same solvent is monitored, and $I_{2\omega}$ is plotted versus concentration, which under normal circumstances yield straight lines. From the known β of the reference compound, the β value of the unknown is determined from the ratio of the slopes of the two straight lines. In other words, a steeper straight line for the unknown compared to that of the reference implies a higher value of β of the former. In the $I_{2\omega}$ vs concentration plot, therefore, the slope of the line is a qualitative measure of the first hyperpolarizability of the solute.

In the present study, we directly monitor $I_{2\omega}$ as a function of concentration − the slopes prior to and after micellization would reflect the β values of the free surfactants and that when incorporated in micellar aggregates, respectively. A plot of $I_{2\omega}$ versus concentration of surfactant **A** is shown in Figure 1a. It is apparent that the variation of the HRS intensity does not follow the simple linear variation one might expect for nonassociating chromophores. The CMC of **A** was determined independently from ionic conductance measurements and was found to be 5.8 mM, which is close to the concentration at which one sees a sudden drop in the HRS intensity. It is possible that the micellization of surfactant **A**, which causes a clustering of the chromophores in the hydrophobic core of the micelle, could lead to a variation of the local dielectric thereby leading to some variation in the HRS signal. To preclude such effects, a second surfactant **B**, in which the chromophores, upon micellization, would be located at the hydrophilic periphery of the micellar aggregate, was examined.

From Figure 1b, it is clear that the variation in the HRS intensity of **B** occurs in much the same way as seen in the case of surfactant **A**.[13] The CMC values of the different surfactants determined by ionic conductance measurements are compared with those determined by HRS in Table 1. It is clear that the first break in the curve—the point where the HRS intensity begins to fall, occurs at the CMC. Interestingly, in both cases, the fall in the HRS intensity at CMC is followed by an increase at some higher concentration.

To further confirm that aggregation was the reason for the observed variation, a model chromophore **C**, which is similar to surfactant **B** but does not possess the long hydrocarbon tail, was examined. The variation of the HRS intensity (Figure 1c) in this case was seen to follow the expected linear behavior with concentration, as in the case of nonaggregating chromophores. This confirms that the first deviation from linearity in the HRS plot is indeed reflective of the onset of micellization.

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(13) The variation in the UV−vis spectra of the surfactant B as a function of concentration, spanning the range above and below CMC, followed the expected Beer−Lambert's law confirming the absence of any specific ground-state interactions between the chromophores above CMC.

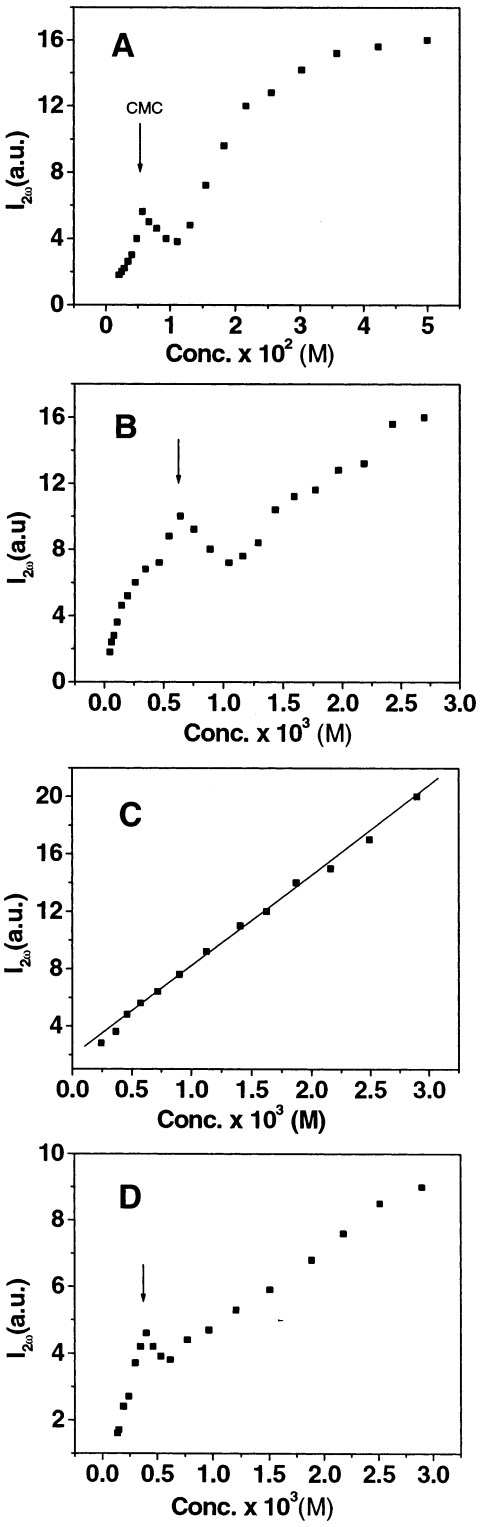


Figure 1. Variation of HRS signal intensity as a function of concentration for molecules A, B, C, and D.

Chromophore as the Counterion. Another surfactant **D**,[10] wherein the NLO chromophore (4-acryloyloxy benzoate) is the counterion, was also examined using HRS. Figure 1d, shows that such a surfactant too exhibits a similar trend showing a fall in HRS intensity at CMC. Thus, it is evident that even the clustering of the NLO chromophores in a nearly spherically symmetric fashion around the aggregated hydrophobic tails of the surfactant causes a drop in the HRS intensity.

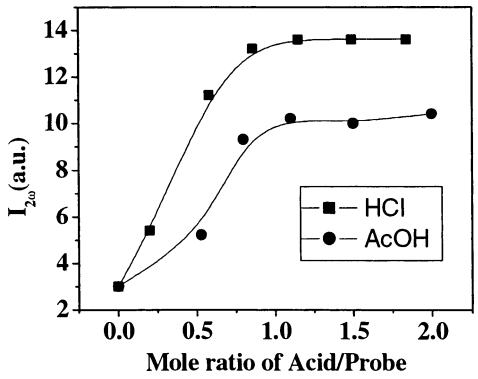
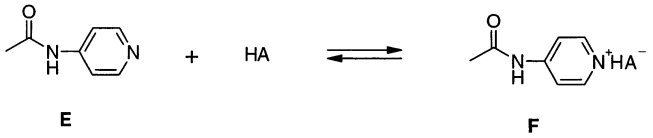


Figure 2. Variation of HRS signal intensity as a function of acid composition.

Table 1. CMC Values Determined by HRS and Ionic Conductance Measurements

surfactant	C M C by conductance	C M C by HRS measurement
A	5.8×10^{-3}	5.7×10^{-3}
B	6.4×10^{-4}	6.2×10^{-4}
D	3.4×10^{-4}	3.9×10^{-4}

Scheme 2



An NLO Probe for CMC Determination. In an effort to further extend this HRS approach for CMC determination, we designed a probe molecule E (Scheme 2) that can detect the micellization of long-chain fatty acids. The probe is designed such that it can be protonated in the presence of an acid to yield a salt F that would have a significantly higher β value. In E, the protonation of the pyridine ring makes it a strong electron withdrawing group resulting in an enhancement of its β.

Figure 2 shows the variation in the HRS signal when the probe molecule is titrated with an acid − both HCl and acetic acid show a saturation in the intensity at an acid:probe ratio of ca. 1:1. A 3−5-fold enhancement in the signal intensity is noticed upon protonation. The absolute value of the signal is higher in the case of HCl, probably reflecting the higher concentration of the protonated form. To test the feasibility of using E as a probe for CMC studies, it was taken along with nonanoic acid in three different acid:probe mole ratios − 1.7:1, 2.4:1, and 3:1. Maintaining these mole ratios, the HRS signal intensity was measured as a function of dilution, and these are plotted in Figure 3. It is clear that the general behavior is similar to the earlier observations, and the CMC of the fatty acid-salt is in the range: 1.8−3.9 mM. At a low acid-to-probe ratio, the CMC is apparently slightly higher but the difference is minimal beyond a ratio of 2.4:1. This is again a reflection of the acid−base equilibrium constant, which approaches a limiting value at high acid concentrations.

Discussion

The most widely accepted rationalization of the micellization process is one where the concentration of the free surfactant remains nearly constant after the onset of micellization—implying that any further addition of surfactant leads only to

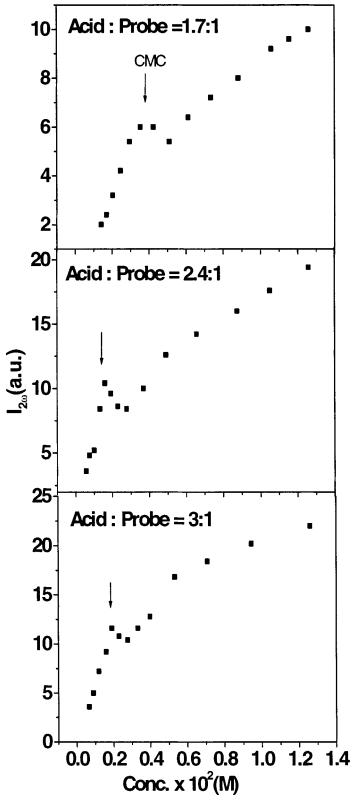


Figure 3. Variation of HRS signal intensity as a function of concentration at different acid:probe ratios.

the generation of more micellar aggregates.[1] This implies that the free surfactant concentration $[S]_{free}$ at any point above CMC is equal to CMC that is

$$[S]_{free} = \text{CMC}$$

This being the case, the slope of the variation of any given property (whose intrinsic value per molecule is lower for the micelle than the free species) as a function of concentration may be expected to decrease after micellization. Even if the micelles do not contribute at all toward the measured property, one might at best expect the value to plateau beyond CMC.

However, our observations show a lowering of the HRS intensity upon micellization, up to a concentration of about 2 times the CMC, before it begins to increase again. This is a rather unprecedented observation, and therefore, we carried out a few control experiments to preclude trivial effects due to simple scattering, etc. In the first control experiment, the HRS signal of a simple chromophore, *p*-nitrophenol (PNP), was monitored in the presence of increasing concentrations of a standard surfactant—cetyl trimethylammonium bromide, CTAB. It is important to note that the primary source of the HRS signal in this experiment is the PNP, which is assumed to be isotropically distributed in the medium, while the micellization of the relatively "HRS-silent" CTAB creates an increasingly scattering medium. The fact that no variation in the SH signal intensity is seen as one traverses through the CMC of CTAB (see Figure 4) clearly demonstrates that trivial scattering effects do not contribute to the drop in the HRS intensity. If linear Rayleigh scattering due to the formation of micellar aggregates were to affect the HRS signal from the chromophore, then we should see a decrease in the HRS intensity from PNP, above

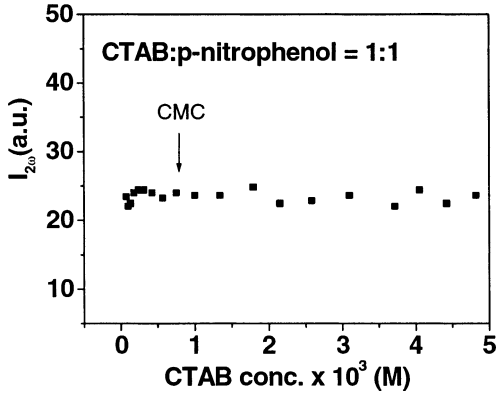


Figure 4. Variation of HRS signal intensity as a function of CTAB concentration at constant *p*-nitrophenol concentration. The arrow indicates the CMC of this mixture.

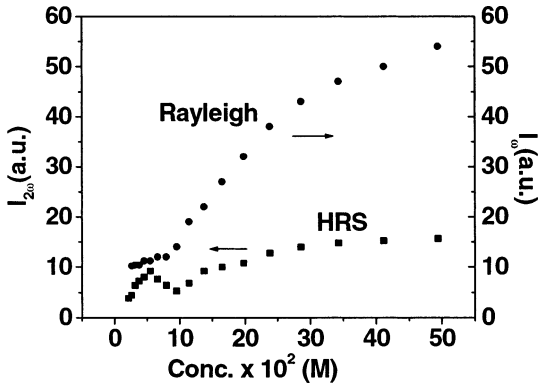


Figure 5. Comparative variation of Rayleigh scattering and HRS signal intensity as a function of concentration of surfactant A.

the CMC of CTAB.[14] The CMC of CTAB in the presence of PNP was independently measured by conductance method and was found to be 0.84 mM. In yet another experiment, both the linear Rayleigh (I_ω) and the hyper-Rayleigh ($I_{2\omega}$) scattering intensities were monitored sequentially from the same sample as a function of dilution. This was done by the use of a series of neutral density filters for measurement of the linear Rayleigh scattering. Figure 5 shows one such representative measurement carried out for surfactant **A**. The expected increase in the Rayleigh scattering intensity after CMC is seen, which is distinctly different when compared to the unusual drop in the HRS intensity. It is, however, apparent that the CMC as detected by the linear Rayleigh scattering is slightly higher than that seen by HRS. It is known that CMC values do vary a little depending on the method employed for its determination.[1] These control experiments confirm that this unexpected variation of the HRS signal is indeed a direct consequence of the near spherically symmetric assembly of the chromophoric units upon micellization.

One plausible explanation for this observation is the formation of smaller pre-micellar aggregates (such as dimers or trimers) in which the chromophoric dipoles could effectively add up leading to a higher effective β value (per chromophore).[15] Above CMC, however, the concentration of such smaller aggregates

(14) As pointed out by one of the reviewers, the PNP could be partitioned into the hydrophobic interior of the CTAB micelles, once formed, and this could result in a change in the HRS signal. We exclude this factor, as its contribution, if any, appears to be minimal as seen from the almost invariant HRS intensity as one traverses through the CMC of CTAB.

may become negligible due to their inclusion into the micelles, thus causing the actual concentration of free surfactants to go down. Under such circumstances one would see a drop in the HRS signal at CMC, which reflects the loss of the smaller aggregates that possess a higher effective β value (per chromophore) compared to the micelles. But as the concentration of micellar aggregates increase the contribution due to these larger aggregates begin to dominate and the HRS signal increases again. The fact that the HRS signal continues to increase at higher concentrations is a clear indication that the contribution from micellar aggregates is nonzero, although the relative slopes before and after, suggest that the effective β value (per chromophore) of the micellar aggregates is indeed lower.

Conclusions

In summary, we have shown that the CMC of surfactants that incorporate a NLO chromophore, either at their tail end, or at their headgroup or even as a counterion, can be readily determined by HRS. The CMC is detected by a rather unprecedented drop in the HRS signal, which we believe could be due to the formation of smaller pre-micellar aggregates whose effective β value is higher than that of the micellar aggregates. It is postulated that the concentration of these pre-micellar aggregates becomes negligible beyond CMC, thus explaining the observed decrease in HRS intensity. At higher concentrations, the contribution from the increasing numbers of micelles dominates causing the HRS intensity to increase again (a simple concentration effect). One advantage of the HRS technique is that the CMC is inferred by a change in the sign of the slope (which appears as a peak) as opposed to, a simple change of slope (sometimes a very small one) typically seen in most of the standard approaches. This makes the detection of CMC using HRS more precise. Furthermore, the sensitivity of this approach to the shape of the micellar aggregate provides an added advantage—for instance, formation of elongated or cylindrical micelles at higher concentrations may be detected.

An NLO chromophoric probe, whose β value increases severalfold upon protonation, has also been developed. This probe was utilized to measure the CMC of a simple long chain fatty acid, which formed a salt with the probe. An interesting feature of this probe is that it can be utilized to study the formation of well-defined secondary structures in macromolecules that have pendant acid functionality. Formation of various types of structures, such as a helix, may be readily detected by HRS using such probes. They could also be used to investigate solvent and temperature-induced conformational transitions in suitable designed polymer systems. Studies along these lines are currently underway and will be reported in the future.

Experimental Section

HRS Measurements. The apparatus used for the HRS measurements has been described in detail elsewhere.[9] In brief, the fundamental (1064 nm) of a Q-switched Nd:YAG laser was focused by a planoconvex lens (f.l. 22 cm) to a spot 5 cm away after passing through a glass cell containing the sample solution. The scattered light in the perpendicular direction was collected, through a set of condensing optics and broadband as well as narrow band filters, on a UV−vis photomultiplier tube (PMT) and averaged in a digital storage oscilloscope. Each data point was averaged over 400 shots. The input power was monitored using a power meter. All data were collected at laser powers $\leq$12mJ/pulse which is below the threshold for stimulated Raman, self-focusing/self-defocusing, Brillouin scattering and dielectric breakdown.

CMC Determination using HRS. A typical procedure is provided here. 113 mg of the cationic surfactant **B**, was dissolved in 100-mL double distilled water to make a 2.7 mM solution. 20-mL of this stock solution was taken for the HRS measurement, and it was diluted in steps by replacing a fixed volume (typically, 2−3 mL) of the solution with the same volume of distilled water, using a micropipet, till the final concentration was well below the CMC (as determined by conductivity measurement). At each concentration, the $I_{2\omega}$ value was measured and was plotted against the concentration to determine the CMC. A similar dilution procedure was followed for the ionic conductivity measurements also. In the case of nonanoic acid, an aqueous suspension of a weighed amount of the acid was taken along with the probe molecule **E** (in the appropriate acid:probe ratio) and sonicated to generate a homogeneous solution of the resulting salt. This solution was diluted in steps with distilled water as described above. At each concentration, the $I_{2\omega}$ was measured and was plotted against the concentration to determine the CMC.

Control Experiment using PNP and CTAB. A 4.83 mM solution of CTAB was prepared using a 10 mM aqueous solution of *p*-nitrophenol. 12 mL of this solution was taken for HRS measurement, and it was diluted in steps by replacing a fixed volume (typically, 1−3 mL) of the solution with the same volume of a 10 mM solution of *p*-nitrophenol (using a micropipet) till the final concentration was well below the CMC of the CTAB-*p*-nitrophenol mixture (as determined by conductivity measurement). This ensured that the concentration of the *p*-nitrophenol remained unchanged after each dilution step. The $I_{2\omega}$ value was measured after each dilution, and plotted against the concentration of CTAB.

HRS Measurements using Mixtures of the Probe and an Acid. Typically, 500 μL of the probe solution (74.6 mM in MeOH) was taken along with varying volumes (250−1600 μL) of the aqueous HCl solution (43 mM) and the total volume was made up to 12 mL with water, for the HRS measurement. In this way, the $I_{2\omega}$ values were measured maintaining a fixed concentration of the probe, whereas the acid:probe ratio was varied. A similar procedure was followed using acetic acid also. Plots of $I_{2\omega}$ versus acid:probe ratio are shown in Figure 2.

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Supporting Information Available: Experimental details for the synthesis of the various surfactants, the model compound, and the probe molecule, along with their 1H-NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) It is not essential that the effective β value (per molecule) of the smaller aggregates be higher than that of the free species. But for this kind of behavior to be seen, this value should certainly be higher than that of the β -value (per molecule) of the micellar aggregates.