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Large Assembly Formation via a Two-Step Process in a Chromonic Liquid Crystal

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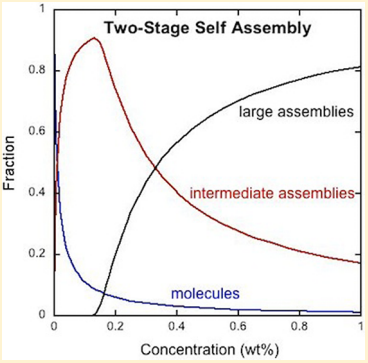
ABSTRACT: IR-806 is a near-infrared cyanine dye that forms assemblies in aqueous solutions which in turn orientationally order into a liquid crystal phase at concentrations as low as 0.5 wt %. Unlike many chromonic liquid crystals, the absorption spectrum of IR-806 changes dramatically with concentration, showing an isodesmic assembly process at lower concentrations followed by a second process at higher concentrations that is not isodesmic. The lower concentration assembly process is characterized by a free energy change per molecule of about 9 k_BT, not unlike other chromonic systems. However, X-ray scattering measurements suggest that the assemblies that form during the higher concentration process are much larger than what is observed for many chromonic liquid crystals. Although there is a transitional region between the liquid crystal and isotropic phases of 10−15 °C, unlike most chromonic liquid crystals, no biphasic region is observed using polarizing microscopy.



INTRODUCTION

The last five years or so has seen increasing interest in chromonic liquid crystals for good reasons. Chromonic liquid crystals possess a nematic phase, which is the phase of thermotropic liquid crystals that has been exploited so successfully for optical applications, including displays. Unlike thermotropic liquid crystals, however, chromonic liquid crystals are aqueous solutions, opening up many new avenues for applications, especially in biology and medicine.[1,2] In addition, the molecules in chromonic liquid crystals spontaneously assemble, a process ubiquitous in living systems. Since dyes, drugs, and nucleic acids comprise most of the molecules forming chromonic liquid crystals, understanding the assembly process in these systems has ramifications throughout biological science. There have been several excellent reviews of what is known about chromonic liquid crystals, among them two recent ones.[3,4]

Chromonic liquid crystals sometimes result when molecules spontaneously form anisotropic assemblies in solution. When the anisotropy and concentration of these assemblies are high enough, the assemblies usually order into a nematic liquid crystal phase with orientational order only, typically followed by a hexagonal phase with both orientational and positional order at even higher concentration. The basic assembly mechanism is a stacking of the molecules, driven by attractive interactions and solvent effects. There is evidence that the process is often isodesmic, meaning that the free energy change when a molecule joins an assembly is independent of the size of the assembly.[5−9] This is only approximately true; for example, there are indications that the free energy change when two molecules stack together differs from the free energy change when a molecule is added to a larger aggregate.[10−12]

X-ray scattering measurements have shown that, for some chromonic liquid crystals, the assemblies are simple stacks of molecules with one or two molecules in the cross section of the assembly. This work has been recently summarized.[13] However, there is also evidence that, for some chromonic liquid crystals, the assemblies are more complicated and larger. Such a cyanine dye was studied by Harrison, Mateer, and Tiddy in 1996,[14,15] and benzopurpurin 4B and pinacyanol acetate have been investigated recently.[9,16] Only for pinacyanol acetate were the data sufficient to indicate a possible structure, in this case a hollow cylinder roughly 4.6 nm in diameter with water inside.[16] Given the large number of possible structures that can be formed by molecules with a tendency to stack, it is likely that other assembly structures form and orient into a liquid crystal phase. Investigating such systems is therefore an important direction for future work in this area.

The use of the term assembly instead of aggregation is intentional. Researchers in protein chemistry sometimes distinguish between the two. The term assembly refers to thermodynamically reversible phenomena and properties that can be characterized by equilibrium. On the other hand, aggregation is used to refer to irreversible self-association processes, usually leading to structures that vary significantly and are not characterized by equilibrium conditions.[17] Since the former describes the processes leading to chromonic liquid crystals much more closely than the latter, it is the more appropriate term to use.

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Most of the chromonic liquid crystals that have been investigated absorb in the visible part of the spectrum. One exception is disodium cromoglycate that absorbs in the ultraviolet. Infrared absorbing dyes have not been the subject of much experimentation at all, yet some of these are very important in medical imaging. For example, the near-infrared cyanine dye IR-806 (structure shown in Figure 3) is used as a contrast agent in some biomedical imaging techniques.[18−20] However, there is a reference to it forming a chromonic liquid crystal at very low concentrations.[21] Hypothesizing that the ability to form an ordered phase at very low concentrations indicates a more complex assembly structure, an investigation of the assembly process and liquid crystal properties of IR-806 was initiated. This investigation utilized polarizing microscopy, laser transmission measurements, absorption spectroscopy, and X-ray scattering.

The results of this investigation demonstrate that the assembly process in IR-806 is quite interesting. While it forms a chromonic liquid crystal phase at concentrations below 1 wt % at room temperature like benzopurpurin 4B and pinacyanol acetate, absorption spectroscopy measurements show that the assembly process leading to the liquid crystal phase proceeds in two steps. The first is an isodesmic assembly process over concentrations up to 0.1 wt %, and the second is a more abrupt assembly process that begins around 0.2 wt %. The free energy change for the isodesmic assembly process is about 9 k_BT, much like other chromonic systems. However, the X-ray scattering measurements provide evidence that the assemblies are much larger than stacks of one or two molecules, and are very similar to what is observed in pinacyanol acetate for which the structure is thought to be a hollow cylinder. Finally, unlike many chromonic liquid crystals, in a polarizing microscope, IR-806 does not show a biphasic region at the liquid crystal to isotropic liquid transition. Instead, the birefringence of the liquid crystal phase simply disappears gradually over a temperature interval about 10−15 °C wide.

SIMPLE THEORIES OF ASSEMBLY

There has been considerable theoretical work attempting to catch the fundamental aspects of the spontaneous assembly of molecules into linear structures in solution. All include the competition between the energy considerations that drive assembly and the entropy considerations that favor disassembly. Two review articles summarize the two equivalent approaches that are used as the starting point for many researchers investigating assembly in solution. The first approach models the assembly as chemical reactions between an assembly of i molecules and a single molecule to form an assembly of $i + 1$ molecules. In principle, the reaction for each size assembly can have a different equilibrium constant.[22]

$$\begin{aligned} A + A &\rightleftharpoons A_2 \qquad K_2 = [A_2]/[A]^2 \\ A_2 + A &\rightleftharpoons A_3 \qquad K_3 = [A_3]/[A_2][A] \\ &\cdots \qquad\qquad \cdots \\ A_{i-1} + A &\rightleftharpoons A_i \quad K_i = [A_i]/[A_{i-1}][A] \end{aligned} \tag{1}$$

Here A represents a single molecule, A_i represents an assembly of i molecules, and K_i is the equilibrium constant for the reaction forming an assembly of i molecules. When all the equilibrium constants are set equal to a single value K_E,

$$K_E = K_2 = K_3 = K_4 = \ldots = K_i \tag{2}$$

then the process is called isodesmic assembly. A slightly more complicated process is when the equilibrium constant for the formation of an assembly of two molecules is different from all the other equilibrium constants

$$K_E = K_2/\rho = K_3 = K_4 = \ldots = K_i \tag{3}$$

where $\rho > 1$ and $\rho < 1$ represent the cases in which the assembly of two molecules forms more or less easily than other sized assemblies, respectively. The case in which there is a nucleation process can be modeled with $\rho \ll 1$.

The second approach recognizes that there are energy considerations for molecules at different locations within an assembly. For an assembly of i molecules, the free energy difference relative to i free molecules, $\Delta\mu_i/(k_BT)$, contains a negative term for all molecules inside the assembly and a positive term representing molecules at the two ends of the assembly

$$\frac{\Delta\mu_i}{k_BT} = -i\alpha + \delta = -(i-1)\alpha + (\delta - \alpha) \tag{4}$$

where α and δ are free energy parameters in units of k_BT. The parameter α represents the "stacking free energy change" and $\delta - \alpha$ represents the "scission free energy" (the free energy difference between the two ends of an assembly in solution relative to the two sides of a molecule in solution). In order for this theory to describe systems such as assembling dyes, δ must be greater than or equal to α.[23] This expression for the free energy is used in the partition function and equilibrium is assumed by equating the free energy per molecule for different sized assemblies. The case in which $\alpha = \delta$ represents isodesmic assembly and a nucleation process can be modeled by $\delta \gg \alpha$.

These two approaches give identical results if the volume of phase space appearing in the partition function is equal to the molecular volume. In fact, the parameters of one approach can be expressed in terms of the parameters of the other approach as follows

$$K_E = e^{\alpha} \qquad \rho = e^{-(\delta-\alpha)} \tag{5}$$

In the case of an isodesmic assembly process, assembly occurs at all concentrations (there is no critical assembly concentration), and the average assembly size increases with concentration. The distribution of assembly size is quite broad, with the width of the distribution on the same order as the average size. When $\rho \ll 1$ ($\delta \gg \alpha$), there is a critical assembly concentration at which molecules begin to form assemblies. Plots showing how the assembly process depends on concentration and the distributions of assembly size for various conditions can be found in the literature.[7,24,25] Figure 1 shows the fraction of molecules in different size assemblies for K_E = 60 wt %$^{-1}$ and a concentration of 0.14 wt %.

Isodesmic assembly should be contrasted to processes in which a structure is formed that requires a specific number of molecules. The best example of this is the formation of spherical micelles by amphiphilic molecules in water. In the chemical reaction model, the reaction and equilibrium constant are now

$$NA \rightleftharpoons A_N \qquad K = K_E^{N-1} = [A_N]/[A]^N \tag{6}$$

where N is the number of molecules in each assembly and K_E plays the same role as in isodesmic assembly. Calculating the fraction of single molecules in solution and the fraction of molecules in assemblies as a function of the total volume

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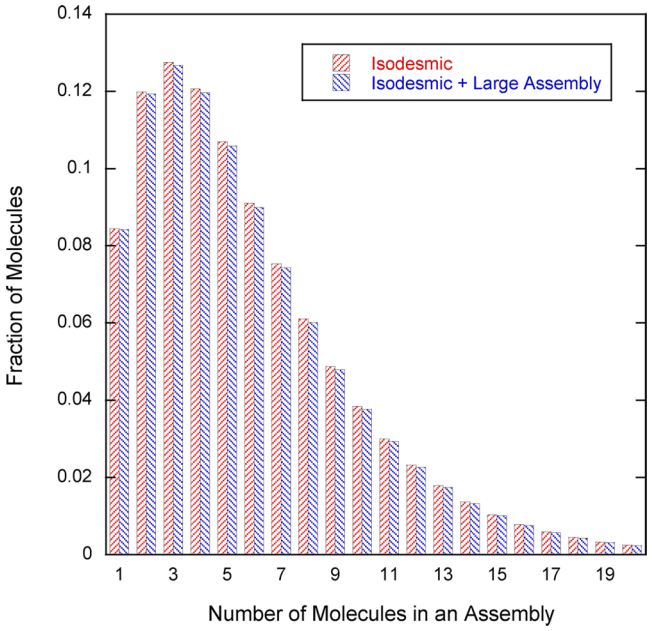

Figure 1. The fraction of molecules in assemblies of each size according to both an isodesmic model and an isodesmic model with the formation of large assemblies at higher concentration. For both models, K_E = 60 wt %$^{-1}$ and the concentration is 0.14 wt %. For the isodesmic model with the formation of large assemblies at higher concentrations, K_N = 1000 wt %$^{-1}$ and assemblies with 9 molecules or more form large assemblies.

fraction, one obtains the well-known behavior with a critical micelle concentration. As can be seen in Figure 2, even when N is small, a critical micelle concentration is evident by a fairly abrupt threshold for the formation of assemblies.

It is possible to combine these two types of assembly in a model with a two-step process in which an isodesmic process forms intermediate assemblies at lower concentrations together with the formation of large assemblies from a specific number of intermediate assemblies at higher concentrations. The set of equations to be solved simultaneously includes those given in eq 1 plus a set of equations resembling eq 6 in which N assemblies with J or more molecules can form large assemblies with an equilibrium constant K_N. This set of many equations can be solved numerically, revealing that the qualitative behavior of the solution changes very slightly when J is varied between 5 and 15. At low concentrations, there are few large assemblies and the distribution of assembly size is very similar to an isodesmic process. This is illustrated in Figure 1 for a specific value of J, where it can be seen that the fraction of molecules for each size assembly is slightly reduced. At concentrations lower than those shown in Figure 1, there is almost no difference in the distribution with and without large assemblies, because there are so few large assemblies. However, at higher concentrations, the large assemblies form in much the same way as in Figure 2, while the number of intermediate assemblies of all sizes decreases, with the total number of intermediate assemblies decreasing similarly to what is shown for single molecules in Figure 2.

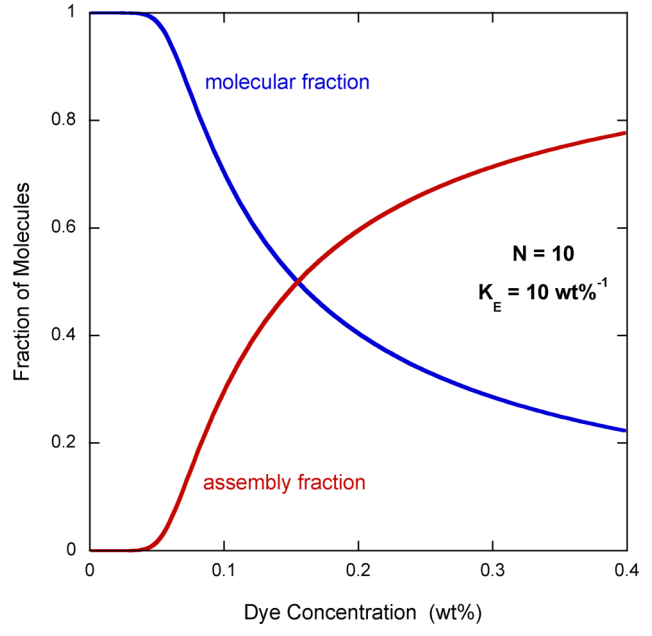

Figure 2. Nature of the assembly process when a specific number of molecules is necessary to form the assembly. Even when this number is small, N = 10 for this plot, an abrupt threshold for the formation of assemblies is apparent.

EXPERIMENTAL PROCEDURES AND RESULTS

Chemical Stability. In working with IR-806 solutions, it was noticed that the solutions lost their color over a period of time when stored in airtight vials. It also seemed that the lowest concentration solutions lost their color the fastest. In order to ascertain whether this was a photoinitiated process or not, identical solutions were divided with some stored in a dark chamber and others stored in room light. Absorption measurements on both sets of samples revealed no discernible difference. In order to obtain quantitative information on the degradation process, the absorption spectrum of buffered solutions with three different pH values was monitored as their color faded. The results of this investigation using a low concentration IR-806 sample are shown in Figure 3, where it is evident that the process is much more rapid in acidic solutions as compared to neutral or basic solutions. These data are important to the study of IR-806, because it means that experiments must be completed within hours of preparing the solutions, especially in the case of low concentration samples. This was the protocol followed throughout the investigation of IR-806.

In order to learn more about the degradation process, proton NMR spectra were obtained for a low concentration sample immediately after preparation and 3 weeks later. Because the concentration was low, the NMR spectra are quite noisy, even after a good deal of averaging. However, many NMR peaks are clearly present in both spectra. Most of the peaks showed modest changes in amplitude and splitting after aging, but a peak with a chemical shift of about −5.9 ppm that was present in the fresh sample is completely absent after 3 weeks. Finally, hypothesizing that oxygen might be involved in a reaction with IR-806, two low concentration pH 8 samples of IR-806 were prepared, but nitrogen was bubbled through one of them for 20 min before sealing the vial. After a month, the nitrogen-bubbled solution still retained its original color while the other sample had lost most of its color.

The tentative conclusion on the degradation process is that a reaction between dissolved oxygen and double-bonded carbons causes a loss of conjugation within the molecule and consequently a loss in color. Such reactions are not uncommon

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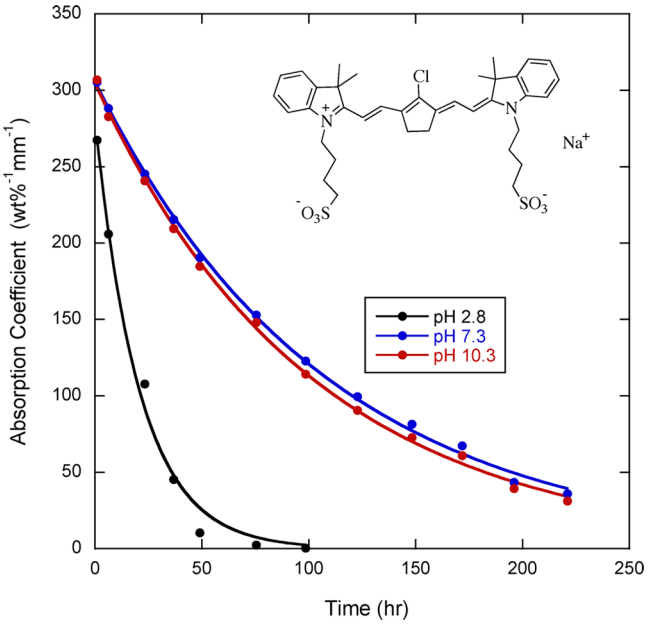

Figure 3. Maximum absorption coefficient vs time for a 0.0005 wt % sample of IR-806 in aqueous solutions of different pH at room temperature. The time constants are 21 ± 2, 108 ± 2, and 101 ± 2 h for the pH 2.8, 7.3, and 10.3 solutions, respectively. The structure of the IR-806 molecule is also shown.

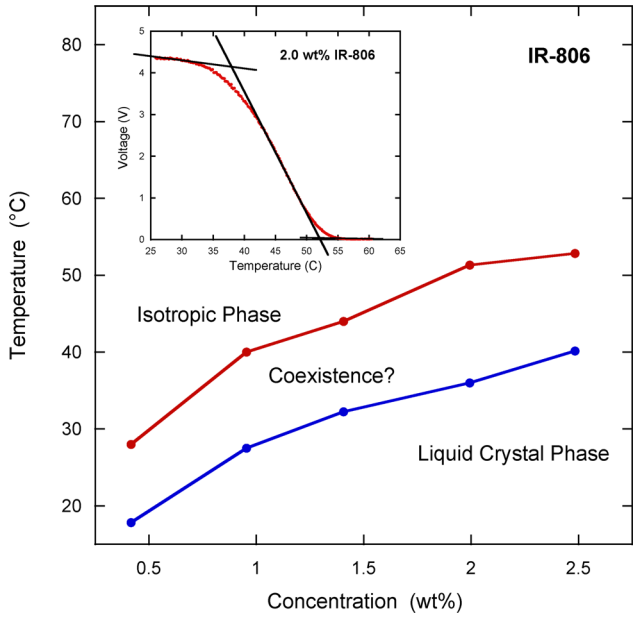

Figure 4. Phase diagram for IR-806. A typical trace of transmitted light as it depends on temperature is shown in the inset. Straight lines are used to describe the three regions, and the temperatures defined by the intersection of these lines are used for the phase diagram. Temperature scans were repeated two or three times, with the averages shown in the main plot.

in dyes. As an example, the cyanine dye studied by Harrison, Mateer, and Tiddy suffered irreversible hydrolytic degradation and experiments had to be performed within 72 h.[14]

Phase Diagram. When examining samples of IR-806 under a polarizing microscope, it is clear that at some concentrations and temperatures the solution is highly birefringent and at other concentrations and temperatures it is not. However, when heating a birefringent sample, the birefringence slowly decreases to zero without showing a biphasic region between the birefringent phase and the nonbirefringent phase. This is not a question of the heating being done too fast for domains of the two phases to form. When the temperature of a sample is held constant so that it remains in a state of intermediate birefringence for many hours, no changes occur to what is seen in the polarizing microscope. Likewise, centrifugation of a sample in an intermediate state of birefringence with subsequent sampling from the top and bottom of the centrifuge tube reveals no differences between the two samples under the polarizing microscope. This situation is very similar to what was reported for benzopurpurin 4B.[9] In addition, the birefringent texture in the polarizing microscope looks quite similar to the images of pinacyanol acetate.[16]

In order to investigate this more quantitatively, IR-806 samples of different concentrations were prepared and placed between crossed polarizers. Light from a HeNe laser passed through this arrangement, and its intensity was measured by a photodiode detector. The light from the laser was chopped so a lock-in amplifier could process the output from the detector. As the temperature of the sample was increased and the birefringence decreased to zero, the detector signal also decreased to zero. A typical trace is shown in the inset of Figure 4. Something resembling a phase diagram was constructed by modeling the dependence with three straight lines, as shown in the inset of Figure 4, and using the intersection of the straight lines to indicate the beginning and end of the change in birefringence. Figure 4 shows the result of this procedure, where the birefringent region is labeled the liquid crystal phase and the nonbirefringent region is labeled the isotropic phase. The intermediate region may be a coexistence region. This is discussed later.

Absorption. For most dyes that form chromonic liquid crystal phases, the UV−vis absorption spectrum does not change dramatically with concentration. If one measures the absorption coefficient as a function of increasing concentration, usually the spectrum grows slightly weaker and broader, often showing an indication of increased absorption in the high wavelength portion of the absorption band. There are counter examples; some are the cyanine dyes investigated by Harrison, Mateer, and Tiddy.[14,15] Only three spectra for one of the dyes are shown, but for all four dyes, the authors ascribe an absorption band to the molecule at low concentrations, a blue-shifted absorption band to an H-aggregate at intermediate concentrations, and a red-shifted absorption band to a J-aggregate at high concentrations. In the one spectrum shown, the molecular peak is a shoulder on the H-aggregate band. Another counter example is pinacyanol acetate, in which molecular absorption bands and a blue-shifted aggregate band are observed.[16]

Absorption measurements were obtained using a Hewlitt-Packard 8453 UV−vis spectrophotometer. The concentration of the samples ranged from 0.0005 to 1 wt %, and the path lengths were varied from 0.01 to 10 mm in order to keep the absorbance within the range of the spectrophotometer. The path length of the 0.1 mm commercial cuvette and the fabricated thinner cuvettes were measured by using potassium dichromate in HCl solution as a calibration sample or by measuring the path length of the empty cell optically.

The absorption coefficient spectra are shown in Figure 5, where three plots are used to show the evolution of the spectrum. At the lowest concentrations, the spectrum is

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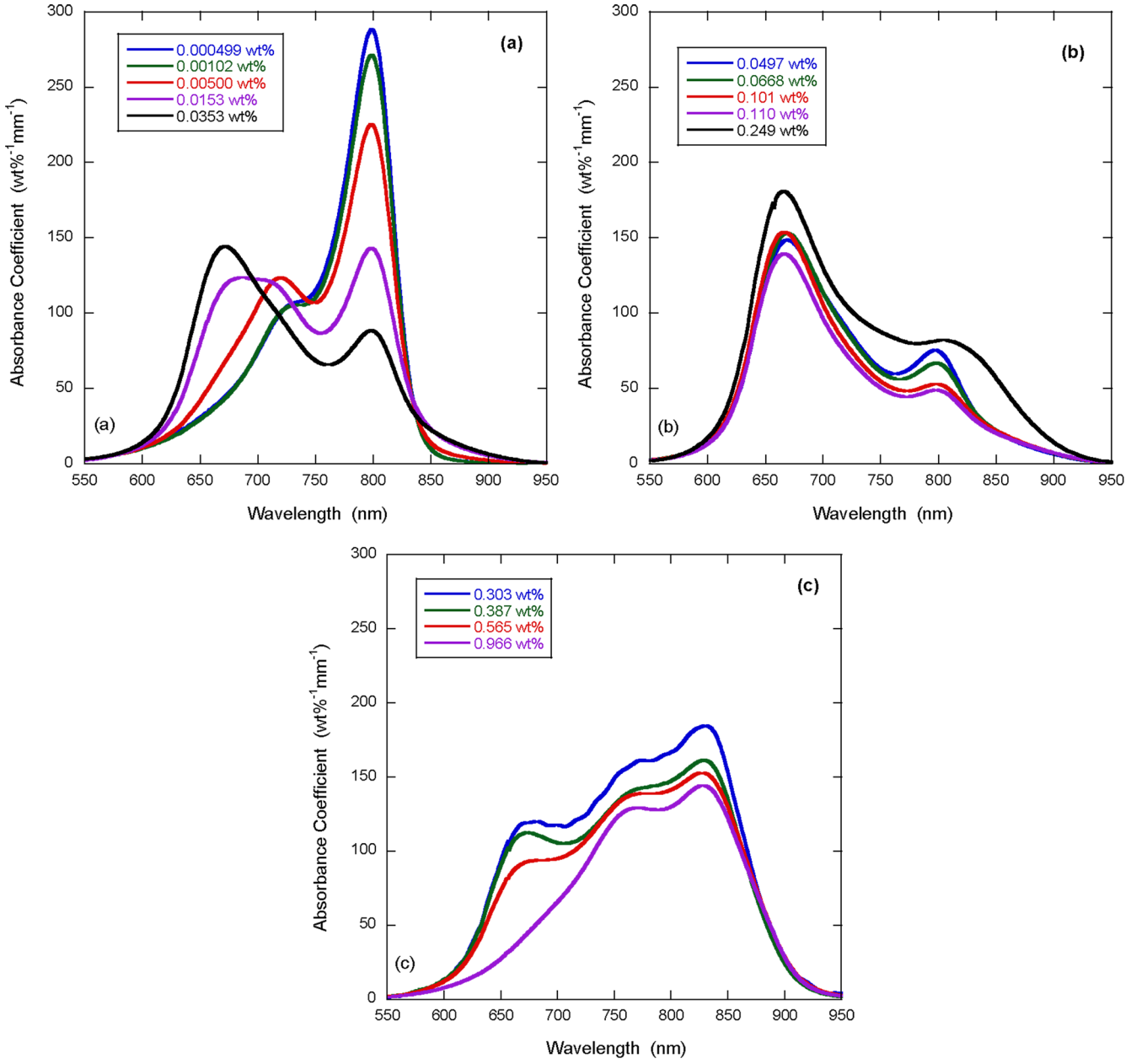

Figure 5. Absorption spectra of IR-806 for different concentrations at room temperature: (a) low concentrations, (b) medium concentrations, and (c) high concentrations. As the concentration is increased, a band at 800 nm gives way to a band at 667 nm, which in turn gives way to a band at 828 nm.

dominated by a band at 800 nm, which we attribute to single molecules in solution. As the concentration is increased, the 800 nm band gives way to a blue-shifted band at 667 nm. As will be shown later, this process appears to be isodesmic, so we attribute the 667 nm band to intermediate sized linear assemblies. Finally, as the concentration is increased even more, the 667 nm band decreases in magnitude and a red-shifted band at 828 nm appears. This last band appears at a slightly lower concentration than when a birefringent phase appears. As is discussed later, this process resembles the formation of micelles, so we attribute the 828 nm band to a large assembly with a structure that requires a minimum number of intermediate assemblies to form.

In order to extract quantitative information from the absorption spectra, the spectra were decomposed into a combination of Gaussian absorption bands. It was found that the spectra could not be successfully decomposed unless six bands were used. Three of these bands correspond to the bands in the spectra described previously and vary strongly with concentration. The other three bands are small and depend very weakly on concentration. Successful decompositions at three concentrations across the full range of concentration are shown in Figure 6, where all six decomposition bands are shown. The center wavelengths and the widths of the decomposition bands did not vary and are given in Table 1. It was necessary to vary only the amplitudes of the decomposition bands in order to successfully fit all spectra. The variation of the amplitudes of the three important decomposition bands is shown in Figure 7, where it is evident that the 800 nm band gives way to the 667 nm band continuously, whereas the 828 nm band does not appear until the concentration is much higher.

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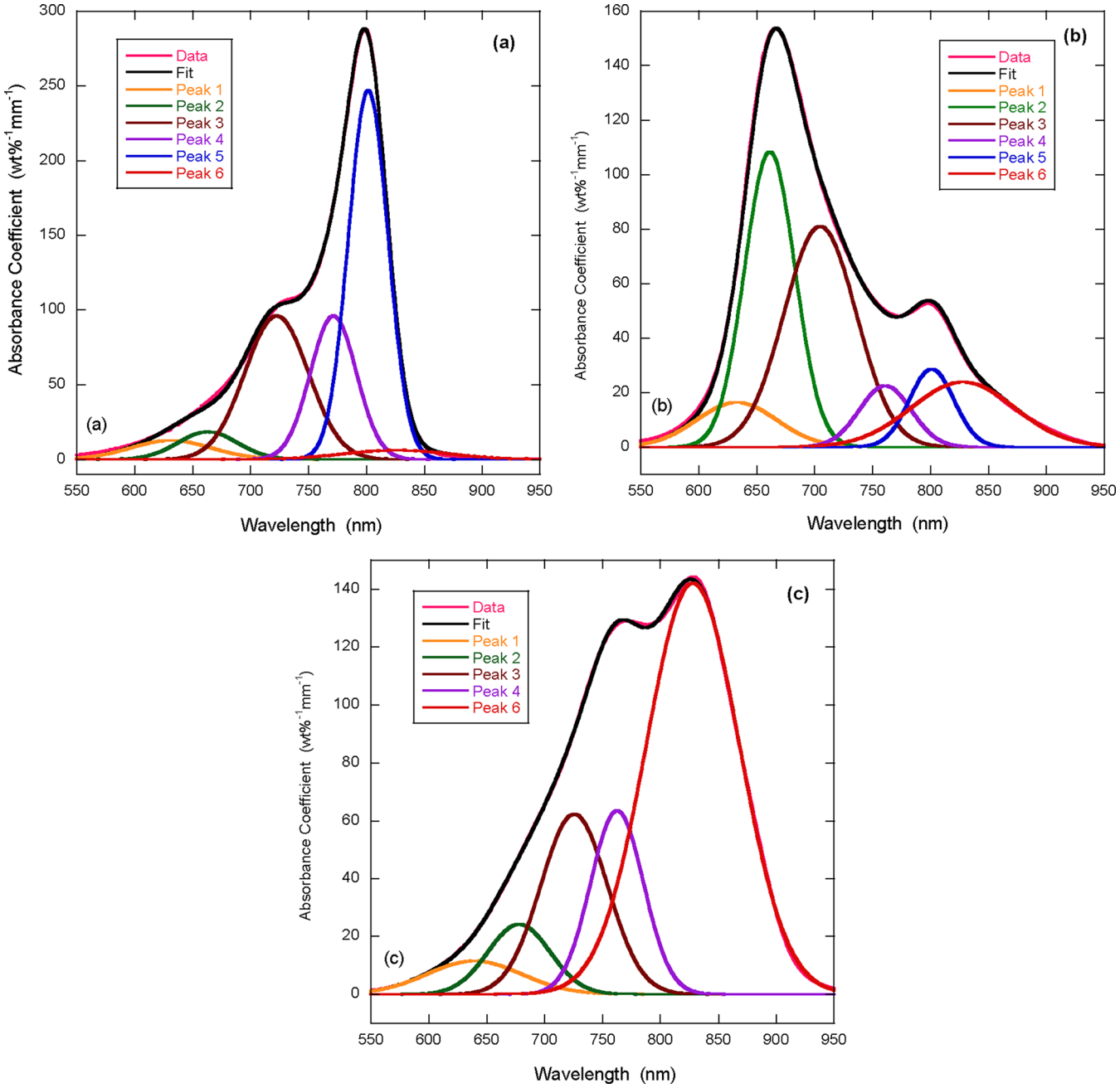

Figure 6. Decomposition of the room temperature absorption spectra of IR-806 into Gaussian bands at three different concentrations: (a) 0.0005 wt %, (b) 0.101 wt %, and (c) 0.996 wt %. The absorption coefficient axes are different to show the decomposition as clearly as possible. In all three cases, the sum of the six Gaussian bands, labeled "Fit" in the plots, agrees with the data extremely well.

Table 1. Gaussian Bands Used in the Decomposition of the Room Temperature Absorption Spectra[a]

band	center (nm)	width (nm)
1	630 ± 2	34 ± 8
2	667 ± 4	24 ± 8
3	716 ± 6	24 ± 10
4	759 ± 10	24 ± 6
5	800 ± 2	18 ± 3
6	828 ± 1	39 ± 10

[a]The uncertainties represent the range of values for which equivalent fits could be found.

In order to uncover the nature of the low concentration assembly process, the amplitudes of the 800 and 667 nm decomposition bands were compared to the prediction of two processes: a simple monomer−dimer reaction and an isodesmic assembly process. The absorption coefficient of the 800 nm decomposition band was assumed to be proportional to the fraction of dye molecules in solution as single molecules. In the monomer−dimer reaction process, the absorption coefficient of the 667 nm decomposition band was assumed to be proportional (with a different proportionality constant) to the fraction of dye molecules in solution as dimers. In the isodesmic assembly process, the absorption coefficient of the 667 nm decomposition band was assumed to be proportional (with a different proportionality constant) to the fraction of dye

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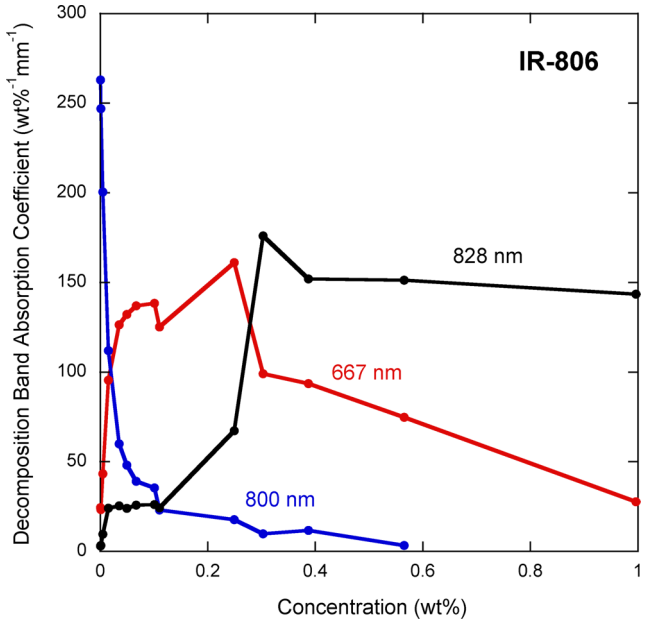

Figure 7. Amplitudes of the three important decomposition bands (800, 667, and 828 nm) vs concentration. The amplitudes of the other three decomposition bands are less and vary by about 30% over the entire concentration range with no systematic trend.

molecules in assemblies of size two or greater. The results of fitting these processes to the data are shown in Figure 8. The

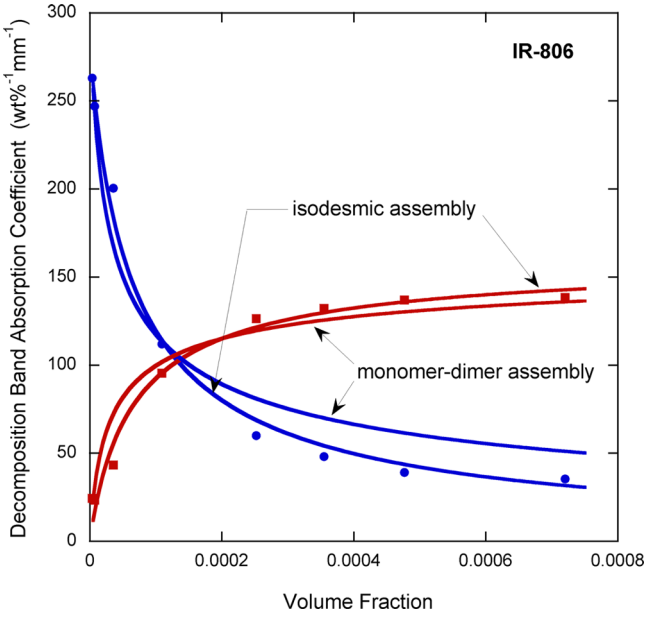

Figure 8. Comparison of fits to the low concentration changes of the decomposition band amplitudes as explained in the text. An isodesmic assembly process is a much better fit to the data and yields a stacking free energy change of 9.0 ± 0.1 k_BT.

monomer–dimer process does not fit the data well at all, while the isodesmic assembly process yields a good fit. The best fit for an isodesmic assembly process indicates that the stacking free energy change is 9.0 ± 0.1 k_BT.

X-ray Scattering. Due to the low concentrations at which assemblies form in IR-806, X-ray measurements are extremely difficult for two reasons. First, the low amount of dye in solution makes the scattering intensity very weak. Second, at low concentrations, the assemblies are far apart, resulting in X-ray reflections at extremely small angles. However, utilizing the high intensity of the National Synchrotron Light Source at Brookhaven National Laboratory (X6B beamline), X-ray measurements did reveal the reflection due to the assembly–assembly distance. A reflection due to a possible stacking of the molecules 0.34 nm apart, which is present in many dyes that spontaneously assemble, was not observed in IR-806, even for extremely long acquisition times.

The IR-806 solutions were enclosed in sealed glass capillary tubes and placed within an oven with windows made of Kapton tape. The wavelength of the X-rays was 0.155 nm, and the distance from the sample to the detector was 1.29 m. Acquisition times were 8 min, and the scattering from a capillary tube of water was subtracted from the IR-806 scattering results before analysis.

Figure 9 shows the X-ray intensity as a function of the scattering wavevector for four concentrations of IR-806. The

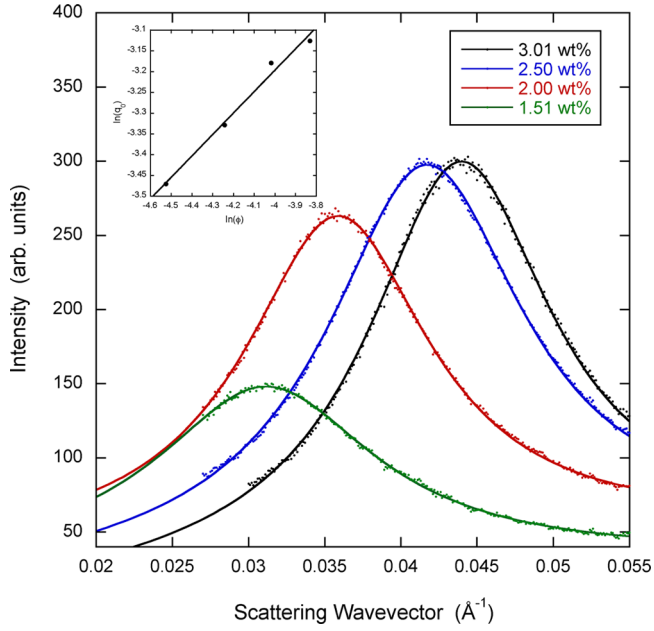

Figure 9. X-ray scattering intensity as a function of scattering wavevector for four concentrations of IR-806 at room temperature. The scattering from water has been subtracted. The lines are fits to Lorentzian functions with linear backgrounds. The inset is a plot of the log of the peak scattering wavevector vs the log of the dye volume fraction. The slope of this plot is 0.52 ± 0.04, consistent with the scattering expected from long assemblies of fixed cross-sectional area.

inset shows a log–log plot of the peak scattering wavevector vs total volume fraction, where a linear fit gives a slope of 0.52 ± 0.04, consistent with scattering from long assemblies of a fixed cross-sectional area. Notice that the X-ray results are for a concentration range in which the larger assemblies are present. So even though the process that forms these larger assemblies may involve a structure requiring a minimum number of intermediate assemblies, as the dye concentration increases, the cross-sectional area of this more complex structure must remain the same and/or these larger assemblies must lengthen without a change in their cross-sectional area.

X-ray scattering for the 2.5 wt % sample was investigated as a function of temperature also. As can be seen from Figure 10,

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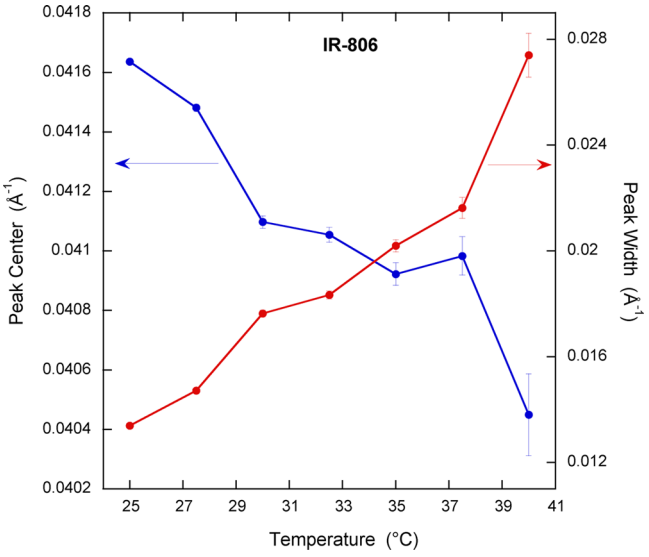

Figure 10. Change in the peak scattering wavevector and the width of the scattering peak for a 2.5 wt % solution of IR-806 as a function of temperature. For a 15 °C increase in temperature, the peak scattering wavevector decreases by about 3%, while the width of the peak increases by a factor of 2.

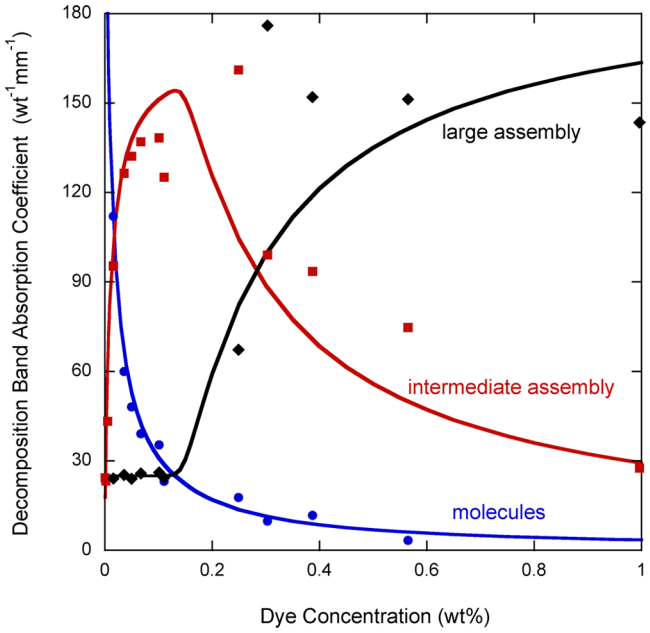

Figure 11. Comparison of the variation of the decomposition band amplitudes with a theoretical two-step assembly process (K_E = 60 wt %$^{-1}$, K_N = 1000 wt %$^{-1}$) in which a specific number, N = 15, of intermediate assemblies with nine or more molecules form large assemblies. The theoretical fraction of molecules in each type of assembly has been multiplied by different scaling factors and in the case of the large assembly shifted up slightly.

with increasing temperature, there was a 3% decrease in the peak wavevector but a factor of 2 increase in the width of the peak. Measurements were halted at 40 °C, since the reflection became too weak to be analyzed at this point. Notice from Figure 4 that, at this temperature and concentration, the sample is entering the region where the birefringence begins to decrease.

DISCUSSION

In previous studies when there has been little change in the absorption spectrum with increasing concentration, it has still been possible to analyze the absorption change in an effort to estimate the stacking free energy change.[5,6,9,13] However, systems in which dramatic changes occur allow for a more rigorous analysis. This has led to the observation that the first step in the assembly process of IR-806 is an isodesmic one, meaning that structures incorporating many molecules are involved. The ability to observe a second step in the process reveals that this step is not isodesmic but proceeds after a threshold dye concentration is reached. While the data are not robust enough to lead to a definite conclusion, it is not difficult to show that a model involving the isodesmic assembly of molecules into intermediate assemblies and the assembly of large structures by intermediate assemblies greater than a certain size is consistent with the data. Such a model fits the data at lower concentrations just as well as the isodesmic model in Figure 8 and, as is shown in Figure 11, also follows the trend of the data at higher concentrations.

Except for the lack of an X-ray scattering peak due to the stacking of molecules, the X-ray data for IR-806 qualitatively resembles what is seen in other systems, even ones in which the assembly structure is quite simple. The shift of the X-ray peaks with concentration (Figure 9) is similar to what one observes in Sunset Yellow FCF.[5] Likewise, the slight decrease in the peak wavevector and the substantial increase in the width of the peak as the temperature is increased also look quite similar to the analogous data for Sunset Yellow FCF.[5] However, there are at least two large quantitative differences. IR-806 forms a liquid crystal at room temperature at a concentration that is roughly 50 times less than what is seen in Sunset Yellow FCF. Also, the peak wavevectors for IR-806 are a factor of 5 smaller than those found in Sunset Yellow FCF. These profound differences point to a different assembly structure in the two systems. In order to make this point stronger, the spacing between the assemblies in two systems can be compared at room temperature in the nematic phase near the coexistence region. For disodium cromoglycate, which forms simple stacks of double molecules, this spacing is about 5 nm,[26] whereas the spacing for IR-806 is about 30 nm. Since significant interactions between assemblies are required to form a liquid crystal phase, it is plausible to hypothesize that significant interactions over 6 times the distance require assemblies with a much larger cross-sectional area. Another way to make the same argument is to realize that the volume fraction in the nematic phase near the coexistence region is about 0.09 in disodium cromoglycate but only about 0.004 in IR-806 if the assemblies contain only dye. Of course, if the assembly length to diameter ratio in IR-806 is larger than that in disodium cromoglycate, it is likely that IR-806 would form a liquid crystal at a lower volume fraction. Still, it is difficult to imagine that the length to diameter ratio is 20 times greater. A more plausible hypothesis is that the assemblies in IR-806 contain water, effectively increasing the volume fraction of the assemblies.

Given the similarities between pinacyanol acetate and IR-806, and given the fact that for pinacyanol acetate there is X-ray evidence for an assembly structure that is a hollow cylinder,[16] it is tempting to hypothesize the same structure for IR-806. If a thin layer of dye molecules separates the water that is inside the assembly from the water that is outside the assembly, then the larger distances between the assemblies and the formation of a liquid crystal phase at very low concentrations can be explained.

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In addition, if the dye molecules that make up the outside of the assemblies are not stacked as neatly and over as large a distance as in the more simple assemblies formed by some dye molecules, then a peak representing the stacking distance would be very weak. This is consistent with such a peak not being observed in IR-806. Such a hollow cylinder model is also consistent with the IR-806 X-ray results showing that the X-ray peak from the assembly−assembly distance varies with volume fraction as expected for linear assemblies. Also, it must be pointed out that light scattering experiments performed by us show almost no scattering, even when the concentration is almost high enough to form the liquid crystal phase. Again, if the water inside and outside the hollow cylinder is separated by a thin wall of dye molecules, one would expect the scattering of light to be extremely weak. Finally, since the higher concentration process behaves similar to a model in which a specific number of molecules is necessary to form an assembly, the larger IR-806 assembly process is consistent with a hollow structure that requires a certain number of intermediate assemblies to close the wall separating the inside water from the outside water.

There have been many reports of investigations on chromonic liquid crystals, and most report a biphasic region between the liquid crystal and isotropic phases. However, for compounds that form a liquid crystal phase at low concentrations, most reports do not address the presence or absence of a biphasic region. One that does states that no biphasic regions were observed for pseudoisocyanine chloride.[27] More recently, no biphasic region was reported for benzopurpurin 4B.[9] At this point in time, it appears that for systems in which the assemblies have a simple stacked structure (Sunset Yellow FCF, disodium cromoglycate, Bordeaux dye), a biphasic region about 10 °C wide is present. However, when indications are that the assembly structure is more complicated (benzopurpurin 4B, IR-806), a biphasic region is not observed. Perhaps the surface tension between the liquid crystal and isotropic phases in systems with these more complicated assemblies is extremely small, meaning that the driving force to form macroscopic domains of each phase is too weak. If the structure has water both inside and outside the assembly, the surface tension between a region of ordered assemblies (liquid crystal) and disordered assemblies (isotropic) might be less than in systems with water only outside the assemblies. Clearly, more work is going to have to be done to understand the lack of an observable biphasic region in some chromonic liquid crystals.

CONCLUSIONS

IR-806, a near-infrared cyanine dye, is a chromonic liquid crystal forming dye that shows no biphasic region in a polarizing microscope, forms a liquid crystal phase at very low concentrations, and possesses no (or a very weak) X-ray peak corresponding to the stacking of molecules. The number of systems known to behave in this way is not large and includes a cyanine dye, benzopurpurin 4B, and pinacyanol acetate. All of the evidence points to the conclusion that, like pinacyanol acetate, IR-806 forms a hollow assembly with water inside a thin veneer of dye molecules. More importantly, the assembly process in IR-806 has been shown to proceed in two steps, with an assembly of molecules forming by an isodesmic process at lower concentrations, and with large assemblies forming at higher concentrations via a nonisodesmic process. It is these larger assemblies that order into the liquid crystal phase.

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Notes

The authors declare no competing financial interest.

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