Quantification of Optical Chirality in Cellulose Nanocrystal Films Prepared by Shear-Coating

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Abstract: Evaporation-induced-self-assembly is widely used to produce chiral cellulose nanocrystal (CNC) free-standing films reflecting left-handed polarized light. Research on supported chiral CNC films is rather scarce. The reflection and/or transmission of unpolarized light are the most common optical techniques used to characterize the selective reflection of CNC films whereas the use of techniques to quantify chiral properties is limited. Here, the fabrication of chiral CNC films supported on glass substrates by a shear-coating method, as well as a full characterization of their polarization properties, are reported. Optical chirality is evidenced in films, showing a brilliant blue structural color when viewed through a left-handed polarizer and darkness through a right-handed polarizer. Mueller-matrix data in the reflection and transmission modes are used to quantitatively characterize the structural origin of color in the films. The quantification of the linear and circular birefringence, as well as circular dichroism, is performed by analytical inversion of the Mueller matrix data in the transmission mode and regression analysis using Tellegen constitutive equations. The equivalence of the two methods to quantify the structural chirality in CNC films is demonstrated. The swelling of films in water and kinetics during drying is studied by reflection spectroscopy.

Keywords: cellulose nanocrystals; chirality; structural color; Mueller matrix; humidity sensor

1. Introduction

Cellulose nanocrystals (CNCs) extracted by acid hydrolysis from cellulose fibrils have seen increasing interest from the scientific community because of their sustainability, large availability, and low cost, as well as for their potential to create nanostructured materials with optical properties that can be exploited as humidity sensors [1], conductive and thermal responsive devices [2], and optical filters [3]. Recent reviews account for the widespread range of potential applications of CNC materials [4–6].

Depending on concentration, a CNC suspension can be in an isotropic in a biphasic or in an anisotropic phase, and at higher concentrations a gel is formed [7]. The anisotropic phase, which corresponds to chiral nematic liquid crystal ordering is of interest for the manufacture of chiral films. In a suspension of CNC above a critical concentration, nucleation of the anisotropic phase occurs forming anisotropic droplets (tactoids) surrounded by an isotropic environment. Gravity causes sedimentation, giving rise to a phase separation, which can be detected macroscopically. The isotropic phase appears dark when observed through crossed linear polarizers, whereas the anisotropy phase appears bright [8]. The
phase separation is a phenomenon that can take anywhere from a few hours to several weeks [9].

CNC films formed from slow evaporation of a suspension in the nematic chiral phase preserve their arrangement [9]. The chiral nematic ordering is characterized by CNC preferentially oriented in pseudo-planes along a certain unit vector (the director). The director of adjacent pseudo-planes is rotated by a small constant angle about the helix axis. The pitch ($\Lambda$) of the structure corresponds to the distance separating two pseudo-planes when the director has completed a full rotation of 360°. The helical microstructure in the chiral film enables selective Bragg reflection, which refers to the reflection of left circular polarized light in a spectral range centered at wavelength $\lambda_{\text{max}}$ [10],

$$\lambda_{\text{max}} = n_{\text{av}} \Lambda \cos \theta_t,$$

where $n_{\text{av}}$ is the in-plane average refractive index and $\theta_t$ the angle of wave propagation in the film and is determined from the law of refraction, $n_a \sin \theta = n_{\text{av}} \sin \theta_t$, where $\theta$ is the angle of incidence and $n_a$ is the refractive index of the surrounding ambient. However, there are many factors involved during the drying process that interfere with the collapse of the microstructure, leading to the production of defects in the films. To achieve a homogeneous pitch and helical axis orientation in areas of hundreds of square micrometers is a current challenge, and is the focus of attention for several research groups. Several approaches have been reported to manufacture homogenous CNC chiral films, like phase separation [11], application of orbital shear flow during drying [12], planar anchoring [13], or promoting tactoid annealing [14]. Obtaining a homogeneous microstructure, controlling both the orientation of the helical axis and the pitch, results in films with a reflection of left circular light in a narrow range of wavelengths, which is a desirable characteristic for their use in applications.

In addition to film casting, the use of a doctor blade method for the preparation of CNC films on glass substrates has been reported [15]. In that work, birefringent films were obtained because of the alignment of the CNC in the direction of the applied shear stress [15]. Other authors used a similar method based on convective-shear assembly to obtain the same alignment effect of CNC on gold and silica substrates [16]. Dip coating of non-sonicated CNC suspensions also produce birefringent films, as reported by our group [17].

To characterize the chiral response of self-assembled CNC films, circular dichroism spectroscopy has been used, but often the strong signal produces saturation in the detector [1]. Furthermore, causality requires that circular birefringence should be determined too. A complete description of the polarization and depolarization properties of a sample is obtained by using the Mueller matrix approach [18]. In this approach, a $4 \times 4$ matrix representing the sample can be determined from measurements in the reflection or transmission modes. Previously, a Mueller matrix approach was used to characterize chiral CNC free-standing films with a mosaic-like texture, that is, films with a wide range in color reflected by each color domain [19].

In this work, we report the fabrication of glass-supported CNC chiral films by shear-coating. The films display an iridescent structural color in areas of cm$^2$. Mueller matrix measurements at several angles of incidence in the reflection mode, as well as transmission at normal incidence, are used to characterize the optical response of the CNC films. The elementary polarization properties of the films are extracted from the experimental Mueller matrix in transmission mode, by analytical inversion and by regression analysis using Tellegen constitutive equations as well. Immersion of the films in deionized water shows the swelling property and the kinetics of the drying is studied by reflection spectroscopy.

2. Materials and Methods

2.1. Preparation of the CNC Suspension

CNC aqueous suspensions were prepared as reported in the literature [9,20]. Ashless filter paper (Whatman40) was chosen as a source of cellulose. An amount of 8 g was milled
on a miller of coffee type to increase the surface area (three cycles of 30 s each). The milled filter paper was hydrolyzed with 70 mL of 64 wt% sulfuric acid (J. T. Baker) at 60 °C for 60 min under mechanical stirring. To stop the reaction, 700 mL of cold distilled water was added and left to rest for 24 h. The top clear layer was decanted, and the bottom part was centrifuged three times at 9000 rpm for 10 min. These washes helped to separate the CNC from the amorphous parts that were hydrolyzed. To continue the isolation of the nanocrystals, the suspension was dialyzed against distilled water with a dialysis tubing cellulose membrane (Sigma Aldrich, St. Louis, MO, USA) for a week with periodic changes of water. The resultant viscous suspension was left to evaporate under continuous stirring until it reached a 6.5 wt% concentration. Ultrasonic power was not applied to disperse the suspension, but it was left undisturbed for 18 days at room temperature before use.

2.2. Preparation of Glass-Supported Films by Shear-Coating

Glass slides of 25 × 75 mm (Corning 2947) washed with water and detergent were used as the substrates and coater plates. The procedure is very similar to that reported by Hoeguer et al. [16]. A schematic representation is shown in Figure 1. The substrate was placed on a horizontal surface, and a 0.5 mL drop of CNC suspension was deposited on one end of the glass slide (Figure 1a). The coater plate was then placed with an inclination of approximately 35° with respect to the substrate, and was put in contact with the suspension, which adhered through capillary forces, as depicted in Figure 1b. Then, the suspension was distributed to the other side of the substrate by moving the coater plate at 5 mm/s in the direction indicated with the red arrow in Figure 1c (the substrate remained fixed). The sample with the suspension distributed was placed inside a Petri dish (90 mm diameter) on a horizontal surface. To promote slow water evaporation, water droplets were added around the coated-glass slide and the Petri dish was covered (Figure 1d). This drying scheme allows for more time for helix ordering before the kinetic arrest of the microstructure [13]. The evaporation took three days at room temperature.

![Figure 1. Schematics of the preparation method: (a) a drop of cellulose nanocrystal (CNC) suspension is deposited onto a glass substrate; (b) another glass slide is placed so that the suspension adheres to it; (c) the coater plate is moved to distribute the suspension; (d) the sample is placed inside a petri dish for the slow evaporation of water in a humid environment.](image)

2.3. Characterization Techniques

The films were imaged using polarized optical microscopy (POM) with an Olympus BX60 optical microscope equipped with a Hitachi KP-D50 color digital camera. Left- and right-handed sheet polarizers (Edmund Optics, Barrington, NJ, USA) were used to evidence optical chirality. Mueller matrix measurements in transmission and reflection modes were performed with a dual rotating compensator spectroscopic ellipsometer (RC2, J. A. Woollam Co., Inc., Lincoln, NE, USA). A collimated beam (4 mm in diameter) was used for measurements in transmission mode at normal incidence in the wavelength (λ) range of 210–1690 nm. For these measurements, the shear direction was placed vertically
(along the y-axis) and the beam path was assumed along the z-axis. In the reflection mode the measurements were taken at angles of incidence (θ) from 20° to 70° in steps of 5° using focusing probes, whereby areas with a diameter below 100 μm in were sampled [19,21]. The use of focusing probes limited the wavelength range to 210–1000 nm. Regression analysis was performed with the WVASE software (RC2, J. A. Woollam Co., Inc., Lincoln, NE, USA). The reflectance spectra of unpolarized light were measured in the wavelength range of 350–1000 nm with a CHEM4 system (Ocean Optics, Inc., Dunedin, FL, USA) using an optical fibre probe. The latter had six illumination fibres around one read fibre. The effective angle of incidence was 25° with a spot size of about 0.4 mm in diameter. A silicon wafer was used to calibrate the measurements. The thicknesses of the films were determined from scanning electron microscopy images using Phillips XL30 ESEM equipment. To avoid overcharging, a thin layer of conductive graphite was deposited using a Denton Vacuum Desk V using argon as the carrier gas.

3. Results and Discussion
3.1. Visual Appearance of CNC Films

Figure 2a shows an image of a CNC film taken through a left circular polarizer. As can be seen, a bright blue color extends over areas of several cm² with border effects. In a visual test of the selective Bragg reflection, it is observed that the sample appears dark when viewed through a right circular polarizer, as shown in Figure 2b. This optical chirality is indicative of a left-handed chiral structure in the CNC film. The reflection of the left-handed polarization implies the transmission of the right-handed complementary polarization. The quantification of these effects is discussed below in Section 3.2. Figure 2c shows a POM image of the center of the film. At this scale, the color domains are blue and cover areas of hundreds of square micrometers. The small variation of the color of the domains is indicative of a small variation in pitch. In summary, although the deposition method used in this work applies a shear stress on the suspension, it is not strong enough to break the tactoids in the chiral nematic liquid crystal phase, but might cause elongation in the direction in which the stress was exerted [7].

![Figure 2](image_url)

**Figure 2.** Photography of a CNC film through (a) left and (b) right circular polarizers. (c) Polarized optical microscopy (POM) image in reflection mode. Scale bars are 1 cm in (a,b), and 250 μm in (c).

The fabrication of supported CNC films by shear-coating methods has been reported to produce films with ordered/oriented CNC [15,16,22]. Doctor blade coating of a highly concentrated CNC suspension (11.8 wt%) aligned the CNC along the direction of the applied stress, producing birefringent films with a thickness of about 30 μm [15]. In another report, ultrathin films (thicknesses in the range 17–85 nm) with ordered CNC were shear-coated using a low volume (10 μL) of a ramie CNC suspension at a concentration 2.5% [16]. Other authors reported a study of blade coated films from sonicated CNC suspensions using hydrolyzed filter paper [22]. In that work, the films prepared from a suspension in the biphasic regime (6.98 vol%) lost the birefringent effect in a few minutes, relaxing to a polydomain structure. However, the spectroscopic properties were not reported. A suspension in the anisotropic phase (11.6 vol%) was required in order to produce a permanent alignment of CNC [22]. In the present work, a non-sonicated suspension in the
biphasic regime (6.5 wt%) stored for 18 days was used to produce CNC chiral films. It is important to emphasize the importance of the storage time of the suspension as the films produced with a fresh suspension did not show colors. This proves that the formation of the anisotropic phase takes time after preparation from a non-sonicated CNC suspension. A detailed study on the effect of the storage time on non-sonicated suspensions will be reported elsewhere.

3.2. Polarization Properties of CNC Chiral Films

A complete description of the polarization and depolarization properties of a linear optical system is given by the Stokes–Mueller formalism [18]. In this approach, light beams are assumed to travel along the z-axis and are represented by column vectors \( \mathbf{S} = [I, Q, U, V]^T \) (T means transpose), where \( I \) represents the total irradiance, \( Q > 0 \) \((-\infty, 0)\) accounts the tendency for linear polarization along the \( x \)-axis \((y\)-axis), \( U > 0 \) \((<0)\) is the tendency for linear polarization at \(+45^\circ \) \((-45^\circ)\) in the \( xy \)-plane, and \( V > 0 \) \((<0)\) the right \((\) left\()\) circular character of polarization. After interacting with a sample, the Stokes vector of the resulting beam is given by \( \mathbf{S}' = \mathbf{MS} \), where \( \mathbf{M} = [M_{ij}] \) \((i, j = 1 \ldots 4)\) is the \( 4 \times 4 \) Mueller matrix representing the sample. In this work, normalized Mueller matrices \( m_{ij} = M_{ij}/M_{11} \) and Stokes vectors \( (S_0 = 1) \) are used. For measurements in the reflection mode at the oblique incidence, the \( xy \) axes were replaced with those parallel \((p)\) and perpendicular \((s)\) to the plane of incidence.

Figure 3 shows Mueller matrices measured in the reflection mode as a contour map representation. The angle of incidence dependence is on the vertical axis and the wavelength dependence is on the horizontal axis. This contour map looks similar to that reported for the beetle \textit{Cotinis mutabilis} [21] and displays the same symmetries among elements, as reported for multidomain free-standing CNC chiral films [19]. In general, the symmetries expected for a chiral system are fulfilled [23], as follows: \( m_{12} = m_{21}, m_{13} = -m_{31}, m_{14} = m_{41}, m_{23} = -m_{32}, m_{24} = m_{42} \), and \( m_{34} = -m_{43} \). As for unpolarized incident light \( \mathbf{S} = [0, 0, 0, 1]^T \), the reflected beam is given as \( \mathbf{S}' = \mathbf{MS} = [1, m_{21}, m_{31}, m_{41}]^T \). The negative values in the \( m_{41} \) element for small angles of incidence account for selective Bragg reflection with left-handed polarization. Homogeneous blue color was perceived on large areas of the CNC film, as shown in Figure 2, so it was expected to find selective reflection at short wavelengths of the visible range. Indeed, for an angle of incidence \( 20^\circ \), \( m_{41} \) had a minimum at about 400 nm and the band of selective reflection extended to the visible range. The spectra of the normalized Mueller matrices are shown in Figure A1 in Appendix A. The minimum in \( m_{41} \) shifted to shorter wavelengths as the angle of incidence increased, which is indicative of iridescence, a property distinctive of self-assembled CNC films.

![Figure 3. Contour map of the reflection Mueller matrix of a CNC glass-supported film. Notice the reflection band with negative values in the \( m_{41} \) element.](image-url)
The experimental Mueller matrix in transmission mode at normal incidence shows selective Bragg reflection in a narrow range, as can be noticed in Figure A2 in Appendix A. Data below a wavelength 300 nm were omitted because of the strong absorption of the glass substrate. To highlight the most important features, Figure 4 shows the experimental data (continuous curves) in the range of 300–600 nm. Thus, for unpolarized incident light the transmitted beam is right-handed polarized (\( m_{41} > 0 \)) in a band centered at a wavelength of about 415 nm. The latter value agrees with that of 400 nm found for the reflection at \( \theta = 20^\circ \), according to Equation (1), taking \( n_{av} = 1.54 \) for cellulose [24]. The mean pitch is about 270 nm. Data represented with dashed curves in Figure 4 correspond to the fit described below in Section 3.4.

![Figure 4](image)

**Figure 4.** Experimental Mueller matrix in transmission mode at normal incidence. The fit corresponds to Tellegen constitutive equations.

To quantify the polarization properties, the capability of the system to depolarize incident polarized light is tested first. This can be done by the polarization index \( P_\Delta \) of a Mueller matrix \( \mathbf{M} \) given by [25],

\[
P_\Delta = \sqrt{\frac{\text{tr}\mathbf{M}^T\mathbf{M} - 1}{3}},
\]

where tr stands for trace. A value of \( P_\Delta = 0 \) corresponds to an ideal depolarizer and \( P_\Delta = 1 \) to a non-depolarizing system. As can be seen in Figure 5, \( P_\Delta \) for \( \mathbf{M} \) measured in the transmission mode shows that the chiral CNC film practically does not depolarize incident polarized light, because the average value of \( P_\Delta \) is 0.998 ± 0.004. On the other hand, \( P_\Delta \) for measurements in the reflection mode appreciably deviates from unity in the band of selective reflection (Figure A3 in Appendix A).

![Figure 5](image)

**Figure 5.** Polarization index from Equation (2) of the experimental Mueller matrix in Figure 4.
The difference in $P_\Delta$ between normal incidence transmission and oblique incidence reflection measurements can be understood in terms of the model developed for the cuticle of scarab beetles with selective Bragg reflection [26]. In that model, non-uniformity in the thickness was assumed and ideal Mueller matrices were calculated for nine thicknesses ($d$) within the interval $d - \Delta d$ and $d + \Delta d$. The Gaussian weighted average of those ideal Mueller matrices represent lateral variations in the chiral structure, which gives rise to incoherent superposition of the light reflected from the non-uniform cuticle causing light depolarization. In the cuticle of beetles, it was found that a non-uniformity in thickness of about 2.4% produced deviations in $P_\Delta$ of the same order as those observed for the CNC chiral films investigated here (Figure A3 in Appendix A). From a cross-sectional SEM image of the film (Figure A4 in Appendix A), a thickness $d = 18.1 \pm 0.4$ $\mu$m was determined. The standard deviation gave a 2.2% non-uniformity, supporting the origin of depolarization in the present case as being analogous to the case of beetles. On the other hand, for measurements in transmission at normal incidence, the emerging wave is built by a single trip across the film thickness and the left-handed polarization component is (partially) filtered out. As the variation in thickness is about 2%, only small differences are expected, and nearly coherent superposition is achieved. That is, the phase difference between the two orthogonal components of the incident polarized light changes continuously, but not randomly. A similar situation was reported for free-standing chiral CNC films, where $P_\Delta$ was noticeably larger for oblique reflection than for normal incidence transmission [19].

### 3.3. Elementary Polarization Properties from Normal Incidence Transmission Mueller Matrix

In the previous section, the reflection of left-handed polarization and the transmission of right-handed polarization in shear-coated CNC films were proven by the sign of the $m_{41}$ element of the measured Mueller matrices. However, the quantification of circular dichroism (CD), circular birefringence (CB), linear birefringence ($LB$, $LB'$), and linear dichroism ($LD$, $LD'$) requires a more complete analysis. The fact that $P_\Delta \approx 1$ in transmission, as shown in Figure 5, makes the use of an analytical expression to calculate the six elementary polarization properties from the experimental Mueller matrix possible [27]. The parameters $LB$ ($LD$) and $LB'$ ($LD'$) are the linear birefringence (dichroism) along the $x$-$y$ and $\pm 45^\circ$ axes, respectively. $LB$ and $LB'$ are related to the linear retardance $\delta$ as $LB = \delta \cos 2\varphi$ and $LB' = \delta \sin 2\varphi$, where $\varphi$ is the angle between the $xy$ frame and the principal axes frame of the sample. $LD$ and $LD'$ obey a similar relationship related to the diattenuation coefficient $p$ (the main property of a linear polarizer). To eliminate such orientational dependence, the linear retardance and linear diattenuation can be derived via $\delta = (LB^2 + LB'^2)^{1/2}$ and $p = (LD^2 + LD'^2)^{1/2}$, respectively.

Figure 6a shows that CD and CB are present for wavelengths below 500 nm. The linear retardance in Figure 6b is attributed to the alignment of some CNC in the direction of the applied shear during deposition. However, the values of $\delta$ are one order of magnitude smaller than those obtained in the dip-coated birefringent CNC films by our group [17]. It can also be noticed that there is a very low linear diattenuation. Data represented with dashed curves in Figure 6 correspond to the analysis described in Section 3.4 below.
Figure 6. Elementary polarization properties determined from the analytical inversion of experimental Mueller matrix data in Figure 4. (a) Circular dichroism (CD) and circular birefringence (CB). (b) Linear retardance ($\delta$) and linear diattenuation ($\rho$). The dashed curves correspond to properties determined using the Tellegen constitutive equations.

3.4. Effective Structural Chirality Parameter Determined from Tellegen Constitutive Equations

The structural chirality can also be assessed using the Tellegen constitutive equations, which has recently been applied to the scarab beetle *Cetonia aurata* [28]. For a reciprocal medium, the electromagnetic coupling is described by the chirality tensor $\kappa$, and Tellegen constitutive equations are given by the following

$$
\mathbf{D} = \varepsilon_0 \varepsilon \mathbf{E} + ie^{-1} \kappa \mathbf{H}
$$

$$
\mathbf{B} = -ie^{-1} \kappa \mathbf{E} + \mu_0 \mu \mathbf{H},
$$

where $\mathbf{D}$, $\mathbf{E}$, $\mathbf{B}$, and $\mathbf{H}$ are the electric displacement field, electric field, magnetic flux density, and magnetic field, respectively. The dielectric tensor $\varepsilon = \text{diag}(\varepsilon_x, \varepsilon_y, \varepsilon_z)$ in the principal axis frame, the complex chirality tensor $\kappa = \kappa \mathbf{I} (\kappa = \kappa' + i \kappa'')$, and the permeability tensor $\mu$, represent the materials properties (for non-magnetic materials $\mu = \mathbf{I}$); fundamental constants of vacuum are the speed of light $c$, permittivity $\varepsilon_0$, and permeability $\mu_0$. The refractive indices were modeled with the Cauchy expression, as follows

$$
n_j = A_j + \frac{B_j}{\lambda^2} + \frac{C_j}{\lambda^4},
$$

where $A_j$, $B_j$, and $C_j$ are fitting parameters and $\lambda$ is given in units of $\mu$m.

The chirality parameter was modeled with gaussian functions

$$
\kappa'' = \sum_{j=1}^{2} A_j \left[ \exp \left( -\left( \frac{E-E_0}{\Gamma_j} \right)^2 \right) - \exp \left( -\left( \frac{E+E_0}{\Gamma_j} \right)^2 \right) \right]
$$

$$
\kappa' = \kappa_0 + \text{KK}(\kappa_2),
$$

where $A_j$, $E_0$, and $\Gamma_j$ are the amplitude, central energy, and broadening, respectively; $\kappa_0$ is an offset; and KK refer to a Kramers-Kronig transformation. The thickness of the film is $d = 18.1 \pm 0.4 \mu$m, as determined from a cross-sectional SEM image (Figure A4 in Appendix A). The values of the fitting parameters in Equations (4) and (5) are shown in Tables 1 and 2, respectively. From the fitting, $\varphi = -180.2^\circ$ is obtained, which is consistent with the sample position during the measurement. As can be seen in Figure 4, Tellegen constitutive equations give a very good description of the experimental Mueller matrix. Furthermore, Figure 6a shows that circular polarization properties and the chirality parameter fulfill the relationship $\text{CB} + \lambda d = 4\pi d k / \lambda$. The retardation obtained from the analytical inversion agrees very well with that determined by the relationship $\delta = 2\pi d \Delta n / \lambda$ (Figure 6b), where $\Delta n = n_y - n_x$ is the film birefringence.
Table 1. Cauchy parameters for \( n_x \) and \( n_y \) in Equation (4).

|   | \( A_j \)       | \( B_j \)       | \( C_j \)       |
|---|-----------------|-----------------|-----------------|
| \( n_x \) | 1.5773 ± 0.0001 | 0.0035 ± 0.002  | 0.00046 ± 5 \times 10^{-5} |
| \( n_y \) | 1.5750 ± 0.0001 | -7.5 \times 10^{-6} ± 0.32 | 5.1 \times 10^{-5} ± 2 \times 10^{-5} |

Table 2. Gaussian parameters for \( \kappa \) (offset \( \kappa_0 = -6.64 \times 10^{-4} \pm 4 \times 10^{-6} \)) in Equation (5).

| \( \kappa \) | \( A_j \)       | \( E_j \) (eV) | \( \Gamma_j \) (eV) |
|---|-----------------|----------------|------------------|
| \( \kappa_1 \) | 0.00055 ± 1 \times 10^{-5} | 2.787 ± 0.008 | 1.41 ± 0.01 |
| \( \kappa_2 \) | 0.00199 ± 1 \times 10^{-6} | 2.982 ± 0.001 | 0.497 ± 0.003 |

3.5. Swelling and Drying of CNC Chiral Films

CNC chiral films have been proposed as humidity colorimetric detectors, taking advantage of the swelling properties of cellulose materials. The swelling of the glass-supported CNC chiral films was investigated by immersing the film in deionized water for 1 min and then measuring the reflectance as a function of the drying time at room conditions (25 °C and 45% relative humidity). Figure 7a shows an image of the sample just after immersion, and as can be seen, the central part looked light green. After 1.3 min of drying, that part turned to light blue (Figure 7b), and finally became a strong blue as shown in Figure 7c. The time evolution of the reflectance spectra is shown in Figure 7d. In these measurements, \( \lambda_{\text{max}} \) in the dry film is located at 395 nm and shifts to 477 nm after immersion in water. This shows that the average pitch increases by about 20% as the film swells. The swelling is similar to that reported for neat CNC free-standing films [1,29]. As the film gets dry, the band of selective reflection monotonically shifts to shorter wavelengths. The experimental time-dependence of \( \lambda_{\text{max}} \) is shown with the symbols in Figure 7e, and the line corresponds to a fitting with an exponential decaying function, \( \lambda_{\text{max}} = \lambda_0 + A \exp(-t/\tau) \), where \( \lambda_0 = 395 \) nm is the wavelength for the reflection of the dry film, \( A = 85.1 \pm 2.1 \) nm and \( \tau = 1.20 \pm 0.06 \) min. The relaxation time \( \tau \) is of the same order of magnitude as reported for free-standing CNC films [30,31]. The introduction of highly hydrophilic molecules like glycerol [1,29] and polyols [32] in free-standing CNC films produces larger swelling. The improvement of the swelling capabilities of glass-supported CNC chiral films is the subject of ongoing research by our group.

![Figure 7](image_url)

Figure 7. (a) Image of a CNC film after immersion in deionized water, (b) after 1.3 min, and (c) after 5.8 min. (d) Time-dependence of the reflectance for unpolarized incident light. (e) Experimental (blue circles) wavelength maxima of selective reflections as a function of drying time; the red curve corresponds to a fitted exponential decay of \( \lambda_{\text{max}} \).
4. Conclusions

Glass-supported CNC chiral films were obtained from non-sonicated CNC suspensions after 18 days of equilibration. The films display a blue color in zones of several cm². Mueller matrix spectroscopic measurements demonstrated iridescence and reflection of left-handed polarized light in a band centered at 400 nm. The transmission of right-handed polarized light was demonstrated at normal incidence. Linear and circular birefringence, as well as linear and circular dichroism, were obtained by analytical inversion of the non-depolarizing Mueller matrix. Those polarization parameters agree with those obtained from the regression analysis of the data using the Tellegen constitutive equations. The glass-supported chiral CNC films swelled when immersed in water and the drying kinetics was studied.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Figure A1. Mueller-matrix spectra at several angles of incidence measured in reflection mode.
Figure A2. Mueller matrix measured in transmission mode at normal incidence. Data below wavelength 300 nm are omitted because of the strong absorption of the glass substrate.

Figure A3. Polarization index of Mueller matrices at several angles of incidence in Figure A1.

Figure A4. Cross-sectional scanning electron microscopy image of the film.

References
1. Xu, M.; Li, W.; Ma, C.; Yu, H.; Wu, Y.; Wang, Y.; Chen, Z.; Li, J.; Liu, S. Multifunctional chiral nematic cellulose nanocrystals/glycerol structural colored nanocomposites for intelligent responsive films, photonic inks and iridescent coatings. *J. Mater. Chem. C* **2018**, *6*, 5391–5400. [CrossRef]
2. Santos, M.V.; Tercjak, A.; Gutierrez, J.; Barud, H.S.; Napoli, M.; Nalin, M.; Ribeiro, S.J.L. Optical sensor platform based on cellulose nanocrystals (CNC)—4’-(hexyloxy)-4-biphenylycarbonitrile (HOBC) bi-phase nematic liquid crystal composite films. *Carbohydr. Polym.* **2017**, *168*, 346–355. [CrossRef] [PubMed]
31. Shrestha, S.; Diaz, J.A.; Ghanbari, S.; Youngblood, J.P. Hygroscopic swelling determination of cellulose nanocrystal (CNC) films by polarized light microscopy digital image correlation. *Biomacromolecules* 2017, 18, 1482–1490. [CrossRef]

32. Meng, Y.; Cao, Y.; Ji, H.; Chen, J.; He, Z.; Long, Z.; Dong, C. Fabrication of environmental humidity-responsive iridescent films with cellulose nanocrystal/polyols. *Carbohydr. Polym.* 2020, 240, 116281. [CrossRef]