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Deriving Binary Phase Diagrams for Chromonic Materials in Water Mixtures via
Fluorescence Spectroscopy: Cromolyn and Water

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Abstract

We report here the *first example of* a new and novel method of determining the binary temperature – composition phase diagram of a chromonic material in water using its intrinsic fluorescence. Disodium cromoglycate, or cromolyn, is an anti-allergy medicine representative of a class of compounds known as the chromonics. We have discovered that cromolyn's fluorescence is very sensitive to the polarity, hence structure, of the phase it exhibits. The fluorescence signal shifts its wavelength maximum and its shape depending on whether the cromolyn is a single phase or in coexisting phases. Since the signal due to individual phases can be identified, the fluorescence signal can reveal the temperature-induced transitions between single phase and phase coexistence regions. By studying such fluorescence data for different compositions, an isobaric temperature-composition phase diagram may be constructed. We present here a phase diagram derived from fluorescence studies that is in agreement with previous determinations using other techniques. Our results suggest that the binary phase diagrams of other intrinsically fluorescent chromonic materials, such as perylene monoimide and bisimide derivatives used in organic optoelectronic devices, solar cells, and light-emitting diodes, can be studied in water using an analogous fluorescence approach.

Key words

Chromonics

Lyotropic

Nematic

Hexagonal

Aggregates

Fluorescence

INTRODUCTION

Molecular structural motifs have both challenged and enabled those interested in liquid crystals since their discovery. Rod-like calamitic molecules exhibit nematic and smectic phases while disc-shaped molecules exhibit columnar phases. More recently plank-like molecules have been observed to exhibit lyotropic phases, usually called chromonic phases after Lydon¹. Excellent introductions to the growing field of chromonics may be found in the literature²⁻⁶. The desire to understand the details of the molecular organization of a pure phase or the details of molecular aggregation formation in lyotropic solute/solvent systems has motivated an immense amount of study. While the details of the molecular organization in a phase are of great interest, so too are the temperature, pressure, and composition regimes that characterize the observed liquid crystal phases. Binary isobaric temperature – composition phase diagrams convey considerable information in a concise manner. Though only recently recognized as a new type of liquid crystal, chromonic systems show tremendous promise for a wide variety of applications dependent on the properties of partially ordered condensed soft matter. A short list of applications would include thin film polarizers and compensators⁷, organic electronics^{8,9}, and biosensors¹⁰. As interest grows in the applications of chromonic liquid crystals, it follows that the availability of accurate temperature – composition phase diagrams [T-X diagrams] is of great importance.

To date T- X diagrams have been studied using a wide variety of techniques from simple visual observation to sophisticated scattering techniques. Isobaric T-X phase diagrams have been published for several chromonic phase-forming molecules following the pioneering work of Cox et al. who first detailed the disodium cromoglycate, cromolyn, [4H-1-Benzopyran-2-carboxylic acid, 5,5'-[(2-hydroxy-1,3-propanediyl)bis(oxy)]bis[4-oxo-, sodium salt (1:2)] [*CAS*

(15826-37-6)] /water diagram in 1971¹¹. Since then water/chromonic diagrams have been published for: xanthone derivatives [CAS 66934-53-0, CAS 33440-58-3, CAS 33459-28-8]¹²; C.I. Direct Blue 67 [CAS 2783-94-0]¹³; perylene derivatives [CAS 1258419-94-1⁸, CAS 232941-48-9¹⁴, CAS 1009098-26-3¹⁷, CAS 1009098-22-9¹⁷, CAS 232941-48-9¹⁷]; Sunset Yellow FCF [CAS 2783-94-0]¹⁵; Optiva Violet 20 [CAS 606125-50-2]¹⁶; Blue 27 [CAS 87092-10-9]¹⁶; Edicol Sunset Yellow [CAS 15790-07-05]¹⁸; Bordeaux dye [CAS 852155-90-90]¹⁹; and Benzopurpurin 4B [CAS 992-59-6]²⁰. In addition, T-X diagrams have been determined in the presence of added salts such as sodium chloride for cromolyn^{21,23} and Sunset Yellow.²² The studies presenting T - X diagrams of the above chromonic materials in water all show a generic phase diagram topology similar to that illustrated in Figure 1.

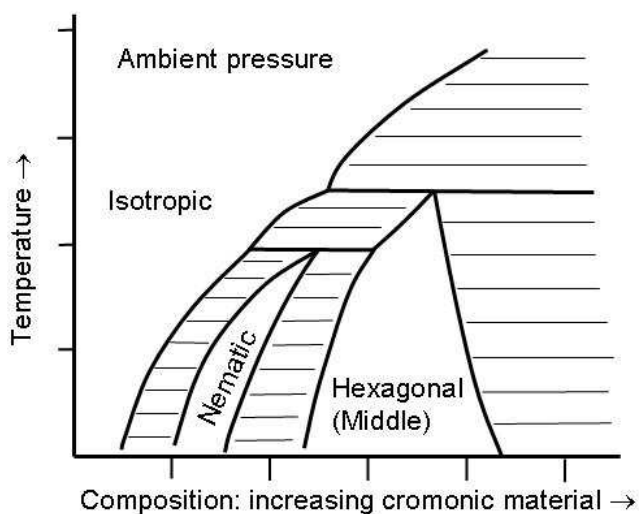


Figure 1. Generic isobaric temperature – composition phase diagram of chromonic materials in water. Typically the two-phase coexistence regions of isotropic–nematic, isotropic–hexagonal, and nematic–hexagonal phases are quite broad. This diagram follows that of Cox et al. who first detailed the cromolyn/water phase diagram.¹¹

Prior methods used to detect the phases present and their transition temperatures have been varied but fairly traditional. Microscopic visual observation of fixed composition samples or polarized microscopy of known compositions combined with solubility measurements were the methods of choice for Cox et al.¹¹ for studying cromolyn in water. Temperature-controlled polarized microscopy of known compositions has been the most commonly used technique for phase diagram determination^{8,11-13,15,16,18,19}. Another approach involved temperature-dependent ²H NMR spectroscopy of fixed compositions of the chromonic material in ²H₂O^{12,17}. The intensity and linewidth of chopped laser light passing through a thin sample [benzopurpurin 4B] was monitored as a function of temperature and composition to estimate the phase diagram²⁰.

Fluorescence is a technique little used to date for phase diagram determination in lyotropic systems. Fluorescence signals from either the chromonic material itself or from an added fluorescent probe offer highly sensitive means to determine the state of aggregation of the studied phase. While the fluorescent probe prodan has recently been used successfully to elucidate the *n*-octyl- β -D-glucoside/water phase diagram²⁴, no elucidation of the phase diagram of a lyotropic system using intrinsic fluorescence has been reported. As a proof of concept but not to be construed as the limiting example, we use the known phase diagram of cromolyn in water and our observed fluorescence emission spectra of cromolyn/water samples to report here the discovery that the intrinsic fluorescence of cromolyn can be used to map out its T – X phase diagram with water. Our work not only reveals the distinct fluorescence signals of cromolyn aggregates but suggests that intrinsic fluorescence may offer a convenient yet powerful means of phase diagram determination for other lyotropic systems.

EXPERIMENTAL SECTION

Sample Preparation. Cromolyn sodium salt was obtained from Sigma Aldrich (95%) and used as received. Samples were prepared by adding an appropriate amount of cromolyn by mass into a custom-blown Vycor glass (Corning) tube. The tube was then centrifuged at 3,000 rpm in a fixed angle centrifuge (SpeedFuge HSC10K, Savang Instruments Inc., Farmingdale, NY) to draw all the powder down. A 1 ml syringe was used to deliver 0.2 ml of deionized water to the vial, rinsing the sides to get any cromolyn powder still near the top. The mixture was then centrifuged as before and 50 μ l of deionized water was then delivered to the vial. The sample was then centrifuged once more as before and then sealed with custom-made stoppers to prevent loss of moisture. This produced samples of similar volume by using a fixed amount of water and a varying amount of cromolyn, ranging from 5.069 mg to 83.344 mg, to achieve the desired composition. The mass of each sample was recorded after sealing.

Teflon rod of a diameter to fit snugly into the Vycor tube was machined with two grooves to hold O-rings that would serve to moisture seal the sample. The top of the rod was threaded and fitted with a machine screw and washer to prevent the rod from going to the bottom when centrifuged and to aid in removal. New O-rings were always used when preparing a new sample tube. Samples were repeatedly heated (80-95 °C) in a sand bath to yield the isotropic phase and then mixed while still isotropic with a Vortex-Genie 2, starting at its lowest setting and increasing as appropriate to mix without splashing the sides and the stopper. To avoid the loss of water at elevated temperatures, samples were heated for a minimum time possible, always kept below 100 °C, and were stored at room temperature once mixed.

After appearing homogenized, at room temperature, the sample was reheated to its isotropic phase and then recooled to room temperature to equilibrate for 1 week before

fluorescence measurements were made. Samples were weighed periodically to check for water loss.

Literature phase diagrams guided the creation of samples such that each phase region was studied.¹¹ In the absence of literature, a penetration study [also known as a contact method], provides sufficient guidance to the number and types of phases exhibited by the compound in water. Simple visual observation of the sample at room temperature showed sufficient variation in physical characteristics to distinguish the three main phases (Figure 2). The hexagonal phase was highly viscous and cloudy to almost opaque. The nematic phase was viscous and cloudy, though it still flowed readily, and was more translucent than the hexagonal. The isotropic phase was clear. High concentrations of cromolyn produced yellow solutions, regardless of the exact phase. No distinct features indicated the presence of any two-phase region to visual inspection.

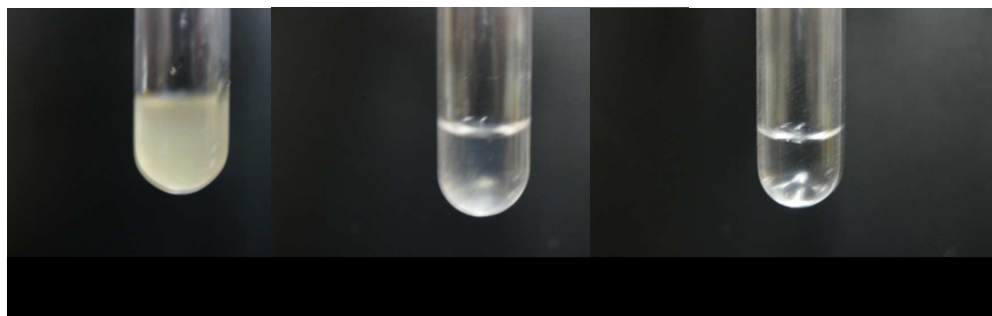


Figure 2 Photograph of aqueous cromolyn samples (left to right) in the hexagonal, nematic, and isotropic phases.

Fluorescence Measurements. Fluorescence studies were conducted using a Horiba Jobin Yvon FluoroMax-4 Spectrofluorimeter. Fluorescence spectra were recorded from 420 nm to 660 nm using an excitation wavelength of 407 nm. Both excitation and emission slit widths were set at 5 nm. Temperature control was achieved using a Wave Electronics 5 amp–40 watt Peltier temperature controller (model LFI-3751). Spectra were collected at 4°C ($\pm 0.5^\circ\text{C}$) intervals with 8 minutes of temperature equilibration before measuring.

Polarized Microscopy. Polarized light microscopy was used to visually inspect samples for characteristic birefringence textures. A drop from a fixed composition sample was placed on a slide, with a cover slip (25 mm x 25 mm) applied so that the drop was centered, and then a minimum of water was applied via syringe to the edges of the cover slip to provide a seal against evaporation while not making contact with the sample to prevent changing its composition.

For the study, an Olympus BH-2 polarizing microscope was used. Temperature control was achieved using an INSTEC STC200 program to heat an INSTEC HS400 hot stage. Sample textures were observed for each composition at various temperatures and compared to literature textures for cromolyn in its nematic and hexagonal phases to confirm phase assignments from the fluorescence data.

RESULTS AND DISCUSSION

Fluorescence Analysis. To construct the phase diagram from the observed fluorescence spectra of cromolyn-water samples at various temperatures, we make use of three fundamental principles dictating fluorescence spectra:

(1) Each lyotropic phase typical of a chromonic system - isotropic, nematic, and hexagonal - is expected to exhibit a unique fluorescence emission spectrum with characteristic shape and peak range. The origin of these distinctive spectra is the dependence of fluorescence emission maxima and quantum yields on the surrounding environment. The distinct molecular assemblies of the isotropic, nematic, and hexagonal phases give rise to these different environments. With varying dye concentration and temperature, the cromolyn molecules self-aggregate into simple columnar assemblies via π - π stacking at a separation of 0.34 nm²⁵. The isotropic (I) phase consists of very short assemblies of cromolyn molecules²⁵. The nematic (N) and hexagonal (M) phases are comprised of orientationally ordered assemblies with hexagonal

packing in the M phase. The temperature-dependent separation between assemblies is on the order of 3.5-4.2 nm in the nematic phase and 2.7-3.2 nm in the hexagonal phase²⁵. These structural variations influence the observed fluorescence signature of each phase.

(2) The presence of a single aggregate in an aqueous cromolyn sample over a range of temperatures is indicated by a series of superimposable normalized fluorescence spectra.

(3) The presence of multiple aggregates in a given aqueous cromolyn sample gives rise to an observed fluorescence spectrum that is the sum of the individual spectra emanating from the distinct aggregates present in the sample.

Normalized fluorescence spectra of the aqueous cromolyn samples, and by extension other chromonic materials, may reveal multiphase regions in one of two primary ways. Case 1: Fluorescence spectra may change in shape (and possibly in λ_{max} value) as the relative contributions of the aggregates present vary as the temperature of the sample is altered. This situation is most common when the spectra of the individual aggregates have significantly separated λ_{max} values and/or distinct relative contributions to the overall spectrum. Case 2: Fluorescence spectra may exhibit similar shapes but shift the observed λ_{max} value as the temperature of the sample is changed. This situation is most common when the distinct aggregates present have similar relative contributions to the overall spectrum.

Determining the Fluorescence Signature of Individual Phases. Cromolyn exhibits each lyotropic phase typical of a chromonic system—isotropic, nematic, and hexagonal. To identify the cromolyn fluorescence spectrum indicative of each of these phases, samples were prepared at those compositions consistent with each phase as suggested by the published phase diagram of Cox et al¹¹. Figure 3 shows the characteristic fluorescence signals of the isotropic phase for a sample of 2.0 weight % from 5 to 25°C, the nematic phase for a sample of 15.0

weight % from 17 to 25°C, and the hexagonal phase for a sample of 25 weight % from 33 to 37°C. As is evident from the superimposability of the multiple curves for each phase, increasing the temperature has no effect on the curves' shape or position. The consistency of the fluorescence signal is strong evidence that the sample remains in only one phase over the given range of temperature and for the given total composition.

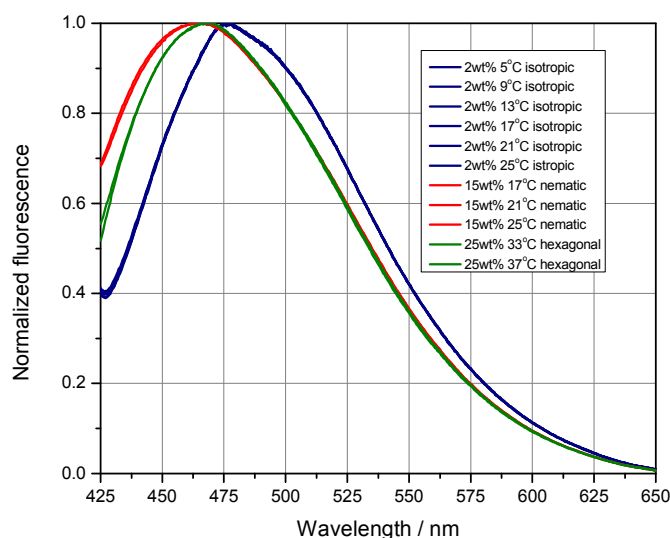


Figure 3. The superimposable fluorescent spectra assigned to the 2 wt% [isotropic], 15 wt% [nematic], and 25 wt% [hexagonal] phases of cromolyn in water sample as a function of temperature.

Thus, each individual phase was detected and found to exhibit a unique fluorescence emission spectrum with characteristic shape and peak range. Table 1 summarizes the peak values for each single-phase region. The isotropic phase exhibits the most distinct spectrum in terms of

both shape and peak emission wavelength, consistent with this phase being distinct from the orientally-ordered nematic and hexagonal phases. Nematic and hexagonal phases show subtle differences in shape and differ primarily in terms of spectral width.

Table 1. Fluorescence peak maxima characteristic of specific phases

Phase	$\lambda_{\text{max}} / \text{nm} (\pm 1 \text{ nm})$
Isotropic	475
Nematic	466
Hexagonal	468

Assigning Phases to Samples of Varying Composition and Temperature. The characteristic spectra of the isotropic, nematic, and hexagonal phases can be used to delineate the phases present in samples of varying composition over a range of temperatures. Figures 4 and 5 illustrate the raw and normalized fluorescence spectra recorded for a 15 wt% sample over the temperature range of 5 to 53°C. The variations in fluorescence intensities and in the shapes of the curves reflect the temperature-induced phase transitions of the sample. By overlaying the observed spectra with the characteristic spectra of the distinct phases, the single-phase regions can be determined. The intervening non-superimposable spectra, such as those recorded between 29 and 41°C, correspond to two-phase regions and reflect the varying contributions of the two phases with changes in temperature. Identification of the two phases in coexistence can be made by simple application of the horizontal transverse rule whereby two single phase regions must be separated by an intervening phase region consisting of the two phases in co-existence.²⁴ Alternatively, the overall spectrum could be deconvoluted to determine the relative contribution of the individual spectra. However, since the fluorescence intensity depends on many factors,

including quantum yield, the relative contributions of each spectrum cannot be used to estimate the relative amount of each coexisting phase.

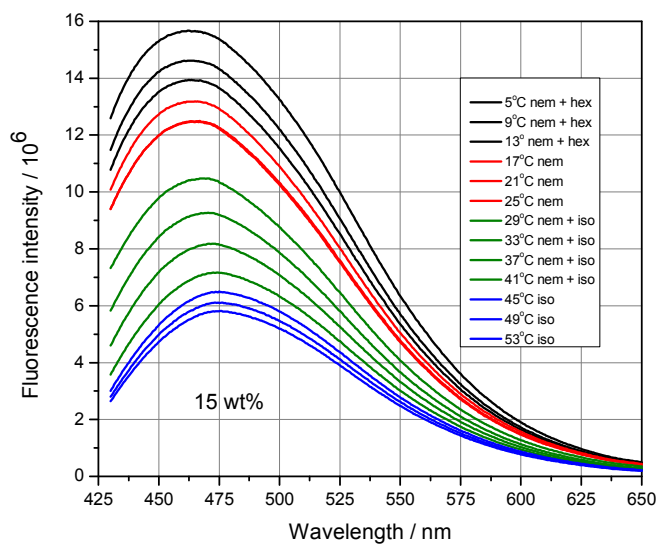


Figure 4. The raw fluorescence spectra of a 15 %wt cromolyn in water sample as a function of temperature.

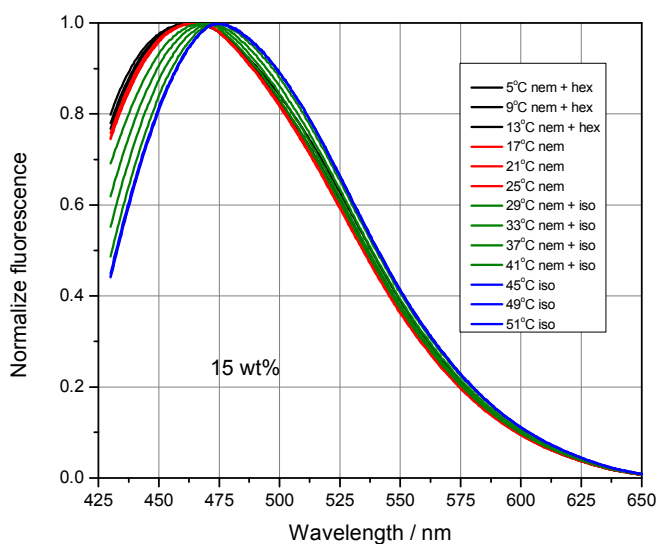


Figure 5. The normalized fluorescence spectra of a 15 %wt cromolyn in water sample as a function of temperature.

Phase Diagram. Using the above analysis of fluorescence spectra, the phase diagram depicted in Figure 7 was constructed. As noted previously, assignments of the single-phase regions were in accordance with the samples prepared at those compositions consistent with each phase as suggested by the published phase diagram of Cox et al¹¹. The different phase regions were demarcated by examining the temperature dependence of the fluorescent spectra for a fixed composition sample. The black phase boundary lines were drawn to map the single and coexisting phases in accordance with thermodynamic criteria for binary phase diagrams²⁴. The diagram almost draws itself by placing the phase lines between the identified single and binary phase regions. Since the temperature data points were collected in 4°C intervals, the precision of

the drawn lines is about ± 2 °C. This precision could be improved by collecting data in shorter temperature intervals around the expected phase transition temperatures.

With the diagram in hand, relative amounts of coexisting phases at any temperature can be estimated using the lever rule²⁶. For example, a 12.5 wt% composition sample at 30°C is in a coexistence region of nematic and isotropic phases. A lever rule at this temperature would estimate the relative masses of the isotropic phase to nematic phase to be in the ratio of 1.7.

The diagram reproduces the features of the generic diagram presented in Figure 1 and agrees with the original diagram of Cox et al.¹¹ as well as the other cromolyn/water phase diagrams found in the literature.

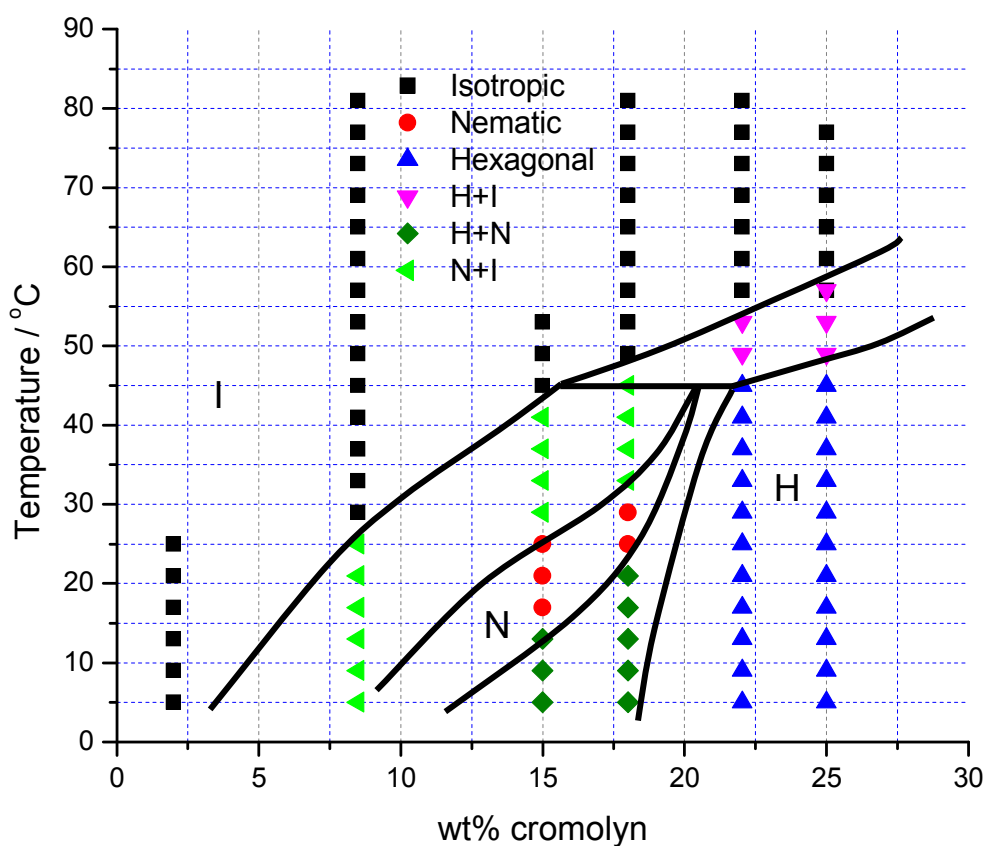


Figure 6. The cromolyn/water temperature–composition phase diagram derived from cromolyn’s intrinsic fluorescence. The colored symbols show the phases change with temperature for fixed composition samples. For example the 8.5 wt% sample shows coexistence of nematic and isotropic from 5 to 25°C, then only isotropic for higher temperatures. The compositions illustrated are 2, 8.5, 15, 18, 22, and 25 wt% cromolyn in water.

CONCLUSIONS

The use of the intrinsic fluorescence of cromolyn has provided a means to construct the mesomorphic portion of the cromolyn/water isobaric temperature-composition phase diagram. The derived diagram agrees with the literature and further supports the developing picture of the generic T – X diagram shown in Figure 1 for chromonic lyotropic mesophase systems. As the first construction of a binary phase diagram of an aqueous chromonic system using intrinsic fluorescence, this work provides a promising new means to construct phase diagrams of other chromonic materials that exhibit intrinsic fluorescence.

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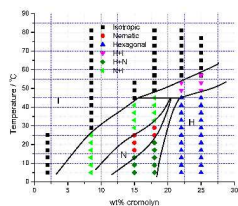
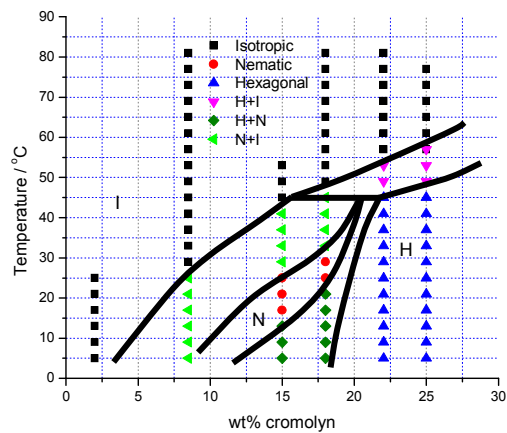
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TOC graphics



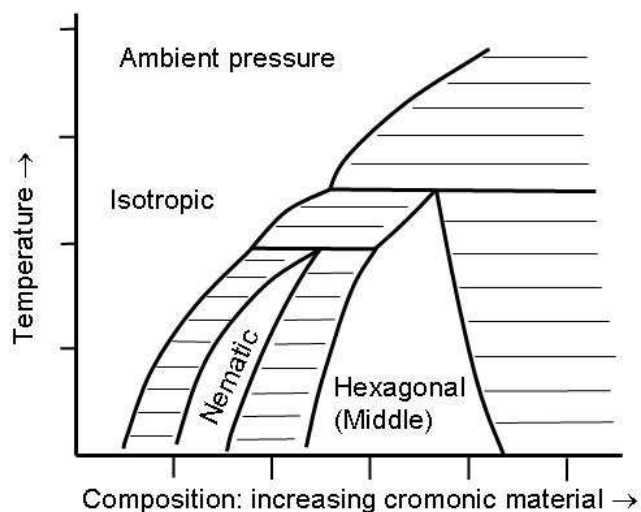


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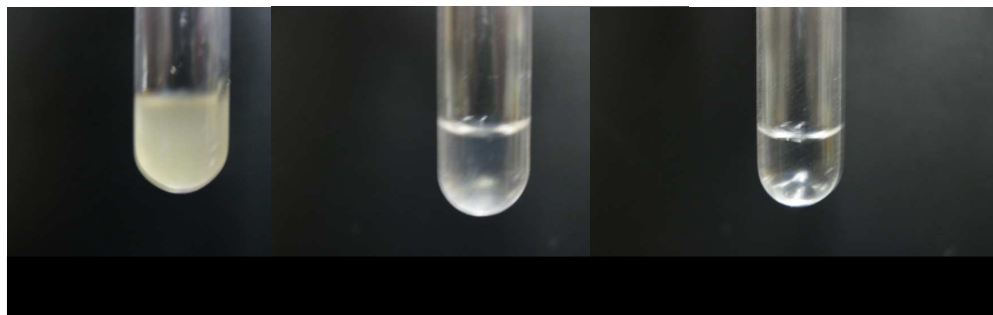


Figure 2 Photograph of aqueous cromolyn samples (left to right) in the hexagonal, nematic, and isotropic phases.

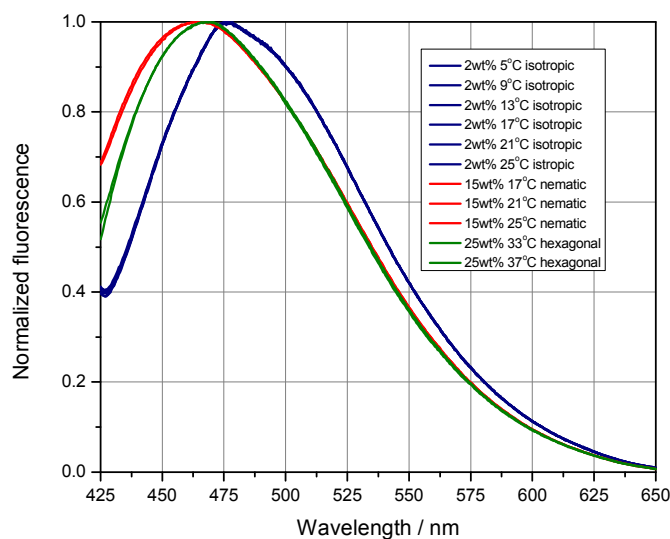


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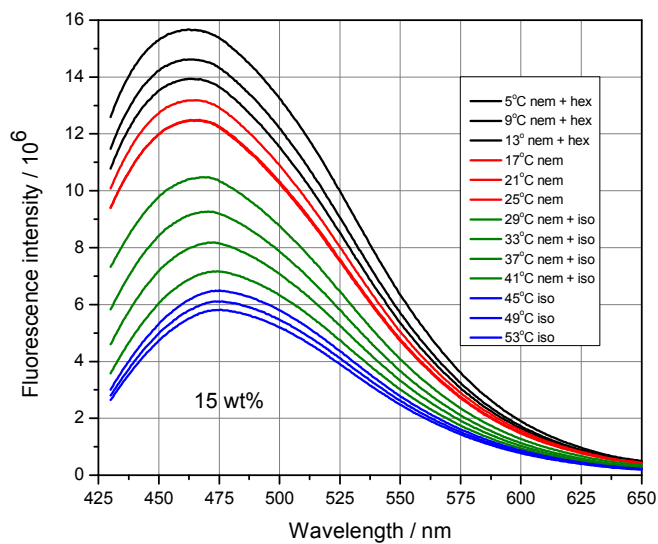


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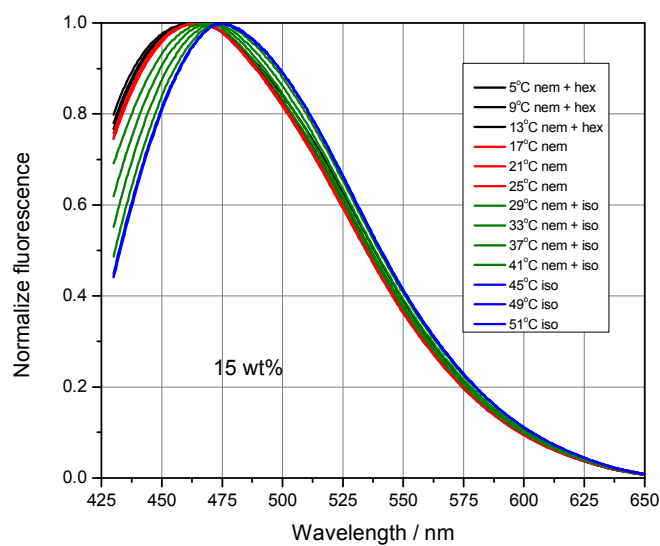


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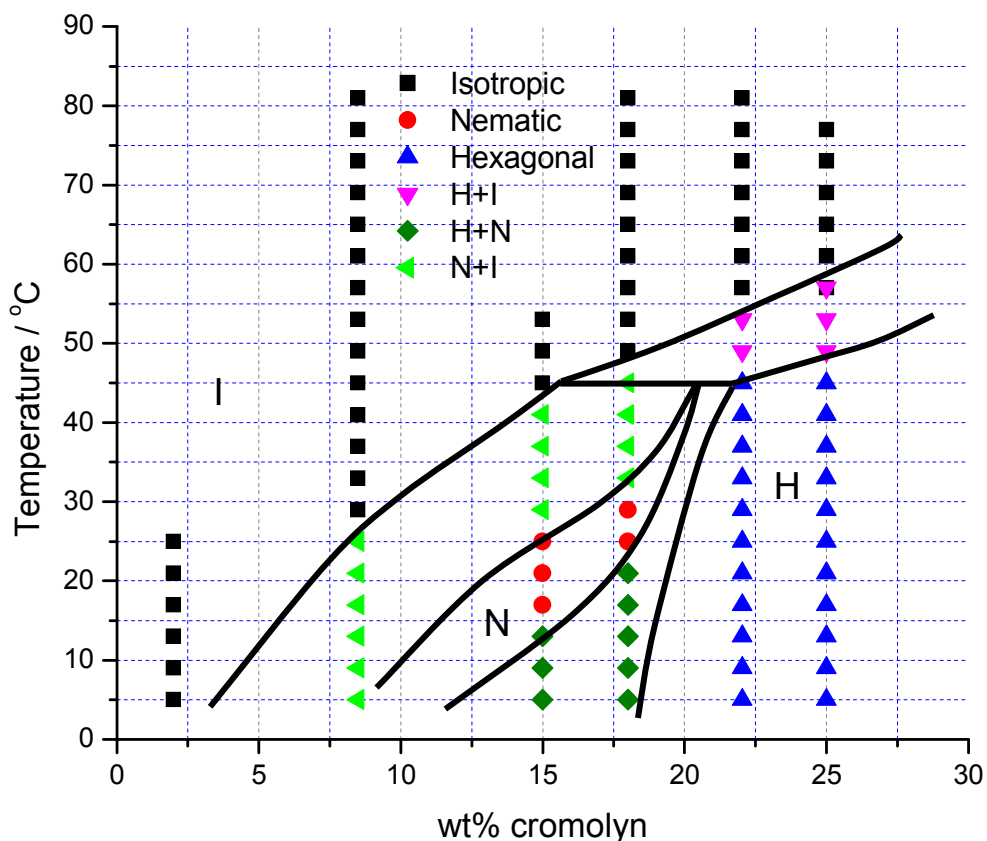


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