Klockars, Konrad W.; Greca, Luiz G.; Majoinen, Johanna; Mihhels, Karl; Rojas, Orlando J.; Tardy, Blaise L.

Drying stresses in cellulose nanocrystal coatings: Impact of molecular and macromolecular additives

Published in:
Carbohydrate Polymers

DOI:
10.1016/j.carbpol.2022.120465

Published: 01/03/2023

Document Version
Publisher's PDF, also known as Version of record

Published under the following license:
CC BY

Please cite the original version:

This material is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.
Drying stresses in cellulose nanocrystal coatings: Impact of molecular and macromolecular additives

Konrad W. Klockars a,1, Luiz G. Greca a,1, Johanna Majoinen a, Karl Mihhels a, Orlando J. Rojas a,b,*, Blaise L. Tardy a,c,**

a Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, FI-00076 Aalto, Finland
b Bioproducts Institute, Department of Chemical and Biological Engineering, Department of Chemistry and Department of Wood Science, University of British Columbia, 2360 East Mall, Vancouver, BC V6T 1Z4, Canada
c Department of Chemical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates

ARTICLE INFO
Keywords:
Cellulose nanocrystals
Drying stresses
Residual stress
Coating
Nanocellulose
Plasticizer

ABSTRACT
The industrial implementation of cellulose nanocrystals (CNCs) in films and coatings requires thorough evaluation of the internal stresses post-consolidation, as they cause fracturing and peeling. Characterizing the impact of plasticizing additives on stress is therefore critical. Herein, we use the deflection of thin glass substrates to measure drying stresses in consolidating CNC films, and benchmark the impact of five additives (glucose, glycerol, poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA) and bovine serum albumin). Glycerol and PEG reduced drying stresses effectively, while PEG of increased molecular weight (from 0.2 to 10 kDa), PVA, and BSA were less effective. We analyzed the temporal aspects of the process, where stress relaxation of up to 30 % was observed 2 years after coating formation. Finally, we provide a framework to evaluate the impact of CNC morphology on residual stresses. The introduced approach is expected to fast-track the optimization and implementation of coatings based on biocolloids.

1. Introduction
Natural biopolymers readily extracted from biomass are formidable building blocks for sustainable materials (Tardy et al., 2021). In particular, cellulose nanocrystals (CNCs) (Vanderfleet & Cranston, 2021) are currently considered for commercialization (Delepierre, Vanderfleet, Niinivaara, Zakani, & Cranston, 2021; Droguet al., 2022) due to their versatility as far as surface chemistry and morphology, remarkable mechanical strength, and optical response of their constructs. CNC suspensions can bare liquid crystalline properties, where the particles form helicoidal assemblies (Liu, Schütz, Salazar-Alvarez, & Bergström, 2019; Schütz et al., 2020). This long-range order can be preserved in the dried, consolidated state, resulting in structurally colored films with finely tunable color and intensity (Frika-Petesci, Kamita, Guidetti, & Vignolini, 2019; Klockars et al., 2018; Parker et al., 2018; Tran, Boott, & MacLachlan, 2020). CNC films bearing a nematic order, with a high relative alignment along a given axis, have also shown remarkable gas barrier properties (Nuruddin et al., 2020).

While the formulations and mechanical properties of CNC films and coatings, with and without plasticizers, have been discussed in the literature (Adstedt et al., 2020; Bardet, Belgacem, & Bras, 2015; de France, Kummer, Ren, Campioni, & Nyström, 2020; Natarajan & Gilman, 2018; Wang & Walther, 2015b; Yao, Meng, Bulone, & Zhou, 2017), the development and characterization of internal stresses (Francis, Mccormick, Vaessen, & Payne, 2002) during drying remains unexplored. This is despite the expectation that the optimization of the internal stresses of a coating system, considering formulation and substrate properties, have a great impact on durability (Leterrier, 2003), gas permeability (Dumont, Tornare, Leterrier, & Månson, 2007) and strength (Tardy et al., 2019). Furthermore, films subjected to internal

Abbreviations: CNC, cellulose nanocrystal; E-CNC, effective CNC; SEM, scanning electron microscopy; PEG, poly(ethylene glycol); PVA, poly(vinyl alcohol); BSA, bovine serum albumin; AFM, atomic force microscopy.

* Correspondence to: O. J. Rojas, Bioproducts Institute, Department of Chemical and Biological Engineering, Department of Chemistry and Department of Wood Science, University of British Columbia, 2360 East Mall, Vancouver, BC V6T 1Z4, Canada.
** Correspondence to: B. L. Tardy, Department of Chemical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates.
E-mail addresses: orlando.rojas@ubc.ca (O.J. Rojas), blaise.tardy@ku.ac.ae (B.L. Tardy).

https://doi.org/10.1016/j.carbpol.2022.120465
Received 1 July 2022; Received in revised form 7 December 2022; Accepted 10 December 2022
Available online 15 December 2022
0144-8617/© 2022 Published by Elsevier Ltd.
stresses fracture more easily than stress-free ones. This occurs as tensile residual stresses pre-load the material and thus facilitate fracture initiation and propagation upon further loading (Chiu, Garino, & Cima, 1993; Tirumukdulu & Russel, 2005). In the case of CNC coatings, residual stresses are far from negligible. For instance, 20-μm films can completely distort fabrics as thick as 100 μm or become fractured when coated on rigid substrates (Tardy et al., 2019). Therefore, there is a need to limit residual stresses, for instance, by the addition of oligomeric or polymeric additives (plasticizers). When considering additives in CNC films, it is essential to not only determine their effect on mechanical strength, but also on the internal stresses arising during drying. The effect of intercalation of polymeric plasticizers between the particles on film plasticity has been discussed in the literature related to CNC films (Adstedt et al., 2020; Bai et al., 2020; Boot, Soto, Hamad, & MacLachlan, 2021; de France et al., 2020; Therien-Aubin, Lukach, Pitch, & Kumacheva, 2015). Moderate additive loadings have been shown not to disrupt the chiral nematic order of the films (Bardet et al., 2015; Bast et al., 2021; Lin, Rahguwanshi, Browne, Simon, & Garnier, 2021; Mu & Gray, 2014; B. Wang & Walther, 2015a; Xu et al., 2018; Yao et al., 2017).

Typically, an increased plasticizer concentration improves film ductility at the cost of strength and stiffness. Furthermore, an increased moisture retention can yield enhanced plasticity. Both the functional groups in the additives, as well as their molecular weight, contribute to the overall properties of the system, although it is not yet known to what extent each of these factors contribute to stress development. Therefore, a simple and reproducible methodology to evaluate the internal stresses would be highly beneficial for future developments of biocolloidal materials.

Herein, we used the deflection of thin glass substrates to quantify the residual stresses of CNC films and to benchmark the impact of additives, such as poly(ethylene glycol) (PEG), glucose, glycercol, poly(vinyl alcohol) (PVA) and bovine serum albumin (BSA). The impact of additive loading was evaluated for PEG and glycercol, which led to a progressive stress reduction as the CNC:additive ratio changed from 10:0 to 10:4. The impact of molecular weight (Mw) was evaluated for PVA and PEG. The lowest Mw PEG (0.2 kDa) generated the highest reduction, while 10 kDa PEG and PVA were the least effective plasticizers even when compared to their higher Mw counterparts (i.e. up to 100 kDa). We used scanning electron microscopy (SEM) to correlate the internal stresses with the morphological features of fractured samples. A framework to normalize stresses to single particles is demonstrated for CNC films and the impact of aging on internal stress evolution was further investigated. The presented approach can be readily implemented across a range of biopolymeric formulations to benchmark the internal stresses that develop in nano- and microstructured films and coatings, facilitating fundamental and applied research that can lead to their commercial deployment.

2. Experimental section

2.1. Suspension preparation

The materials and the characterization methods used in this work are described in Experimental S1 and S3, respectively. Here, we briefly describe the preparation of the film-forming precursor. CNC suspensions were mixed with various water-soluble additives. In general, the additive was first fully dissolved in Milli-Q water and then mixed with a concentrate of the CNCs to obtain 10.37 wt% CNC aqueous suspension using a vortex stirrer (Scientific Industries Vortex genie 2), reaching CNC and additive concentrations of 5.5 wt% and 1.65 wt%, respectively. All the concentrations used in this work are expressed in weight percentage (g/g).

2.2. Drying stresses

In a typical experiment, a glass cantilever was coated with the CNC suspension and allowed to dry at 22 ± 1 °C and 20 ± 1 % RH to produce cantilever-supported CNC films (Experimental S2). The cantilever underwent deflection upon drying and, for selected samples, the full vertical deflection profile was measured over time by imaging the free end of the cantilevers (Video S1), using either an optical contact angle goniometer (KSV Instruments CAM 200) or a tensiometer (Biolin Scientific Attension Theta Flex). For other samples, the equilibrium deflection of the coated cantilevers was measured 20 ± 4 h after casting. Selected samples were also imaged (at 22 ± 1 °C and 20 % RH) after storage in ambient conditions for >2 years. Note: The relative humidity may vary between 10 % in the winter and 50 % in the summer. All measured cantilever deflections were normalized to the specific areal dry density of the CNC fraction in the film. Details are found in supporting information, Experimental S4.

For the control CNC sample, the residual stress was estimated from the cantilever deflection using Eq. 1 (Corcoran, 1969):

\[
\sigma = \frac{dE; t^2_i}{3L^2(t_i + t_f)(1 - \psi_f)^3} \frac{dE; (t_i + t_f)}{L^2(1 - \psi_f)}
\]

where d is the deflection of the cantilever (m), σ is the stress in the film (N/m²), E is the elastic modulus (N/m²), t is the thickness (m) (Table S1), L is the length of the coated area of the cantilever (m) and ψ is the Poisson’s ratio. The subscripts f and s stand for substrate (glass cantilever) and film, respectively. Only the first term in Eq. 1 was used, as the other represents <1 % of the stress value. Experimental S6 details the measurement of stress per single CNC in a film without additives. The assumptions associated with this estimate and related equations are presented in Experimental S6.

3. Results and discussion

3.1. Drying stresses in CNC films

In the formation of particulate films and coatings, internal stresses (Francis et al., 2002) arise when solvents evaporate from particle suspensions. The dried film retains these stresses to an extent which depends on the film components and the supramolecular bonds that formed during the drying. The stress develops upon gelation during CNC assembly (Honorato-Rios et al., 2016, 2018; Mu & Gray, 2014), and result from capillary bridges between CNCs, combined with the effect of pinning on the substrate. At the macroscopic, this is also seen as the elastic response of the colloidal gel. The consolidation of such a colloidal gel involves increasing capillary pressures, which promote CNC-CNC interfacial interactions (Ogawa, Nishiyama, & Mazaue, 2020; Tardy et al., 2021) that provide cohesion to the film to resist the formation of fractures.

Here, the cantilever deflection method (Stoney, 1909; Tardy et al., 2019) was employed to assess stress development, and the final residual stress. In brief, a suspension was poured onto a glass cantilever with one fixed and one free end. As the suspension dries into a solid film, the free end of the cantilever moves upwards, and the vertical position of the cantilever is monitored with time (Fig. 1a). The stress is calculated using the measured deflection with Eq. 1, with the deflection being directly proportional to the stress in the film. More information on the measurement of drying stresses is found in Discussion S1.

Three main assembly stages can be identified based on the deflection of the films. In the first, “initial drying” stage (Fig. 1a), the aqueous suspension is cast onto the substrate and water immediately starts to evaporate. With time, the interparticle distance is reduced, as the concentration increases, and the CNCs adhering to the glass substrate help to restrict the shrinkage of the contact line. This leads to a decreasing contact angle over time. The CNCs self-assemble into liquid crystalline domains (Klockars et al., 2018; Schütz et al., 2020; Wang, Hamad, & MacLachlan, 2016) and experience kinetic arrest upon gelation (Honorato-Rios et al., 2016, 2018; Mu & Gray, 2014), thereby maintaining the developed long-range chiral nematic architecture.
In the second stage, triggered by gelation, “increasing capillary forces” (Fig. 1a) acting at the menisci between CNCs in the consolidated network cause the onset of residual stresses (Ogawa et al., 2020; Tirumkudulu & Russel, 2004). Thereafter, the capillary forces drive the shrinkage of the CNC hydrogel, which can be described as being analogue to a shrinking spring whose ends are fixed to the glass substrate. Strong CNC-glass and CNC-CNC interactions restrict shrinkage, with further drying leading to increased tensile stresses in the film (Fig. 1b). As the stresses are coupled to the substrate, the cantilever experiences compressive and tensile stresses at the upper and lower sections, respectively. A bending moment is thus generated, and, because of the high length-to-width aspect ratio and low thickness of the substrate, the cantilever experiences an upward bending.

In the third, “final drying” stage (Fig. 1a), an equilibrium internal stress and the final mass of the film are reached as a function of relative humidity, as water cannot be further removed from the system. In this condition, the film is considered “dried” (Natarajan et al., 2017; O’Neill et al., 2017). The stress is retained in tensile mode due to the strong self-cohesion of the CNC network. This contrasts with other rigid particles, such as calcium carbonate (Price, Wu, McCormick, & Francis, 2015; Tirumkudulu & Russel, 2005; Wedin, Martinez, Lewis, Daicic, & Bergström, 2004), which tend to relax after reaching the maximum stress, due to a lack of strong interparticle interactions. In the case of silica/alumina particles, the maximum stress coincides with the formation of a consolidated network (Price et al., 2015), whereas for CNCs the stress only starts to build up around the consolidation. This is because consolidation, or kinetic arrest (Honorato-Rios et al., 2016, 2018; Mu & Gray, 2014), occurs at a lower concentration than for silica particles. Some stress relaxation mechanisms, which may have started at the second stage (increasing capillary forces), may take place through the onset of fractures, peeling-off, or rearrangement of the CNCs within the film (Chiu et al., 1993; Francis et al., 2002; Tirumkudulu & Russel, 2005). In the case of dried CNC films, in the absence of additives, the stress relief mechanisms are limited to fracture initiation and propagation as well as film peel-off.

3.2. Impact of additives on drying stresses

Various plasticizing additives have been used to increase the flexibility and toughness of colloidal films and coatings, including those
formed from CNCs, thereby reducing their brittleness. Despite generating similar stress reduction, their working mechanism greatly differs. Small molecules and certain polymers work by retaining water in the film (Kapanya, Somsunan, Phasayavan, Molloy, & Jiranusornkul, 2021; Kassem, Mattha, & El-Khatib, 1984). Some macromolecules, on the other hand, reduce the surface tension (Bhattacharya & Ray, 2004; Meriaux, May, Mansour, Chen, & Kaluza, 2018; Suttiprasit, Krisdhasima, & McGuire, 1992; Vagapova et al., 2013) and, consequently, capillary forces between the CNCs. In addition, the additives might reduce the interparticle interaction forces between the CNCs, thereby allowing some capillary-induced tensile stress to be relaxed as it develops.

Fig. 1a provides the residual stresses of a drying film as a function of time. Such evaluation, in general, is method-dependent (Dumont et al., 2007; Leterrier, 2003). Hence, Figs. 2–4 use the values of normalized residual stress, where the deflection is used “as is” without conversion to stress (Eq. 1), after normalization to the deflection of the CNC film without additives. This enables accurate evaluation of the effect of the additives on the stresses of CNC films. All deflection data was normalized to the areal density (kg/m²) of CNCs in each film, not the overall areal density (CNCs + additive) (Experimental S4).

Fig. 2b compares the deflection profiles of CNC films in the absence (gray) and presence of PEG100k (the subscript refers to the molecular weight of the polymer) at a CNC:PEG ratio of 10:3. The overall stress was significantly reduced for CNC films containing PEG100k, but the stress remains after reaching the “final drying” stage (Fig. 1a). The jagged data in the profile of the additive-free film shown in Fig. 2b, between 50 and 90 min, was likely caused by small fractures (Chiu et al., 1993; Tirumukudulu & Russel, 2005; Wedin et al., 2004), although cracks are not macroscopically visible in the fully dried film (Fig. 2c). In contrast, the slope for the PEG100k-containing film (Fig. 2b) is smoother, indicating that PEG may prevent microfracture formation. PEG100k itself did not significantly contribute to residual stresses (Fig. S4) and experienced comparatively negligible self-cohesion. Although PEG may decrease capillary forces by increasing the CNC interparticle distance and by reducing interfacial tension, the consequent reduction in residual stresses is minor considering the more than three-fold reduction of residual stress in relation to the neat CNC film. Therefore, PEG reduces the overall CNC-CNC supramolecular interactions, most noticeable after the onset of gelation. Importantly, PEG may not adsorb on CNC surfaces in the dispersion (Reid, Marway, et al., 2017) and interacts only weakly with CNCs in the dried film.
To investigate whether the presence of PEG reduces the residual stress by effectively lubricating the glass surface and reducing the attractive CNC-glass interactions, glass cantilevers were coated with a monolayer of PEG100k (Mathur & Moudgil, 1997). Deflection measurements were then carried out as for the reference CNC film without additive. As shown in Fig. S5, a PEG100k monolayer on the glass substrate did not reduce the residual stress.

Fig. 2c2 reveals the impact on the internal stresses of dried films of different additives added to the CNC suspensions prior to drying. Here, the equilibrium residual stress is reported for each additive and based on measurements performed ca. 20 h after the film was dried at given relative humidity (RH = 20 ± 1%) and temperature (T = 22 ± 1°C). A solids weight ratio of CNCs to additive of 10:3 was used for all films (23 wt% additive loading). Adstedt et al. (2020) predicted through modeling that a 10–25 % additive concentration is optimal to improve the mechanical performance while preserving structural color.

PEG100k, glucose and glycerol were the most effective stress reducers (>3-fold reduction). From a practical point of view, however, PEG100k is a preferred choice given the possible leaching of small molecules (Pongjanyakul & Puttipipatkhachorn, 2007), which is further discussed in Section 3.5. Note that the central section of the film did not develop vivid structural color (Fig. 2c2) which is likely a result of the light reflection occurring mainly in the UV region, as discussed in Section 3.7. Considering glucose and glycerol, the former was found more detrimental to the optical properties of the films, as can be seen in their edges, Fig. 2c2. This is possibly caused by a higher suspension viscosity when glucose was added (Telis, Telis-Romero, Mazzotti, & Gabas, 2007), which may cause early gelation (Mu & Gray, 2014), and reduce the time during which liquid crystal domains can reorient and merge into the well-ordered lamellar structures of the CNC films (Klockars et al., 2018). The network consolidation, i.e. gelation, at lower solids content could also lead to higher stress, as discussed by Croll (1979) and hence explain the more limited stress reduction of glucose compared to that of glycerol.

Both PVAs85–124k and BSA significantly reduced the stress, although the reduction was only about 30 %, compared to the >65 % reduction observed for glucose, PEG100k and glycerol. The contribution of self-interactions of PVA14k to the stress in PVA14k-containing CNC films was 11 % (Fig S4). Although BSA is not generally used as a plasticizer, it provided a similar stress reduction as PVAs85–124k. This might stem from a delayed gelation concentration (Croll, 1979). The hazy reflection of the BSA-containing CNC film (Fig. 2c2) might be linked to stress relief mechanisms. Ridge-like microstructures in the BSA-containing film were observed, whereas they were absent in the CNC reference film (Fig. S6). The CNC-BSA suspension used for casting had a pH of 7.08 (anionic BSA in its native, folded state) (Barbosa et al., 2010). Importantly, the standard deviation was significantly reduced in CNC films containing additives, possibly as a result of a higher thickness homogeneity and fewer defects, such as fractures and peeling. This greater homogeneity highlights the importance of using plasticizers and reducing stresses to attain more consistent films.

### 3.3. Effect of additive loading on residual stresses

PEG100k and glycerol were added at varied concentrations to CNC suspensions (Fig. 3). This has also been shown in previous work for CNC films containing PEG10k (Tardy et al., 2019). According to modelling by Adstedt et al. (2020), a 10 % additive loading (10:1 in Fig. 3) has no effects on the optical or mechanical properties, as the additives only fill the voids between the CNCs, which explains the similar residual stress measured at 10 wt% for these significantly different additives. The reduction in residual stresses at 10 wt% loading (Fig. 3) also showcases the difference between mechanical properties and residual stresses of CNC films. On the other hand, the same authors found that at above 30 % loading (10:4) the mechanical performance deteriorated and was combined with reflectance peak broadening (Bardet et al., 2015; Huang, Chen, Liang, Yang, & Shen, 2020; Lin et al., 2021; Xu et al., 2018). This places a CNCadditive ratio of 10:3 as suitable in terms of preserving mechanical and optical properties while reducing the residual stress.

### 3.4. Effect of polymeric additive molecular weight

The effect of molecular weight of the plasticizing additives on residual stresses was evaluated using PVA and PEG. PVA (Fig. 4a) exhibited a slight reduction in stresses when increasing the molecular weight of the additive from ca. 10 kDa to ca. 100 kDa. For PEG (Fig. 4b), using the same Mw range, the effects were similar. On relative terms, however, increasing the molecular weight of PEG reduced the stress more than in the case of PVA in the 10 to 100 kDa range. Specifically, the increase in molecular weight reduced the stresses by ca. 17.6 % and 10.6 % using PEG and PVA, respectively. Very high molecular weight PEG and PVA may disrupt the chiral nematic order formation in the dried CNC films (Lin et al., 2021). Larger macromolecules lower the overlap concentration (Chong, Smith, & Zelikin, 2013), leading to larger capillaries in the CNC film and thus, by impeding the chiral nematic order, reduces drying stress. However, applications that require good chiral nematic order would not benefit from this type of order-
suppressing stress reduction. SEM images (Fig. 5) reveal chiral nematic ordering in CNC films containing large polymers. Also, the above trends might fall below statistical significance given the small differences compared to the standard deviations.

In contrast to the previous trend, when decreasing the molecular weight of PEG from 10 to 0.2 kDa (Fig. 4b), the related stress reduction of 34.5 % is significant. This reduction can be associated to the higher efficiency of the smaller PEGs to disperse CNC aggregates (Cheng et al., 2015). This is possibly caused by a reduced depletion flocculation (Hubbe, Nanko, & McNeal, 2009), which would increase the extent to which smaller PEGs reduce the CNC-CNC interparticle interactions. Small molecules are also more effective in lowering the viscosity than macromolecules and thereby increase the network consolidation concentration, which further reduce the elastic response of the drying hydrogel (Groll, 1979). Therefore, small PVAs would also be expected to reduce the stress more than large ones. Kapanya et al. (2021) found that hydrogels based on poly(sodium 2-acrylamido-2-methylpropane sulfonate) and gelatin containing 5 % PEG 1.3k swelled 9 % more in water than those containing 5 % PEG 1.3k. This difference in hydration could also partially explain the lower reduction of stress for PEG 1.3k compared to PEG 0.2k.

3.5. Analysis of film cross-sections

To better understand the stress reduction mechanisms of different plasticizing additives, scanning electron microscopy was employed to observe structural differences between films containing different additives. Fig. 5a shows a jagged cross-section of a CNC film in the absence of additives, indicating the brittleness of the film and the presence of locally ordered large domains. The inset shows that the CNCs are organized in a chiral nematic manner. For PVA_{124k}-containing CNC films, Fig. 5b, a sharp and uniform fracture surface is obtained. At higher magnification, the upper inset reveals some plastic deformation with stretched-out polymer domains, possibly due to plastic deformation of additive-rich areas within the film. Yet, periodic chiral nematic order can also be observed (lower inset), indicating that PVA_{124k} does not adversely affect the formation of chiral nematic domains.

Fig. 5c displays a CNC film containing PEG_{100k} with roughly the same molecular weight as PVA_{124k} (Fig. 5b). The fracture surfaces are different even though the values of the molecular weight are similar. The PEG_{100k}-containing film has a rougher fracture surface with pulled-out fibrillar features. Nevertheless, the inset indicates well-ordered chiral nematic architecture. Overall, PEG seems to be distributed within the film more effectively than PVA, at least at a high molecular weight. Furthermore, the CNC film with PEG_{0.2k} (Fig. 5d) has the most distinct fracture surface. Some smooth domains formed from the oligomers are visible across the cross-section and a more extensive plastic deformation is also noticeable. Furthermore, a separate top segment lacking periodic ordering, most likely consisting of PEG_{0.2k}, indicates that it might diffuse over time (de Cuadro et al., 2015; Pongjanyakul & Puttipipatkhachorn, 2007) given the small size of the molecule.

Finally, the residual stress state observed for the different films (Fig. 2) correlated qualitatively with the degree of plastic deformation observed in the SEM images of the fractured surfaces (Fig. 5). Furthermore, the observations from the cross-sections support the use of PEG as an effective plasticizer. PEG_{0.2k}, however, behaved similarly to small plasticizers, which may diffuse out of the CNC film.

![Fig. 5. SEM images of cross-sections of films on glass cantilevers. The cross-section is along the width and in the middle of the film. a) CNC film without additives, where the inset shows periodic chiral nematic order. b) CNC film containing PVA_{124k}, where the upper inset shows stretched-out domains of PVA_{124k} while the bottom inset shows the preservation of periodic order in the film, despite the presence of PVA_{124k}. c) CNC film containing PEG_{100k}, where the inset shows the preservation of periodic order in the film, despite the presence of PEG_{100k}. d) CNC film containing PEG_{0.2k}. The ratio of CNCs to additive (CNC:additive) is 10:3 in b), c) and d), and 10:0 in a). The dashed blue frame is used to indicate what is expected to be a PEG-rich layer.](image-url)
3.6. Effect of aging

The cantilever-supported CNC films were stored for at least 2 years in ambient indoor conditions. Fig. 6 shows the % change in stress during this time, with most samples shown to lose approximately 20–30 % of the initial stress. Plastic deformation is not expected as a stress relief mechanism for the additive-free CNC film that consists only of rigid particles. Therefore, the stress reduction could result from submicrometric fractures. However, their macroscopic visual appearance was retained, with no apparent fracturing.

The BSA, PVAs and glucose additives caused slightly lower reductions in the stresses of the CNC films. They may have mitigated microfracture formation and propagation, which would decrease the reduction. The effect of molecular weight was minor for the PVAs, whereas it was considerable for the PEGs, as also shown in Fig. 4a. While all PEGs relaxed the stresses, the rate of the stress relaxation may depend on the molecular weight, where smaller molecular weight PEGs relaxed the stress faster. In other words, larger PEGs do not reach all their stress relaxation potential immediately but over time. Interestingly, the stress increased in the CNC film containing PEG0.2. This may be caused by the oligomeric PEG0.2 leaching out of the CNC film, as discussed in Section 3.5 and shown in the cross-section image of Fig. 5d. This would potentially induce a form of drying stresses, occurring over a long time compared to the evaporation of water, thus leading to increased stress in the film. The films containing glycerol also experienced an increased stress, where mechanisms similar to PEG0.2-containing films may have taken place.

Besides the effect of aging, other factors such as the drying rate (Beck, Bouchard, Chauve, & Berry, 2013; Natarajan et al., 2017) and thermal annealing (D’Acier et al., 2021) are expected to affect the residual stresses. Therefore, this same methodology may serve as a valuable tool to further investigate CNC film formation and their mechanical integrity under various conditions.

3.7. Stress distribution within CNC films

As biocolloidal particles are generally polydisperse, we herein analyzed the particle size distribution and discuss the contribution of morphological aspects to residual stress development. The dimensions of the CNCs used in this work were assessed by atomic force microscopy (AFM), Fig. S7. Fig. 7a shows a representative AFM micrograph where MountainsSPIP Expert software was used to differentiate CNCs from the background. The convexity ratio (Experimental S5) was used to differentiate single CNCs (shown in yellow) from aggregates (shown in blue). The yellow population is assumed to describe the degree of individualization present in the suspension, whereas the blue population are assumed to be drying-induced aggregates. The single CNCs, however, may also be aggregates, and are therefore called “effective” CNCs (E-CNCs). For the purposes of EISA, however, the E-CNCs may function as individual CNCs, or chiral dopants, as discussed by Parton et al. (2022) and are therefore considered the elementary particles forming the CNC-additive assemblies in this work. Small CNCs may appear shorter in the images due to the low resolution imaging. Nevertheless, due to the large quantity of data (474 particles), the results are statistically sufficient.

Fig. 7b shows the length and height data for all E-CNCs. The corresponding size distributions are displayed with a log-normal fit. Fig. 7c shows the corresponding average and median values. The CNCs are relatively long, as a result of software interpretation, with a higher aspect ratio compared to the average CNCs used in previous work (Delepierre et al., 2021; Reid, Villalobos, et al., 2017). Higher aspect ratio CNCs self-assemble at lower concentrations, with lower pitch values for the chiral nematic structures in dried films, thereby reflecting blue or UV wavelengths (Honorato-Rios & Lagerwall, 2020; Parton et al., 2022; Schütz et al., 2020). This matches the weak blue or colorless reflection of the films in Figs. 2, S4. This would also explain their blue coffee-rings, which reflect redshifted or longer wavelengths compared to the central region of the film (Klockars et al., 2019; Mu & Gray, 2015).

Fig. S8 shows the length and height data for all the identified particles (E-CNCs + Aggregates).

---

Fig. 6. Change (%) in residual stress between 20 h and 2 years after casting. The numbers inside the graph represent the molecular weights of PVA and PEG. The bars show average values whereas the black dots represent individual measurements and their number vary depending on the availability of stored samples.
The dimensions of the E-CNCs were used to estimate the residual stress for each E-CNC, depicted in Fig. 7b as a background color scale, with the corresponding values shown in Fig. 7d. The stress per E-CNC was shown to increase both as a function of length and height (and width), but the effect of the height was much greater. When increasing the height, the stress increases semi-linearly, whereas the volume of the CNC increases quadratically. Therefore, the stress per CNC unit volume is always greater at smaller widths. Hence, lower aspect ratio CNCs are subjected to a greater stress per unit volume than larger aspect ratio ones. This can be followed visually from the orange arrow in Fig. 7b, where the stress per CNC unit volume increases by 75% while no absolute stress increase occurs. As discussed in previous work (Honorato-Rios & Lagerwall, 2020; Klockars et al., 2018), the lower aspect ratio CNCs are more likely to exist in the top sections of the films having chiral nematic order. Consequently, the stress is expected to be concentrated in the top side of the films.

4. Conclusions

We monitored the deflection of cantilevers after deposition of aqueous CNC suspensions to investigate the stresses that develop during drying and arise from interparticle capillary forces, as well as their related residual stress state. PEG, PVA, glucose, glycerol and BSA were used as additives, resulting in films having decreased residual stresses and brittleness. The main factors affecting the residual stress state of the films can be summarized as follows:
1) Plasticizer type: glycerol produced the best plasticizing effect, followed by PEG$_{20K}$ and glucose. The latter, however, adversely affected the optical properties of the CNC film, which became more inhomogeneous.

2) CNC-to-plasticizer ratio: by increasing the CNC-to-additive ratio, from 10:1 to 10:4, the residual stresses decreased for both the polymeric and the molecular additives tested, i.e., PEG and glycerol.

3) Plasticizer molecular weight: the stress was reduced with an increased PVA and PEG molecular weight, from ca. 10 to 100 kDa. The CNC-PEG film developed decreased stresses when using PEG of lower molecular weight, from 10 to 0.2 kDa. When observing the cross section of the films, however, the low molecular weight PEG apparently diffused out from the film, as evidenced by a distinct polymeric phase at the top of the CNC film. The chiral nematic order apparently diffused out from the film, as evidenced by a distinct polymeric phase at the top of the CNC film.

4) Aging: in general, the internal stress within most films decreased 30% after storage for 2 years in ambient conditions. A smaller reduction was observed when using PEG of low molecular weights (the film with PEG$_{20K}$ even experienced an increase in stress).

5) Colloidal particle morphology: smaller particles experience lower stresses, in absolute values. However, they contribute more to the stress development when normalizing to their mass and volume.

The method used herein can be easily reproduced with any conventional optical tensiometer and is thus expected to facilitate the study of the effect of interactions between biocolloids and biopolymers. This is expected to enable a rapid benchmarking of their potential for application as coatings and films.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbpol.2022.120465.

CRediT authorship contribution statement

Konrad W. Klockars: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Luiz G. Greca: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Johanna Majoinen: Writing – review & editing. Karl Mihelš: Investigation, Writing – review & editing. Orlando J. Rojas: Funding acquisition, Resources, Supervision, Writing – review & editing. Blaise L. Tardy: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We acknowledge funding support by the European Research Council under the advanced grant 79849 BioElCell. Luiz G. Greca & Karl Mihelš acknowledge funding from Aalto University School of Chemical Engineering and Konrad W. Klockars acknowledges funding from the Walter Ahlström Foundation. We acknowledge the support by Aalto University at OtaNano — Nanomicroscopy Center (Aalto-NMC). The authors are also grateful for the support of the Academy of Finland through its Centres of Excellence Programme (2014–2019) under Project 264677 “Molecular Engineering of Biosynthetic Hybrid Materials Research” (HYBER). We thank Prof. Olli Ikkala for his insightful comments. BLT is the recipient of the Khalifa University of Science and Technology (KUST) Faculty Startup Project (Project code: 84741140-FSU-2022-021).

References


Bast, L. K., Klockars, K. W., Greca, L. G., Rojas, O. J., Tardy, B. L., & Brooks, N. (2021). 30 % after storage for 2 years in ambient conditions. A smaller reduction was observed when using PEG of low molecular weights (the film with PEG$_{20K}$ even experienced an increase in stress).

Conclusions

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We acknowledge funding support by the European Research Council under the advanced grant 79849 BioElCell. Luiz G. Greca & Karl Mihelš acknowledge funding from Aalto University School of Chemical Engineering and Konrad W. Klockars acknowledges funding from the Walter Ahlström Foundation. We acknowledge the support by Aalto University at OtaNano — Nanomicroscopy Center (Aalto-NMC). The authors are also grateful for the support of the Academy of Finland through its Centres of Excellence Programme (2014–2019) under Project 264677 “Molecular Engineering of Biosynthetic Hybrid Materials Research” (HYBER). We thank Prof. Olli Ikkala for his insightful comments. BLT is the recipient of the Khalifa University of Science and Technology (KUST) Faculty Startup Project (Project code: 84741140-FSU-2022-021).

References


Bast, L. K., Klockars, K. W., Greca, L. G., Rojas, O. J., Tardy, B. L., & Brooks, N. (2021). 30 % after storage for 2 years in ambient conditions. A smaller reduction was observed when using PEG of low molecular weights (the film with PEG$_{20K}$ even experienced an increase in stress).

Conclusions

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We acknowledge funding support by the European Research Council under the advanced grant 79849 BioElCell. Luiz G. Greca & Karl Mihelš acknowledge funding from Aalto University School of Chemical Engineering and Konrad W. Klockars acknowledges funding from the Walter Ahlström Foundation. We acknowledge the support by Aalto University at OtaNano — Nanomicroscopy Center (Aalto-NMC). The authors are also grateful for the support of the Academy of Finland through its Centres of Excellence Programme (2014–2019) under Project 264677 “Molecular Engineering of Biosynthetic Hybrid Materials Research” (HYBER). We thank Prof. Olli Ikkala for his insightful comments. BLT is the recipient of the Khalifa University of Science and Technology (KUST) Faculty Startup Project (Project code: 84741140-FSU-2022-021).

References