

Induced phase separation in cellulose nanocrystal suspensions containing ionic dye species

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Abstract

Suspensions of negatively charged cellulose nanocrystal are obtained by sulfuric acid hydrolysis of cotton cellulose. Within a specific concentration range, the suspensions spontaneously phase separate to give isotropic and chiral nematic phases. Added anionic dyes of varying charge cause separation of an isotropic phase from the highly concentrated, completely anisotropic suspensions; these dyes have a much greater effect on the suspensions than the equivalent ionic strength of a simple 1:1 electrolyte (sodium chloride). Neutral, cationic and cellulose-binding anionic dyes do not cause phase separation in the anisotropic cellulose suspensions.

Abbreviations: AO – Acridine Orange hydrochloride hydrate; AR 66 – Acid Red 66; AR 112 – Acid Red 112; CB – Cibacron Blue 3G-A[®]; DB 1 – Direct Blue 1; DR 2 – Direct Red 2; H – Hematoxylin; PhB – Phloxine B; RR 8 – Reactive Red 8

Introduction

Above a critical concentration, suspensions of neutral rigid rod-like colloidal particles undergo spontaneous entropically driven phase separation (Onsager 1949). The number density of particles at which phase separation first occurs depends on their geometric parameters (length L and diameter D) and is proportional to $1/(L^2D)$ (Onsager 1949). Nanocrystals prepared by sulfuric acid hydrolysis of native cellulose contain negatively charged surface sulfate ester groups, stabilizing the aqueous colloidal suspensions of the cellulose nanocrystals. Rodlike polyelectrolytes can be modelled as hard rods with an effective diameter D_{eff} , which depends on the surface charge of the rod and the ionic properties of the solution (Onsager 1949; Stroobants et al. 1986). Suspensions of cotton

cellulose nanocrystals free of added electrolyte separate into liquid crystalline chiral nematic and isotropic phases in a limited concentration range beginning around 5–7 wt% cellulose (Marchessault et al. 1959; Dong et al. 1998). Adding electrolytes to cellulose nanocrystal suspensions suppresses the anisotropic phase formation (Dong et al. 1996). As the ionic strength increases, the cellulose nanocrystal electrostatic double layers are screened and D_{eff} decreases, approaching the hard rod diameter D .

Dong et al. found that the nature of added simple electrolytes is not important, neutral NaCl and KCl, and acidic HCl having the same effect on the phase equilibrium behaviour of cotton cellulose suspensions (Dong et al. 1996). However, the nature of the cationic counterions present has been found to influence several suspension properties

including phase equilibrium and stability (Dong and Gray, 1997). When organic counterions were used, the critical concentration increased with increasing counterion size (increases were on the order of 1 wt%). A possible explanation was offered, based on a strong hydrophobic interaction between the alkyl chains of the organic counterions (Israelachvili and Pashley 1982). The balance between the attractive hydrophobic interaction of the alkyl chains, which reduces the excluded volume of the particles, and the steric repulsion between the counterions, which magnifies the excluded volume, may be important contributions to the free energy of the system and hence the phase equilibrium. If the hydrophobic–hydrophobic attraction dominates the electrostatic interparticle repulsive force of the suspensions, the result would be an increase in the critical concentration for ordered phase formation (Dong and Gray 1997).

Phase separation occurs over a period of several days when the macromolecule blue dextran is added to anisotropic cellulose nanocrystal suspensions (Edgar and Gray 2002; Beck-Candanedo et al. in press). Blue dextran contains a sulfonated triazine dye, Cibacron Blue 3G-A[®] (CB), bound to random hydroxyl groups on the carbohydrate chain. (Böhme et al. 1972) It has been found that the trivalent ionic CB dye attached to the dextran is responsible (for the most part) for the induced phase separation (Beck-Candanedo et al., in press), rather than depletion attraction caused by the macromolecular dextran, as was previously thought. In this article, we report on the induced phase separation behavior of concentrated (~ 14 wt%) cellulose nanocrystal suspensions caused by a range of anionic and cationic dye molecules with varying total charge. In these suspensions, the major contributors to the ionic strength are the sodium counterions associated with the surface sulphate groups on the nanocrystals.

Materials and methods

Materials

Acridine Orange hydrochloride hydrate (AO), Phloxine B (PhB), Cibacron Blue 3G-A[®] (CB) and Reactive Red 8 (RR 8) were purchased from Aldrich. Acid Red 66 (AR 66), Acid Red 112 (AR 112), Direct Blue 1 (DB 1) and Direct Red 2 (DR 2)

were purchased from Matheson Coleman & Bell. Hematoxylin (H) was purchased from Anachemia. All dyes were used without further purification; see Chart 1 for structures and purities. Whatman ashless cotton cellulose powder was purchased from Cole-Parmer. Sulfuric acid (95–98%) for hydrolysis was purchased from Fisher Scientific. Sodium hydroxide, sodium chloride, and sulfuric acid volumetric standards for conductometric titration were purchased from Aldrich. All water used was deionized (Millipore Milli-Q purification system).

Suspensions

Cellulose nanocrystal suspensions were prepared as described previously, and neutralized to pH ~7 with sodium hydroxide (Revol et al. 1992; Revol et al. 1994; Dong et al. 1998; Beck-Candanedo et al. 2005). Whatman ashless cotton cellulose powder (40 g) was hydrolyzed with sulfuric acid (700 ml, 64 wt%) at 45 °C for 45 min and then quenched by 10-fold dilution in cold distilled water. Excess acid was removed by centrifugation and dialysis against deionized water until the suspension pH was around 4–5. The suspension was then sonified (Vibracell, Sonics & Materials, Inc., Danbury CT) in 7-min intervals for a total of 35 min, taking care to ensure that the temperature did not exceed 50 °C. The suspension was placed over a small amount of mixed bed ion-exchange resin (Sigma-Aldrich) for several hours with gentle shaking in order to remove excess ionic materials, and the resin was removed by filtration and centrifugation if necessary. The final aqueous suspensions were 1–2% cellulose by weight.

The concentration of cellulose in the samples was determined by gravimetry. Conductometric titration was used to determine the quantity of sodium hydroxide required to neutralize the H⁺ counterions associated with the sulfate ester groups on the nanocrystal surfaces. A Contiburette μ 10 (Ing. CAT, Staufen, Germany) was used for all titrations. Titrations were performed under a flow of nitrogen using an Orion conductivity cell 018010 (cell constant $K = 0.987 \text{ cm}^{-1}$) attached to a Fisher Scientific accumet pH meter 50. Following neutralization with sodium hydroxide, the suspension was concentrated by evaporation at ambient conditions.

Chart 1. Dyes used.

Dye	Structure	FW	Purity ^a
H	<p>The structure of Hematein (H) is a complex polycyclic molecule. It features a central indole-like ring system fused to a benzene ring. The indole ring has a hydroxyl group at the 3-position and a methyl group at the 2-position. The benzene ring has two hydroxyl groups at the 4 and 5 positions. The indole ring is further substituted with a hydroxyl group at the 1-position and a methyl group at the 3-position. The structure is shown with stereochemistry at the 2-position.</p>	302.27	— ^b
AO	<p>The structure of Auramine O (AO) is a triphenylmethane dye. It consists of a central carbon atom bonded to three phenyl rings. The central carbon is also bonded to a nitrogen atom that is part of a quaternary ammonium salt, with a chloride ion counterion. The nitrogen atom is bonded to three methyl groups. The phenyl rings are substituted with methyl groups at the 2 and 6 positions.</p>	301.85	— ^b
MG	<p>The structure of Methylene Blue (MG) is a phenothiazine dye. It consists of a central carbon atom bonded to three phenyl rings. The central carbon is also bonded to a nitrogen atom that is part of a quaternary ammonium salt, with a chloride ion counterion. The nitrogen atom is bonded to three methyl groups. The phenyl rings are substituted with methyl groups at the 2 and 6 positions.</p>	514.61	98%
PhB	<p>The structure of Phenolphthalein (PhB) is a complex polycyclic molecule. It features a central carbon atom bonded to three phenyl rings. The central carbon is also bonded to a nitrogen atom that is part of a quaternary ammonium salt, with a chloride ion counterion. The nitrogen atom is bonded to three methyl groups. The phenyl rings are substituted with methyl groups at the 2 and 6 positions.</p>	829.63	92%
AR 66	<p>The structure of AR 66 is a complex polycyclic molecule. It features a central carbon atom bonded to three phenyl rings. The central carbon is also bonded to a nitrogen atom that is part of a quaternary ammonium salt, with a chloride ion counterion. The nitrogen atom is bonded to three methyl groups. The phenyl rings are substituted with methyl groups at the 2 and 6 positions.</p>	556.50	— ^b

Chart 1. Continued

Dye	Structure	FW	Purity ^a
CB		840.12	55%
AR 112		744.58	– ^b
DR 2		724.76	– ^b
CR		696.70	91%
DB 1		992.84	– ^b

^a Dye purity given by manufacturer

^b Dye purity not given; assumed to be 100%

Samples

Samples were prepared by adding solid dye to aliquots of concentrated liquid crystalline cellulose nanocrystal suspension and vortexing until homogeneous dispersion was achieved. Suspensions were allowed to equilibrate over a period of at least two weeks.

Results and discussion

Effect of dyes on phase behaviour

To demonstrate the effects of charged groups on dye-induced phase separation, neutral, cationic and anionic dyes were added to anisotropic cellulose suspensions as shown in Table 1.

Table 1. Effect of neutral, cationic and anionic dyes ($\sim 0.80 \mu\text{mol}$ per ml 14 wt% suspension) on induced separation of an isotropic phase.

Dye	Type	Cellulose binding?	# Charged groups	Ionic strength (μM)	$\phi_{\text{iso phase}}$
H	Neutral	No	0	0	–
AO	Cationic	No	1	0.77	–
MG	Cationic	No	1	0.77	–
PhB	Anionic	No	2 ^a	2.01	0.17
AR 66	Anionic	No	2	2.03	0.17
CB	Anionic	No	3	3.90	0.27
AR 112	Anionic	No	4	6.72	0.27
CR	Anionic	Yes	2	1.88	–
DR 2	Anionic	Yes	2	1.90	–
DB 1	Anionic	Yes	4	6.38	–

^a Phloxine B dye carries two negative charges at pH values greater than 5 (Birkedal-Hansen 1973).

The neutral dye hematoxylin did not induce phase separation, presumably since it has no charge to alter the ionic strength. The cationic dyes AO and MG did not dissolve completely in the nanocrystal suspensions; a small amount of solid remained at the bottom of the sample vials. No phase separation was observed in the suspension above the undissolved solid (which contained dissolved dye). However, this may be explained by the significantly lower nominal ionic strengths of the samples containing cationic dyes compared to the other samples (Table 1). In addition, the positively charged dye molecules may be attracted to the negatively charged cellulose nanocrystals, impeding phase separation.

Dyes having different numbers of anionic groups were added to vials of anisotropic cellulose suspension (14 wt% cellulose) at a concentration of $0.8 \mu\text{mol}$ per ml suspension. AR 66, PhB, CB and AR 112 induced phase separation according to the number of anionic groups they contain; the volume fraction of isotropic phase was identical for the samples containing the divalent species AR 66 and PhB, and somewhat larger for CB and AR 112. However, the extent of phase separation is the same for these last two dyes, which is unexpected, as the ionic strength due to AR 112 is much higher (Table 1). This may be explained in one of two ways: (a) there is a real plateau in the extent of the induced phase separation above a certain ionic strength, or (b) the purity of AR 112 is significantly lower than

the assumed value of 1.00. The anionic direct dyes CR, DR 2 and DB 1 bind strongly to cellulose in neutral aqueous solution and are used extensively in the textile industry (Giles 1989). Direct dyes have been used to determine pore structure and accessible surface area of cotton fibres and microcrystalline materials for many years (Inglesby and Zeronian 1996). Congo Red in particular is known to have a very high affinity for cellulose; it has been used in histochemical studies of plant cell walls and as an additive to “separate” fibres in the paper industry (Lipic and Bravar 1972). CR is also known to interact with starch and amylose as well as β -(1 \rightarrow 3)-D-glucans as demonstrated by absorption and circular dichroism studies (Carroll and Cheung 1962; Ogawa and Tsurugi 1973; Trisnadi et al. 1974; Ogawa and Hatano 1978; Wood, 1980). None of the direct dyes induced phase separation (Table 1), presumably because they bind to the surface of the cellulose nanocrystals and therefore do not compress the electrostatic double layer (they contribute to it and increase the repulsive forces between the cellulose nanocrystals, which will prevent phase separation).

A comparison of samples containing PhB at various concentrations (Table 2) shows that increasing the dye concentration (and hence the ionic strength) results in the formation of greater volume fractions of isotropic phase. Increasing the concentration of the cellulose-binding dye Congo Red does not lead to phase separation.

Purity of the dyes

Commercial dyes are rarely sold in a pure form; in addition to isomers of the main product, they often contain stabilizing and diluent agents such as salts, sugar, dextrin, urea, boric acid, as well as

Table 2. Effect of increasing dye concentration on induced phase separation.

Dye	Cellulose binding?	# Charged groups	Ionic strength (μM)	$\phi_{\text{iso phase}}$
PhB	No	2	1.11	0.11
PhB	No	2	1.56	0.14
PhB	No	2	2.01	0.17
CR	Yes	2	0.97	–
CR	Yes	2	1.52	–
CR	Yes	2	1.71	–

dispersants and anti-dusting agents (Kissa 1977). In the case of Cibacron blue 3G-A[®], the impurities are likely to be salts such as NaCl or Na₂SO₄, urea, and dodecyl alcohol (D. Lewis, personal communication). Because the exact quantity of “ionic material” in each batch of dye is unknown (and indeed likely varies from batch to batch), the final ionic strength of a solution of a given dye cannot be accurately calculated.

Phase separation mechanism

Phase separation of an anisotropic suspension to yield an isotropic phase can be interpreted as an increase in the critical concentration for phase separation (or equivalently, an increase in the upper concentration limit required for the formation of a completely anisotropic sample). The system is effectively shifted into the isotropic-chiral nematic coexistence region.

Therefore, if adding the dye causes this phase separation, it is because of (a) increased ionic strength, which alters the effective dimensions/diameters of the cellulose nanocrystals; (b) a change in the force balance of the system such as the hydrophobic attraction and steric repulsion referred to by Dong and Gray for large organic counterions (Dong and Gray 1997); or a combination of (a) and (b).

In light of the above results, it is interesting to note that simple 1:1 electrolytes such as NaCl do not appear to have the effect on phase separation that an equivalent ionic strength of added dye does. Even at up to 1000 times the nominal added ionic strength of samples containing free CB dye or blue dextran, added NaCl does not induce phase separation in an identical cellulose nanocrystal suspension.¹ The reason for these apparently inconsistent results is not entirely clear. The high cellulose concentration of the suspensions relative to the added dyes or electrolytes may provide a partial explanation. The electrostatic repulsive force in the suspension originates from the surface sulfate ester groups on the nanocrystals. In this case, the cellulose suspensions have been neutralized

to pH \sim 7 with sodium hydroxide. All the surface acid groups (sulfate ester, carboxylate) therefore have sodium counterions; the surface charge density of the nanocrystals is maximized (0.12 e/nm²). A “back-of-the-envelope” calculation gives the concentration of sodium counterions after neutralization as approximately 22 mM, a much larger value than the concentrations of added NaCl or dye. As a rule, when the ionic strength of the suspension is increased, the electrical double layer is compressed and the particles can be packed more closely, thereby increasing the critical concentration for phase separation. However, because of this relatively high surface charge density, the contribution of the cellulose nanocrystals to ionic strength becomes important as the concentration increases, which results in double layer compression and diminishes the effect of added salt on the critical concentration. Li et al. observed this phenomenon for chitin crystallite suspensions (Li et al. 1996). This may explain the failure to induce phase separation by modest concentrations of sodium chloride. According to this argument, the dye-induced phase separation cannot be attributed to ionic strength alone.

If the phase separation is not due to the (calculated) ionic strength, could it be caused by size effects? The polyvalent molecular dyes ($z = 2-4$) used in these experiments are much larger than the simple monovalent sodium chloride. However, the dye molecules themselves are not large enough to induce depletion attractions, as even dextran T-2000 ($R_g = 34$ nm (Nordmeier 1993)), has not been found to do so for these suspensions (Beck-Candanedo et al. in press); neither does the neutral dye hematoxylin cause any phase separation.

The above results suggest that the polyvalence and/or the distribution of the charges on the dye species may account for their greater effectiveness in inducing phase separation in our cellulose suspensions. For example, the hydration radius may be greatly enlarged as compared to a simple ionic salt, due to the “cloud” of two to four counterions spread over the larger molecule. The repulsive electrostatic forces contributed by the negative charges on the anionic dye molecules are longer range than, for example, the van der Waals forces contributed by the neutral hematoxylin. It is also worth remembering that dyes are known to stack in solution above certain concentrations (5 μ M for CB

¹Blue dextrans were prepared according to literature methods (Mayes et al. 1992; Tang et al. 2004). The final products were purified to remove excess salt and free dye after preparation. Gel filtration indicated the absence of low-molecular-weight material of high absorbance.

dye), which would lead to an effective increase in size of the ionic species in suspension (Federici et al. 1985; Mayes et al. 1992).

Conclusions

Adding ionic dyes of varying charge caused phase separation of an isotropic phase in initially anisotropic cellulose nanocrystal suspensions. Anionic dyes induce phase separation at much lower ionic strengths than simple 1:1 electrolytes (e.g., sodium chloride), possibly because of their polyvalence and larger hydration radius. Cationic and cellulose-binding anionic dyes do not cause phase separation in the cellulose suspensions, presumably because of their electrostatic attraction and chemical binding, respectively, to the cellulose nanocrystal surfaces.

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