

Polysaccharide-mediated stabilization of bacterial nanocellulose dispersions: Experimental and molecular dynamics study

Ricardo Silva-Carvalho^{a,b,1}, Daniela Martins^{a,b,1}, Tarsila G. Castro^a, Ana Cristina Rodrigues^{a,b}, Bruna Costa^{c,d}, Paula Parreira^{c,d}, Artur Cavaco-Paulo^{a,b}, Fernando Dourado^{a,b}, M. Cristina L. Martins^{c,d,e}, Miguel Gama^{a,b,*}

^a CEB – Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal

^b LABBELS – Associate Laboratory, Braga, Guimarães, Portugal

^c i3S – Instituto de Investigação e Inovação em Saúde, Universidade Do Porto, Rua Alfredo Allen, 208, 4200-135, Porto, Portugal

^d INEB – Instituto de Engenharia Biomédica, Universidade Do Porto, Rua Alfredo Allen, 208, 4200-135, Porto, Portugal

^e ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade Do Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313, Porto, Portugal

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ABSTRACT

Colloidal dispersions of pristine cellulose (nano- or micro-sized; fibrillar or particulate), are typically unstable in water due to interfiber/interparticle interactions. Carboxymethyl cellulose (CMC) is known to effectively stabilize these dispersions, allowing for full recovery of the properties upon redispersion after drying. However, the underlying mechanism has been unclear. This study investigated why CMC effectively stabilized cellulose and whether other polysaccharides could perform similarly. Mixtures of bacterial nanocellulose (BNC) with chia mucilage (ChM), hyaluronic acid (HA), pectin (Pec), xanthan gum (XG), locust bean gum (LBG), salep, alginate (Alg), and polyol glycerol (Gly) were compared to mixtures of BNC:CMC. The stability and redispersibility after drying of each mixture were analyzed. Only ChM produced homogeneous and stable BNC dispersions similar to CMC. The polysaccharides' adsorption onto cellulose films was studied using quartz crystal microbalance with dissipation (QCM-D). CMC and ChM showed stronger and more stable adsorption, with higher retained mass after washing (10.0 ± 0.3 and 7.8 ± 2.3 mg/m², respectively), compared to Alg, HA, or XG (3.6 ± 0.6 , 1.0 ± 0.3 and 0.2 ± 0.1 mg/m², respectively). Molecular dynamics simulations confirmed the stronger interactions of CMC and ChM with cellulose surfaces, characterized by hydrogen bonding and perpendicular orientation.

1. Introduction

Cellulose is a ubiquitous material, with multiple applications in different areas: food, cosmetics, composites, biomedical devices and pharmaceutical industry. Many formulations of plant-derived cellulose, such as microcrystalline and hydrocolloidal dry celluloses, available in different size ranges (from submicron to tens of microns), are used as thickeners and stabilizers in food and cosmetic products (El Bourakadi et al., 2024; Lupidi et al., 2023). Manufacturers normally recommend an activation step involving high-shear homogenization to fully take advantage of the properties of these dry celluloses (Tuason et al., 2009; Wüstenberg, 2014). This activation step helps to eliminate clumps, ensures thorough dispersion and homogenization of the powders, leading to improved stability and performance in the final product. Owing to the

high shear forces provided by this process, aggregates present in those suspensions are broken, and the particles reach their minimum size and desired performance.

Bacterial Nanocellulose (BNC), a 3D nanofibrillar network of interconnected fibers, is produced by fermentative processes by using e.g., *Komagataeibacter* spp. bacteria. Compared to plant cellulose, BNC features thinner fibers (fiber diameter of up to 100 nm (Rodrigues et al., 2023)), higher purity, crystallinity, degree of polymerization, mechanical performance and water retention capacity. These attributes have sparked even greater interest from diverse industries, including biomedical, pharmaceutical, energy, bioelectronics, food, cosmetics, pulp & paper and textiles (Choi et al., 2022; Klemm et al., 2018). To the best of our knowledge, no dry formulations of BNC are available on the market, in contrast with their plant-derived counterpart.

* Corresponding author. Department of Biological Engineering University of Minho Campus de Gualtar, 4710-057, Braga, Portugal.

E-mail address: fmgama@deb.uminho.pt (M. Gama).

¹ These authors contributed equally to this work.

Nanocellulose particles obtained via acid hydrolysis, particularly with sulfuric acid, are highly water-dispersible due to the introduction of negatively charged sulfate groups during hydrolysis. This provides strong electrostatic repulsion, besides the intrinsic hydrophilicity of cellulose hydroxyl groups (Sonowal et al., 2025; Vasconcelos et al., 2017; Zhu et al., 2025). Contrarily, aqueous dispersions of pristine cellulose, as is the case of BNC, exhibit pronounced aggregation due to physical entanglement and interfibrillar van der Waals attraction, including hydrogen bonding (Agarwal et al., 2018). These interactions, combined with the long aspect ratio of the BNC fibers, lead to the formation of extended networks upon dispersion in water. These highly heterogeneous dispersions consist of fiber flocs or bundles ranging from tens to hundreds of micrometers, depending on the concentration and shear homogenization conditions (Martins et al., 2020). Upon drying, the aqueous redispersion of these micro/nano-sized cellulose fibers is a challenging task given the well reported hornification phenomenon (Butchosa et al., 2014; Klemm et al., 2011). The total or partial disruption of the dried aggregates requires the use of high-energy shearing, as referred above.

The ability of CMC to efficiently disperse BNC (fibers or nanocrystals) has been reported by different authors (Tajima et al., 2020; Winter et al., 2010; Xiang et al., 2019). As also demonstrated in our previous work, BNC:CMC mixtures can be further redispersed in water after drying, recovering the properties of the non-dried mixture (Martins et al., 2020, 2021). We have demonstrated for the first time the preparation of BNC formulation that, after drying, can be redispersed in just a few minutes at room temperature, and using low energy mixing. Such formulation is potentially useful for the cosmetics and food industries, as it proved to be a very efficient stabilizer of oil-in-water and particle-in-water dispersions, owing to the stabilization of high-energy interfaces as well as the structuring of the continuous phase in such biphasic systems (Martins et al., 2020). Intriguingly, only CMC, a chemically modified cellulose, has so far been described as effectively producing homogeneous dispersions and to recover the properties of wet BNC, after drying. Polysaccharides that exhibit adsorption related features such as a strong and stable surface-binding affinity to BNC are here hypothesized to effectively prevent fiber aggregation during drying, enabling homogeneous and stable dispersions upon rehydration, with low energy input. In this work, we systematically tested the potential of several other polysaccharides, such as chia seed mucilage (ChM), xanthan gum (XG), alginate (Alg), pectin (Pec), locust bean gum (LBG), Salep and hyaluronic acid (HA), as well as the polyol glycerol (Gly), for similar application. Our choices were made considering: i) natural origin; ii) reported evidence of interaction with cellulose, and iii) inclusion of both neutral and charged polysaccharides, thus covering a wide range of structural and physicochemical properties. HA, Pec, Alg and XG were selected due to their negative charge (Bueno et al., 2014; Di Mola et al., 2022; Lara-Espinoza et al., 2018; Yang et al., 2011) similar to CMC; LBG and salep were selected for their high content in galactomannans (Dos Santos et al., 2015) and glucomannans (Kurt, 2021), respectively. ChM was included based on previous reports indicating its affinity to cellulose (Gorges et al., 2025; Griffiths et al., 2017; Ralet et al., 2016; Yu et al., 2018) and Gly, due to its reported ability to facilitate the redispersion of dried cellulose (Moser et al., 2018). For the first time, we identified ChM as a fully natural alternative to CMC, which may allow the design of clean label formulations with fewer artificial or synthetic ingredients.

2. Materials and methods

2.1. Materials and reagents

Bacterial Nanocellulose (BNC) wet membranes (purity: >99 %, molecular weight: 585000 g/mol) were obtained from HTK Food CO., Ltd (Ho Chi Minh city, Vietnam), a “Nata de Coco” manufacturer, and further purified as described in Silva F et al., 2024. Trimethylsilyl cellulose (TMSC, purity ≥99 %, degree of silylation: 2.55, molecular

weight: 28000 g/mol; repeating unit molar mass: 346 g/mol) was purchased from Posanova GmbH (Weimar, Germany). Chia seeds (*Salvia hispanica*) were purchased in a local store in Portugal and Salep (mainly a glucomannan extracted from the tubers of the Orchidaceae family) was purchased in a local store in Iran. Sodium carboxymethyl cellulose (CMC, molecular weight: 90000 Da, degree of substitution: 0.7) was supplied by Acros Organics (Geel, Belgium). Xanthan Gum (XG, viscosity: 1450–2000 mPa s at 1 % w/v in 1 % KCl, molecular weight: 241.1 g/mol) was purchased from TCI (Tokyo Chemical Industry Co., Ltd, Tokyo, Japan). Hyaluronic Acid (HA, Sodium Hyaluronate Primahyal 300, viscosity: 0.08 cPs at a concentration of 1 %, molecular weight: 300000 g/mol) was gifted from Soliance SA (Pomacle, France). Glycerol (Gly), absolute ethanol (99.8 %), hydrochloric acid (HCl), and sulfuric acid (H₂SO₄, 95 % vol.) were purchased from Fisher Scientific (Geel, Belgium). Pectin (Pec, USP grade, from apple, purity: ≥95 %; molecular weight: 711900 g/mol; degree of methyl esterification: 50–75 %; galacturonic acid content: 80.39 % (Liu et al., 2022)), Locust Bean Gum (LBG, from Ceratonia siliqua seeds, molecular weight: 310000 g/mol; viscosity: 2300–5000 cPs at a concentration of 1 %), dopamine hydrochloride and Trizma® base were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Sodium Alginate (Alg, Aquatic™ N100, purity: microbiological and heavy metal content within pharmacopeia limits - total bacterial viable count ≤5000 cfu/g, yeast and mold ≤200 cfu/g, *E. coli* and *Salmonella* absent; heavy metals: arsenic ≤3 ppm, cadmium ≤1 ppm, lead ≤5 ppm, mercury ≤1 ppm, G/M range: 30–40/60–70, viscosity: 90–150 mPa s at a concentration of 5 %) was purchased from Dupont Nutrition & health (Copenhagen, Denmark). Sodium Hydroxide (NaOH) was purchased from Labkem (Barcelona, Spain). Toluene (anhydrous) was obtained from Chem-Lab Analytical bvba (Zedelgem, Belgium). Hydrogen peroxide (H₂O₂, 30 % vol.) was acquired from Merck (Darmstadt, Germany).

2.2. Chia seed mucilage (ChM) extraction

The extraction of ChM was conducted based on previous reports with some modifications (Feizi et al., 2021; Tavares et al., 2018). Chia seeds (30 g) were soaked in 500 mL of distilled water under magnetic stirring (700 rpm) for 1 h at room temperature (RT). After that, the beakers containing the hydrated seeds were placed in a water bath at 50 °C for 2 h. The mucilage was recovered by vacuum filtration (using a vacuum pump) through a stainless-steel mesh until the filtrate volume was approximately 2/3 of the total volume. The extract was frozen at –80 °C overnight, freeze-dried for 4 days at –100 °C and approximately 0.05 mbar, and stored in a desiccator until use.

2.3. BNC-polysaccharide mixtures

2.3.1. Mixing BNC with polysaccharides

BNC mixtures with different polysaccharides (Chia seed mucilage (ChM), CMC, XG, Alg, Pec, LBG, Salep and HA) as well as with the polyol Gly, were prepared following a protocol similar to the one previously reported for BNC:CMC (Martins et al., 2021). BNC membranes were purified with a 0.1 M NaOH solution for 3 days (with daily exchanges) and then washed with distilled water to remove the alkaline solution (daily exchanges), until the pH of the BNC washing water was the same as that of distilled water. The washed BNC was cut into small cubes and disintegrated with a hand blender at 9000 rpm (Sammic blender, model TR250, Sammic S.L.), until a homogeneous pulp was obtained, as observed by visual inspection (absence of visible unfragmented membrane pieces). The solid fraction of the obtained pulp was determined by drying samples of known mass in an oven at 100 °C, and then adjusted to 0.5 % (w/v) by adding distilled water. Solutions of the above referred polysaccharides were prepared in distilled water at a concentration of 0.5 % (w/v). These solutions were mixed with the 0.5 % BNC pulp at mass ratios of 1:1 and the resulting mixtures were left under magnetic stirring (900 rpm) overnight at RT. The selected working concentration

and BNC:polysaccharide ratio were chosen based on our previous studies with CMC (Martins et al., 2020, 2021, 2023). The pH of the polysaccharide solutions and of BNC:polysaccharide dispersions was measured. On the next day, they were wet-ground in a high-speed blender (Moulinex Ultrablend 1500 W, Écully, France) at 24 000 rpm for 1 min. Finally, a sample of each BNC mixture was freeze-dried and stored in a desiccator, with the remainder being stored at 4 °C until further use.

2.3.2. Evaluation of dispersibility and colloidal stability

To evaluate the dispersibility of the freeze-dried BNC-Polysaccharide mixtures, samples were prepared at 0.5 % (w/w) in distilled water, at RT, using a magnetic stirrer plate at 900 rpm for 10 min. Then, 10 mL of the obtained dispersions were spread over 60 mm petri dishes, observed with the naked eye, and photographed. The colloidal stability of the dispersions was evaluated by dilution to 0.25 % (w/w) (to decrease the viscosity, facilitating any potential sedimentation), followed by centrifugation at 7000×g for 5 min, at 25 °C (Centrifuge 5430 R, Eppendorf).

2.4. Cellulose thin films preparation

2.4.1. Substrate cleaning and spin coating

Gold (Au) substrates (1 × 1 cm) were cleaned with “piranha” solution (7 parts sulfuric acid 95 % vol. and 3 parts hydrogen peroxide 30 % vol.) for 5 min and then thoroughly rinsed with ethanol, ultrasonicated (Bandelin Sonorex Digitec Bath 35 kHz, Berlin, Germany) for 3 min with ethanol (twice) and ultrapure water (Type I water, Milli-Q®) (once), and then dried with a gentle stream of Argon.

Gold-coated quartz crystal sensors (QXS301-Standard Gold, 4.95 Hz, 78 mm² active sensor area; Biolin Scientific) were cleaned as described above after 10 min in an UV/ozone cleaner.

After cleaning, substrates were immersed in a dopamine solution (2 mg/mL in 10 mM Tris-HCl pH 8.5), incubated for 2 h under agitation (70 rpm), and protected from light to allow the polymerization of dopamine into a polydopamine (pDA). The obtained pDA-coated substrates were then rinsed in a sonication bath for 1 min with Tris-HCl (10 mM pH 8.5) buffer (twice) and with Milli-Q® water (once) and then dried with a gentle stream of argon (Costa et al., 2019).

Cellulose thin films were prepared from a soluble cellulose to study its interaction with polysaccharides, using a quartz crystal microbalance, since the preparation of such films using insoluble BNC fibers is challenging. Trimethylsilyl cellulose (TMSC) was dissolved in toluene at 1 % w/v and subsequently regenerated into cellulose. The regenerated cellulose is essentially similar to BNC, although differences in crystallinity and degree of polymerization may occur. In more detail, 100 µL of TMSC solution was placed on the center of the pDA-coated substrates that were then placed in a spin coater (model WS-650-23, Laurell Technologies Corp., North Wales, PA, USA) and spun at 4000 rpm with an acceleration of 2500 rpm/s for 60 s. For the regeneration of TMSC to pure cellulose (CE), each TMSC-coated substrate was placed into a polystyrene Petri dish with a diameter of 5 cm. Two milliliters of 10 % HCl were placed beside the substrate, and the Petri dish was covered with its cap. TMSC was regenerated to pure cellulose during exposure to HCl vapors for 20 min at RT without affecting the film morphology.

2.5. Cellulose thin films characterization

2.5.1. Ellipsometry

The thickness of CE thin films in the pDA-coated Au substrates was determined using an ellipsometer (model EP³, Nanofilm Surface Analysis, Accurion, Goettingen, Germany). The ellipsometer was operated in a polarizer-compensator-sample-analyzer mode (null ellipsometry) with a 532 nm solid-state laser as a light source. The Au substrate refractive index ($n = 0.673$) and extinction coefficient ($k = 2.483$) were determined by using a delta and psi spectrum with an angle variation between 64 and 74° and a Brewster angle of 69°. Measurements were performed

on four zones on the surface for correction of any potential instrument misalignment. The thickness of the pDA and CE were determined using $n = 1.46$ and $k = 0.2$ (Andrianov et al., 2015). Results are presented as the mean ± standard deviation from three independent measurements across three different samples.

2.5.2. Fourier Transform-Infrared reflection Absorption Spectroscopy (FT-IRRAS)

Spectra of CE films produced in the pDA-coated Au, before and after regeneration, were obtained on a Fourier transform infrared spectrophotometer (model 2000, PerkinElmer, Waltham, MA, USA) coupled to a VeeMax II Accessory (PIKE Technologies, Madison, WI, USA) and a liquid-nitrogen-cooled mercury cadmium telluride (MCT) detector. Dry nitrogen was purged into the instrument for 5 min before and during the measurement of each sample, to ensure that there was no water vapor adsorption. An Au substrate was used as a background. Incident light was p-polarized, and spectra were collected using the 80° grazing angle reflection mode. For each sample, 100 scans were collected with 4 cm⁻¹ resolution.

2.5.3. Water contact angle

Static contact angle measurements were performed using the sessile drop method with an Optical Contact Angle system (OCA 20, Data-Physics) and a SCA 20 Software module 22 (DataPhysics). Drops of 2 µL of Milli-Q® water were deposited on top of the films with a Hamilton 500 mL syringe and left in contact for 60 s while recording with the equipment's video camera. Movie records were analyzed frame by frame, and the contact angle of the drop was determined by the instrument's software through shape analysis, using the Young-Laplace fitting method. All measurements were carried out at RT on pure TMSC and regenerated CE films. Results are the average of two measurements of five replicates.

2.6. Polysaccharide adsorption studies

2.6.1. Quartz Crystal Microbalance With Dissipation (QCM-D)

A QCM-D system (Q-Sense E4 instrument, Biolin Scientific, Gothenburg, Sweden) was used to monitor in real time the frequency (Δf) and dissipation (ΔD) shifts on the sensors related to polysaccharide adsorption. Coated sensors were pre-incubated with Milli-Q® water in static conditions for 15 min to achieve a stable baseline. Afterwards, ChM, CMC, XG, Alg and HA at 0.5 % (w/v) in Milli-Q® water were individually injected in the QCM-D modules at a flow rate of 100 µL/min and allowed to react under static conditions for 1 h at 25 °C. Then, the films were rinsed with Milli-Q® water for 15 min (100 µL/min) to remove the unattached polysaccharides. The adsorbed mass of the polysaccharides was estimated using the Viscoelastic model (Broadfit, Fit quality >0.9; Software: Q-Sense D-Find, version Q.Sense D-Find 1.2.7), since high dissipation changes were observed in the system. Data from the 5th to the 13th harmonics were collected and used in the analysis. A density of 1000 g/L was set for all the polysaccharide solutions. Results are presented in average mass per area (mg/m²) and thickness (nm) before and after washing the films. It is important to note that the average mass calculated using the viscoelastic model includes the mass of the polysaccharides as well as the water that is associated with the adsorbed layer and is thus not equal to the dry mass of the adsorbed polysaccharide.

2.7. Molecular Dynamics (MD) simulations

2.7.1. Systems Setup

A cellulose nanocrystal (CNC) was built by recurring to the Cellulose-builder toolkit (Gomes et al., 2012). This CNC comprises 36 chains with 8 cellobioses per chain (Fig. 1). Polysaccharides were designed on PyMOL (Schrödinger, LLC., 2020) and underwent an optimization step by using gaussian09 (Frisch et al., 2009) and semi-empirical PM6

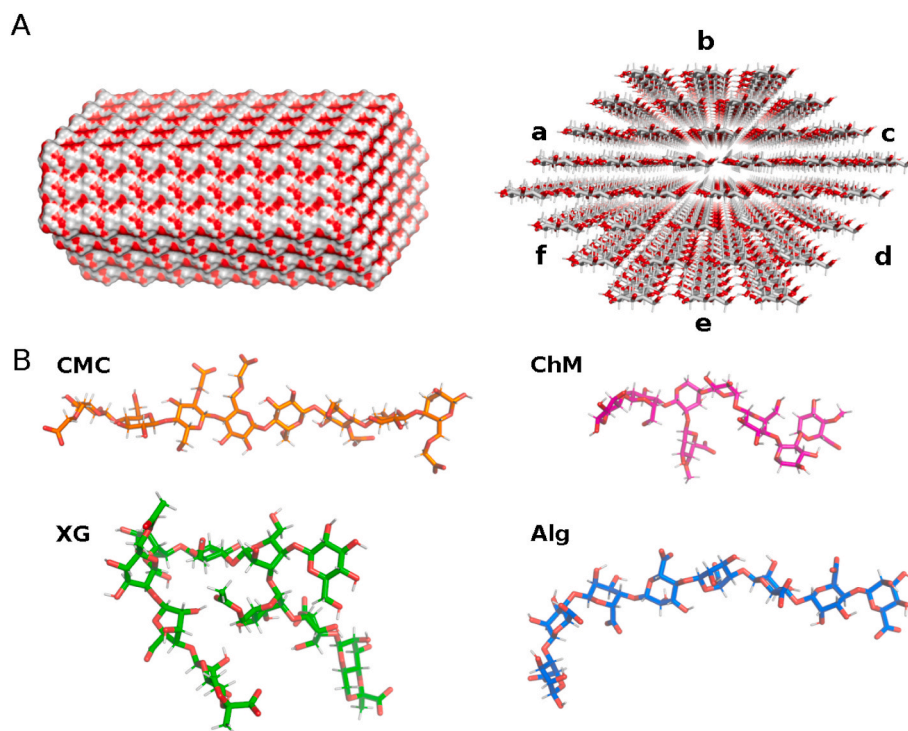


Fig. 1. A) Different perspectives of the cellulose nanocrystal (CNC) model, with 36 chains having 8 cellobioses per chain – ‘a, c, d, f’ are hydrophilic surfaces, and ‘b, e’ hydrophobic ones; B) Representative 3D structures of the polysaccharides CMC (carboxymethyl cellulose), ChM (Xylose, Glucose, 4-O-methyl- α -D-glucuronic acid (2:1:1)), XG (glucose, mannose and glucuronic acid (2:2:1)), Alg (α -L-glucuronic and β -D-mannuronic acids). Oxygen atoms are represented in red and hydrogens in white sticks. Carbons are colored orange, magenta, green or blue, on each polysaccharide.

calculations (Stewart, 2007). Then, these structures were submitted to the SwissParam server (Zoete et al., 2011) to obtain compatible CHARMM36 topologies.

After careful evaluation of experimental results, the four polysaccharides under *in-silico* investigation were: CMC, ChM, XG and Alg. The polysaccharides were designed similarly in the number of saccharide rings and overall size, with the following molecular weight: CMC -1600.28 g/mol, ChM - 1217.04 g/mol, XG - 1887.52 g/mol, and Alg - 1418.95 g/mol. Their 3D structures are represented in Fig. 1.

2.7.2. MD simulations and analysis

To validate the CNC model, molecular dynamics (MD) simulations were performed on the GROMACS 2021 series (GROMACS development team, 2021) within the CHARMM36 forcefield (Huang et al., 2013). First, the CNC was embedded in a 10.5 x 12.0 x 12.0 nm simulation box, giving reasonable space around the crystal but limiting the edges. Hence, the system represents and feels a fibrillar continuity. The box was filled with TIP3P water molecules (Mark et al., 2001) and ions were added to achieve system neutrality. Energy minimization was carried out using the steepest descent algorithm until the atoms felt a maximum force no greater than 1000 kJ mol⁻¹ nm⁻¹. After that, two initialization steps were carried out: the first, during 500 ps with an NVT ensemble (number of particles, volume and temperature, constant), followed by an NPT ensemble (number of particles, pressure and temperature, constant), during 500 ps. The V-rescale algorithm (Bussi et al., 2007) set the temperature at 298K (~25 °C) and the Parrinello-Rahman algorithm (Martoňák et al., 2003) kept a pressure of 1 atm. Both steps were performed with position restraints on carbon atoms, with a force of 1000 kJ/mol/nm⁻² in all directions.

Production runs were performed in two distinct ways: 1) with position restraints and 2) without position restraints, during 60 ns. This was done to compare both systems, their overall stability and whether it would be reasonable to perform polysaccharide-CNC simulations on the ‘carbon frozen’ system.

The polysaccharides were also evaluated free in solution during 60 ns, following the same *in-silico* protocol, but without position restraints in the production run stage. Root Mean Square Deviation (RMSD) analysis and clustering analysis were employed to comprehend structural features. The clustering analysis uses the single-linkage method to indicate a middle structure, the one which minimizes the RMSD variance amongst the sample, from the last 15 ns of simulation time. The representative structures are shown in Fig. 1.

The polysaccharide-CNC systems underwent the same procedure as described above, where 10 units of polysaccharides were randomly added to the simulation box. Spontaneous adsorption was evaluated over 60 ns of simulation time. Many properties were calculated using the GROMACS analysis tools: hydrogen bonding, radial distribution function (RDF), and number of contacts up to 0.5 nm distance over time.

2.8. Statistics

The experimental results are presented as means with standard deviations. The significance of differences between mean values was assessed using a paired *t*-test or one-way ANOVA followed by a post hoc Bonferroni’s test using the GraphPad Prism 5 (GraphPad Software, California, USA). Significance was defined as **p* < 0.05 and ****p* < 0.001.

3. Results and discussion

3.1. Preparation, dispersibility and colloidal stability of dried BNC formulations

The capacity of different polysaccharides to reduce the aggregation of BNC, yielding homogeneous dispersions upon rehydration, was evaluated qualitatively. HA, Pec, Alg and XG were selected due to their negative charge (Bueno et al., 2014; Di Mola et al., 2022; Lara-Espinoza et al., 2018; Yang et al., 2011) similar to CMC, which is known to favor

cellulose fibers dispersion and colloidal stability due to electrostatic repulsion (Martins et al., 2020). LBG and salep were selected for their high content in galactomannans (Dos Santos et al., 2015) and glucomannans (Kurt, 2021), respectively, which, according to the literature, have affinity to cellulose (Vilaró et al., 2020; Yoshimi et al., 2025). ChM was included based on previous reports indicating that seed mucilage from different sources shows binding affinity to cellulose, in addition to also carrying negative charge (Gorges et al., 2025; Griffiths et al., 2017; Ralet et al., 2016; Yu et al., 2018). Gly, a polyol characterized by its low molecular weight and high hygroscopicity, was included in this study for its reported ability to facilitate the redispersion of dried cellulose (Moser et al., 2018). The BNC:PS dispersions presented pH values of 4.65 (for Pec), 6.43 (ChM), 6.36 (HA), 5.62 (XG), 5.41 (LBG), 5.93 (Alg) and 6.51 (CMC), suggesting that, with the exceptions of LBG and Salep (not measured), which are neutral, all others bear a negative charge owing to the presence of uronic and carboxymethyl groups, which have pKa values < 4.5 (Bueno et al., 2014; Fernandes et al., 2023; Zhivkov, 2013). As observed in Fig. 2, only BNC:CMC and BNC:ChM mixtures showed good redispersion, as no visible fiber aggregates or bundles were observed. The redispersed mixtures had the same visual aspect as the never-dried ones. Also, only these two mixtures showed high colloidal stability after centrifugation, with all the remaining BNC:polysaccharide mixtures displaying sedimentation. Actually, the never-dried BNC-formulations containing XG, HA, Pec, Alg, LBG, salep and Gly were already inhomogeneous (not fully dispersed), despite these polysaccharides having good solubility in water; expectably, drying and redispersion did not improve their homogeneity. These results demonstrate that the homogenous dispersion of BNC is a challenging task, since most of polysaccharides failed to perform satisfactorily. In general, the effectiveness of a polysaccharide in stabilizing and/or dispersing cellulose in aqueous medium may depend on several factors, including its composition, molecular weight, degree of substitution, degree of branching, presence and distribution of charged groups, conformation of the polymer in solution and chain flexibility, ability to form hydrogen bonds with cellulose, concentration, and capacity to increase the viscosity (Garcia-Valdez et al., 2018; Izydorczyk et al., 2005; Lefebvre et al., 2006; Naidjonoka et al., 2020). Several studies addressed the adsorption

of CMC onto cellulose (Arumugan et al., 2021, 2023; Liu et al., 2011). It has been proposed that the pectin's neutral sugar side chains could bind to cellulose (Zykwiniska et al., 2005). Nevertheless, it remains unclear why CMC and ChM perform better than all other polysaccharides. To further understand this result, adsorption studies with some selected polysaccharides (effective stabilizers: CMC, ChM; partially effective stabilizer: XG; poor stabilizer: Alg, and HA) on thin films of cellulose were carried out using QCM-D.

3.2. Characterization of the cellulose (CE) thin films

The first step to produce cellulose film was the deposition of a uniform polydopamine (pDA) coating on the Au substrate. This was achieved through a simple dip-coating of the substrate on a dopamine solution, whose catechol groups allow linkage on virtually any organic or inorganic surface. A uniform 13.6 ± 0.2 nm thick pDA film, as measured by ellipsometry, was spontaneously deposited, which lies within the range described in the literature (Costa et al., 2019; Lee et al., 2007). pDA-Au substrates were then used to fabricate the cellulose (CE) coating, since the exposed reactive groups enable covalent grafting of polymers (Teunissen et al., 2023). Thin and homogenous cellulose films were obtained through the deposition of a TMSC solution by spin coating, followed by regeneration to CE with wet gaseous hydrochloric acid. This process promoted a significant increase in the surface thickness to 35.0 ± 1.5 nm, around 21.4 nm corresponding to the CE layer, which is within the range of values described in the literature for CE films (15–25 nm) obtained using spinning speeds above 1000 rpm (Kontturi et al., 2003). Considering the compact nature of the final films the regeneration method here employed was effective in removing the bulky trimethylsilyl side chains and forming a tight hydrogen-bond network of cellulose.

To confirm the regeneration of TMSC to CE films, FT-IRRAS and static water contact angle were employed. Fig. 3 shows the spectra of CE films produced in the pDA-coated Au, before and after regeneration. A band around 3258 cm^{-1} , that corresponds to O-H stretching vibration, is visible in all the spectra. Due to the high degree of substitution (DS) with trimethylsilyl groups, O-H vibrations are generally not visible in pure

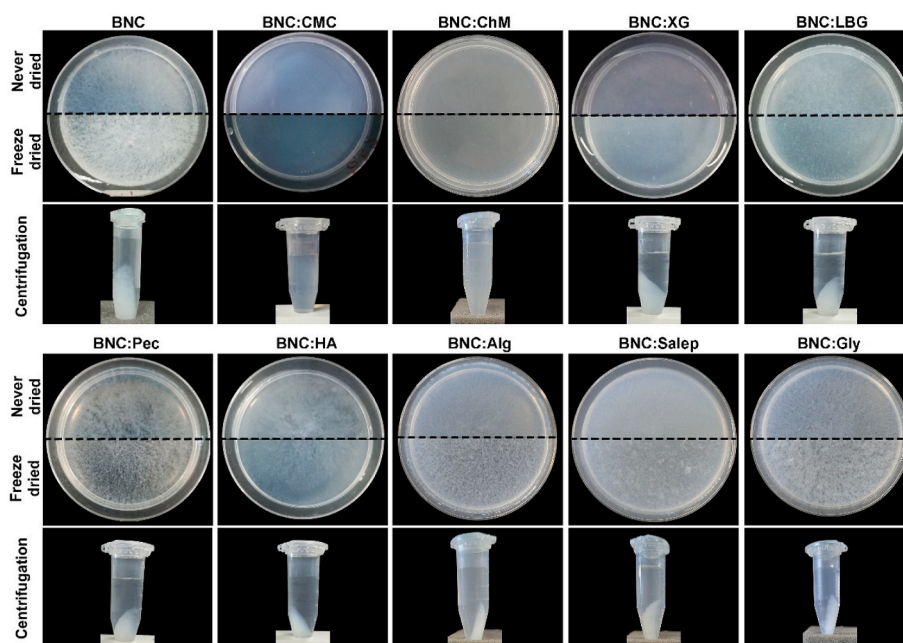


Fig. 2. Petri dish photographs of 0.5 % (w/w) aqueous dispersions of never dried and freeze-dried Bacterial Nanocellulose (BNC) and BNC mixtures (1:1 ratio) with chia seed mucilage (BNC:ChM), carboxymethyl cellulose (BNC:CMC), xanthan gum (BNC:XG), locust bean gum (BNC:LBG), pectin (BNC:Pec), hyaluronic acid (BNC:HA), glycerol (BNC:Gly), alginate (BNC:Alg) and salep (BNC:Salep). The lines labelled “centrifugation” show images of microcentrifuge tubes with the same mixtures, at a concentration of 0.25 % (w/w), following centrifugation.

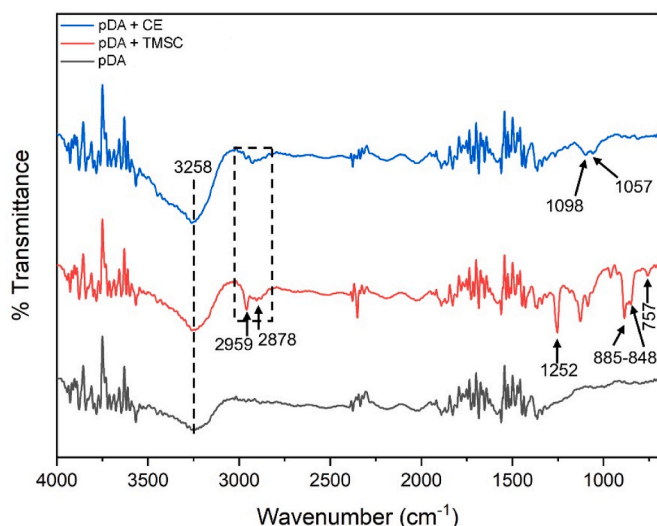


Fig. 3. FT-IRRAS spectra of Au substrates coated with polydopamine layer (pDA), a pDA layer plus TMSC and a pDA layer plus CE.

TMSC (Mohan et al., 2011). However, this modification in the spectra could be due to the incomplete substitution of the hydroxyl groups in the commercial TMSC (Kontturi et al., 2003) (DS being 2.4–2.6 out of the possible 3) and an influence of the pDA layer.

As described in the literature, the observed peaks in the spectra of pure TMSC are C–Si rocking vibrations at 757, 848–885, and 1252 cm^{-1} (Schlemmer et al., 2018). The symmetric (2878 cm^{-1}) and asymmetric (2959 cm^{-1}) stretching bands of the CH_3 group, as well as the typical C–Si rocking vibration peaks, disappeared after regeneration (blue line) and typical features of the glucopyranose ring of cellulose became visible (with small variation in the wavenumber) such as C–O stretching at around 1098 and 1057 cm^{-1} (Kontturi et al., 2003).

The wettability of the films is also affected by the regeneration process. Similar to the values described in the literature (Mohan et al., 2011, 2012; Schlemmer et al., 2018), TMSC films are highly hydrophobic, displaying a contact angle of 93.8 ± 1.5 (Fig. 4). Exposure of the TMSC films to wet gaseous hydrochloric acid promotes a gradual

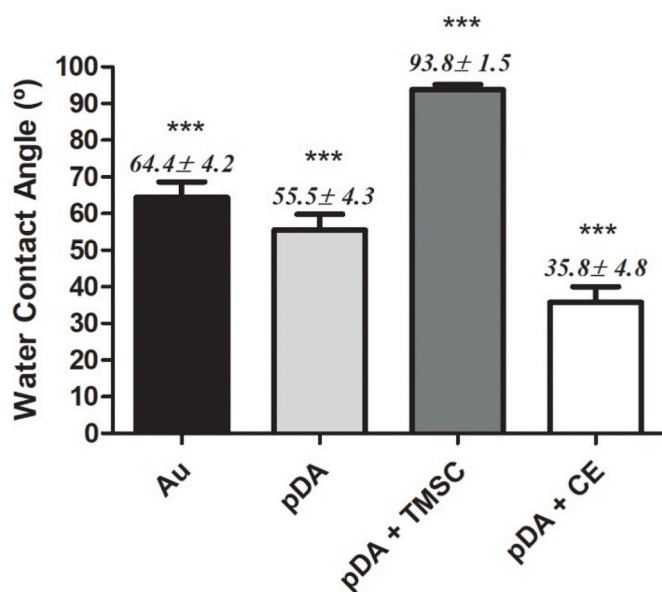


Fig. 4. Static water contact angles determined for Au substrate, pDA, pDA + TMSC and pDA + CE. Statistical differences were observed between all the tested layers using Bonferroni's multiple comparison post-test (***) $p < 0.001$.

desilylation leading to a more hydrophilic surface, as confirmed by the significant reduction in the water contact angle to 35.8 ± 4.2 (Fig. 4). These results clearly demonstrate that a regenerated CE nanometric thin film was successfully obtained, which was then used to perform polysaccharide adsorption studies.

3.3. Interaction of polysaccharides with cellulose thin films: QCM-D analysis

The adsorption of CMC, ChM, Alg, HA and XG to cellulose (CE) was studied using QCM-D. To replicate the conditions used in the assessment of the homogeneity of the mixtures (Fig. 2), only Milli-Q® water without any additional electrolytes was used to solubilize the polysaccharides and perform the analysis. The frequency (Δf) and dissipation (ΔD) shifts (5th, 7th, 9th, 11th and 13th overtones of oscillation) that resulted from the interaction of different polysaccharides with a pure cellulose film are depicted in Fig. 5. A strong deviation between the overtones of frequency and dissipation reflects a low coating rigidity and, therefore, the viscoelastic model was employed, as described in other studies (Liu et al., 2011; Naidjonoka et al., 2020), to estimate the adsorbed mass of polysaccharides before and after rinsing, as well as the films thickness (Table 1). In the tested conditions, high changes in frequency after introduction of CMC, ChM, Alg and HA were observed (Fig. 5A, B, C and D), meaning that the polysaccharides adsorbed to some extent to the cellulose film. Yet, the adsorbed mass decreased upon washing, as Δf increased after rinsing with Milli-Q® water, thus the polysaccharides were reversibly adsorbed to the cellulose layer (Table 1).

Nevertheless, this decrease is more pronounced for Alg, XG and HA (>80 % of desorption). In contrast, for CMC and ChM the detachment after rinsing was only 48 and 39 %, respectively, suggesting a more stable interaction of those polysaccharides with cellulose, in the tested conditions. Furthermore, the thickness reduction was lower in these films (33 ± 14 and 48 ± 23 %, for CMC and ChM respectively). Contrarily to the results obtained by Sorvari et al. (2014), where it was stated that CMC does not adsorb significantly onto nanofibrillated cellulose surfaces in electrolyte free-conditions, we were able to detect around 10 mg/m^2 of CMC in the tested conditions. Apart from the source of cellulose used in film production, the primary differences between the two studies lie in the CMC concentration (0.5 % in this study vs. 0.01 % reported in reference (Sorvari et al., 2014)), molecular weight (90 kDa in this study vs. 700 kDa in reference (Sorvari et al., 2014)), and degree of substitution (0.7 in this study vs. 0.9 in reference (Sorvari et al., 2014)). The higher CMC concentrations and lower molecular weight of the CMC used in our work could have potentiated the contact with cellulose, allowing a cooperative hydrogen bonding between the polysaccharides, which present structural similarities (Martins et al., 2020; Orelma et al., 2012). Indeed, the supramolecular structure of cellulose consists of crystalline assemblies of molecules stabilized by intermolecular hydrogen bonds. Therefore, it is conceivable that carboxymethylated cellulose can also interact with cellulose molecules on the surface of a fiber.

The addition of salts can modulate an irreversible adsorption of anionic CMC on cellulose surfaces. Liu et al. (2011) suggested that the replacement of the monovalent cations, such as Na^+ (that are normally present around the CMC chains) by divalent cations, such as Ca^{2+} , potentiate an electrostatic bridging between the polysaccharide and the regenerated cellulosic surface and consequently increase the adsorbed mass. Furthermore, due to the stronger interaction with carboxylic groups, the adsorbed CMC/ Ca^{2+} complex exhibits greater stability, even after rinsing with water. In a recent study, it was also suggested that, by screening negative charges, Ca^{2+} ions potentiate multichain association of CMC, increasing its hydrodynamic diameter, reducing the polymer-solvent interactions and contributing to the driving force of adsorption. Also, an entropy-driven mechanism has been suggested, where the release of water from the cellulose surface, in the presence of the Ca^{2+} ions, is driving the adsorption of CMC (Arumughan et al.,

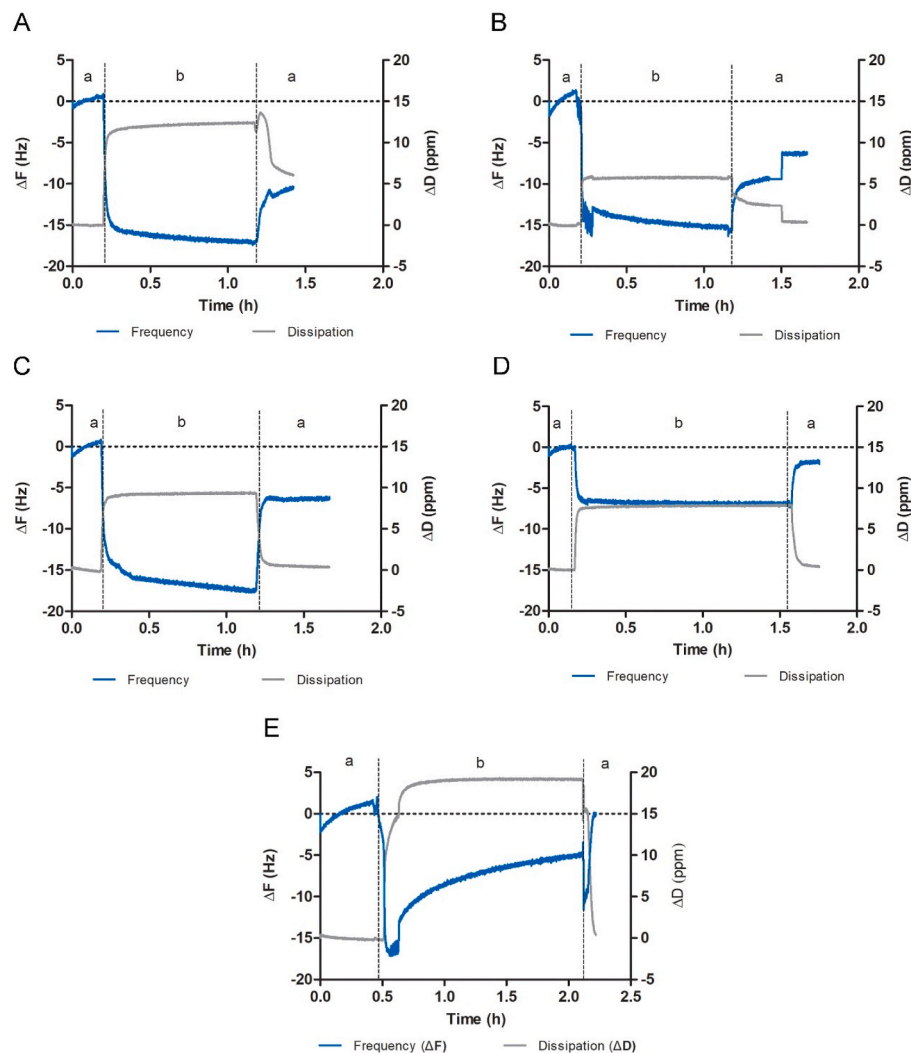


Fig. 5. Representative plots of frequency (Δf) and dissipation (ΔD) changes of the 5th overtones for the adsorption of (A) carboxymethyl cellulose, (B) chia seed mucilage, (C) alginate, (D) hyaluronic acid and (E) xanthan gum on CE thin film. **Legend:** a –films rinsed with Milli-Q® water; b - films incubated with the respective polysaccharide.

Table 1

Adsorbed mass (mg/m^2) and thickness (nm) of 0.5 % carboxymethyl cellulose (CMC), chia seed mucilage (ChM), xanthan gum (XG), alginate (Alg) and hyaluronic acid (HA) on cellulose thin films after 60 min adsorption and 15 min rinsing, calculated using the QCM-D technique. Results for the adsorbed mass are the average of 4 samples and for the thickness the average of 3 samples.

Polysaccharides	Adsorbed mass (mg/m^2)		Detached in rinsing (%)	Thickness (nm)	
	Before rinsing	After rinsing		Before washing	After washing
CMC	20 ± 2	10.3 ± 0.3	48 ± 4	37 ± 12	24 ± 9
ChM	13 ± 2	8 ± 2	39 ± 17	16 ± 6	8 ± 2
Alg	21 ± 2	4 ± 1	82 ± 4	46 ± 14	14 ± 4
HA	17 ± 1	1.0 ± 0.3	94 ± 2	20 ± 5	5 ± 2
XG	15 ± 1	0.2 ± 0.1	98.8 ± 0.4	35 ± 6	3 ± 2

2022).

Despite a partially reversible adsorption being here observed, the obtained results for CMC were similar to the ones obtained in more favorable conditions by Orelma et al. (2012). The authors measured around $11 \text{ mg}/\text{m}^2$ adsorption of salt-free CMC (obtained through a 10–12 kDa mesh dialysis) on cellulose surface when using 0.02 % CMC and 100 mM CaCl_2 .

Therefore, by using CMC at 0.5 % it was possible to obtain (comparatively to the other polysaccharides tested in this work) more stable adsorption (higher amount adsorbed upon washing). Similarly, although the adsorption of ChM was not as high as that of other tested polysaccharides, a higher amount remained adsorbed after washing, thus ChM, as CMC, interacts strongly with cellulose. XG seems to have the lower affinity to cellulose, as depicted by the higher detachment percentage (98.9 %), the higher decrease in the film thickness (90 ± 5 %) after rinsing, as well as by the lower changes in both dissipation and frequency after its contact with cellulose film (Fig. 5E). Despite the chemical similarities with CMC, this polysaccharide has a very distinctive behavior, which could be related to its side chain preventing the contact of the backbone with cellulose (Sorvari et al., 2014), even at high polysaccharide concentrations.

The herein presented results demonstrate that, under the tested conditions, the obtention of a dry and redispersible formulation of cellulose through the addition of a polysaccharide as a dispersing agent is not a straightforward process, since most of the tested polysaccharides failed to perform satisfactorily. Given the diversity of polysaccharides that have been tested unsuccessfully (with different ionic charges, size, structural composition, and ramification), the increase in viscosity of the suspending medium and decrease of inter-fibers contacts, as described by Sorvari et al. (2014), seems not to be enough to obtain a dispersion

effect. We hypothesize that the stability of the polysaccharide adsorption is key to lower the friction between the fibers and prevent mechanical flocculation, achieving an effective dispersion of cellulose (Zauscher et al., 2001). Furthermore, to the best of our knowledge, this work demonstrates for the first time that plant mucilages performance as a potential redispersion aid is comparable to that of CMC.

3.4. Molecular dynamics (MD) simulations: polysaccharide-cellulose systems

Classical MD simulations were conducted as an attempt to better understand the interactions between polysaccharides and cellulose (one of the poorly interacting polysaccharides, HA, was not included in this assessment). First, our cellulose nanocrystal (CNC) model was validated by simulating its molecular motions with and without position restraints. Through a visual inspection of both trajectories, one could observe that in the nanocrystal without position restraints only one or two cellobiose units on the edges were more mobile/flexible, but the core remained very stable. Thus, we opted to proceed with the CNC with position restraints on carbon atoms, which allowed for a more efficient and less time-consuming production runs.

Polysaccharides were also evaluated in solution. Although different conformational states are expected when free in solution versus when in the presence of other units or CNC, this step is important to infer the robustness of the generated parameters and conformational features. The RMSD analysis (Fig. S1) indicates that CMC and Alg maintain a structure closer to that calculated through PM6 calculations, while ChM and XG explore different conformational states. This was somehow expected as ChM and XG present ramifications. Cluster analysis confirmed that the structure which is sampled more frequently is linear and stable in the case of CMC and Alg, whereas more flexible for ChM and XG (Fig. 1).

The radial distribution function (RDF) was one of the descriptors used to evaluate polysaccharide-cellulose interaction. This analysis indicates at which distance from the cellulose surface there is higher probability of the polysaccharide to be located (Fig. 6A). Additionally, the number of contacts up to 0.5 nm was calculated using the CNC as a reference (Fig. 6B). Both analyses indicated that all polysaccharides interacted with cellulose surface to some extent, and reversibly. These interaction features corroborate experimental adsorption studies (Table 1), which showed that all polysaccharides interacted with cellulose to some extent and partially in a reversible way. Nevertheless, our *in-silico* analysis revealed that ChM and XG stood out. It is important to take into consideration two factors: first, the polysaccharide orientation

can interfere on any quantity measured between itself and the cellulose nanocrystal, as in a longitudinal or lateral binding, the interactions are potentiated (larger contact area). Second, the charge of each polysaccharide could facilitate the placement in the surrounding water. ChM and XG are less negatively charged (-2.0 and -4.0 e, respectively), which can facilitate binding to the hydrophobic and hydrophilic faces of the crystal, whereas CMC and Alg are slightly more negative (-5.0 and -8.0 e, respectively).

The number of contacts between polysaccharide units was estimated as well (Fig. 6C), revealing that all of them also interact with other units, but CMC and XG are more prone to it.

Considering all the above, the number of hydrogen bonds established between polysaccharides-CNC, and polysaccharides-water, were calculated to shed light on the interaction pattern of each polysaccharide (Table 2).

The hydrogen bond analysis reinforces the previous results, which indicate that ChM and XG interact more often or using more atoms with cellulose than CMC, as they establish more hydrogen bonds with CNC. Importantly, the high number of hydrogen bonds between XG-H₂O and Alg-H₂O might explain the experimentally determined rapid detachment after rinsing, as indicated by a strong placement and interaction with water. On the other hand, the lower number of ChM interactions with water may imply greater permanence of this polysaccharide on cellulose surfaces, agreeing with RDF and the number of contacts analyses, as well as with the experimental findings.

Nevertheless, the CMC shows an intermediate behavior compared to the ChM and XG, with a reasonable number of hydrogen bonds towards the CNC, and placing their units in the vicinity of the crystal (pairs

Table 2

Average number of hydrogen bonds between polysaccharide-CNC, polysaccharide-H₂O, and polysaccharide-polysaccharide, calculated for the 60 ns of simulation time. Polysaccharide is shortened to "poly" in table columns.

Polysaccharides	Number of hydrogen bonds poly-CNC	Pairs within 0.35 nm poly-CNC	Number of hydrogen bonds poly-H ₂ O	Number of hydrogen bonds poly-poly
CMC	$4.07 \pm 1.16e^{-01}$	$9.65 \pm 2.74e^{-01}$	$696.42 \pm 7.07e^{-01}$	$55.53 \pm 1.78e^{-01}$
ChM	$6.51 \pm 1.59e^{-01}$	$17.14 \pm 3.44e^{-01}$	$457.70 \pm 5.45e^{-01}$	$10.73 \pm 1.34e^{-01}$
XG	$10.51 \pm 2.53e^{-01}$	$10.51 \pm 2.53e^{-01}$	$712.93 \pm 7.00e^{-01}$	$32.72 \pm 2.00e^{-01}$
Alg	$3.16 \pm 1.06e^{-01}$	$6.38 \pm 2.05e^{-01}$	$801.20 \pm 6.16e^{-01}$	$39.79 \pm 1.68e^{-01}$

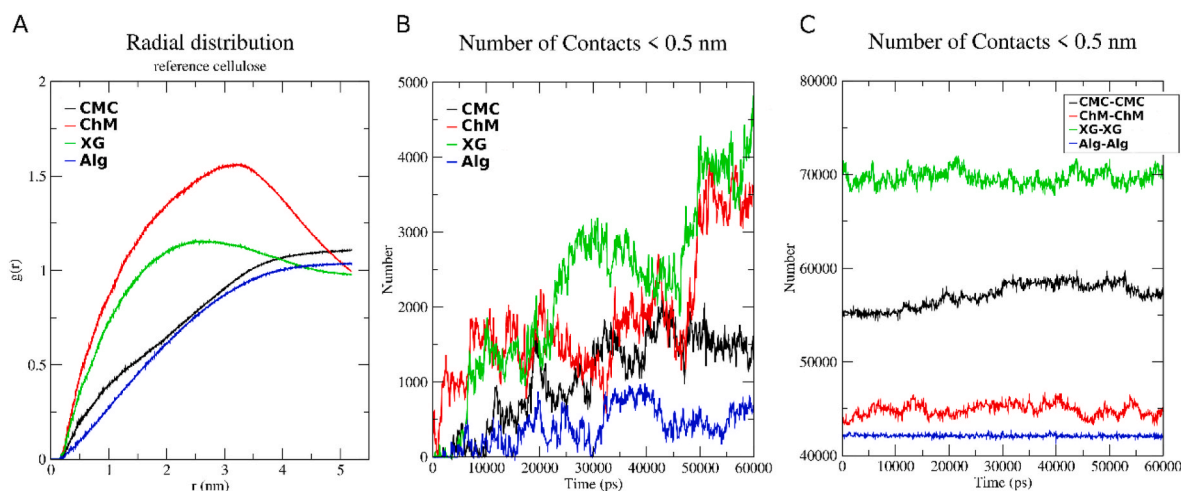


Fig. 6. – (A) Radial distribution function, $g(r)$, of polysaccharides near the cellulose surface, (B) Number of contacts down to 0.5 nm of polysaccharides towards CNC, and (C) Number of contacts down to 0.5 nm amongst polysaccharides units.

within 0.35 nm), but also interact strongly with water molecules and with other units (higher number of hydrogen bonds between units). In addition, CMC has also an intermediate number of contacts and probability of interaction (RDF) towards cellulose. CMC's charge and linearity (less flexible) can contribute to that.

Looking at Alg, the most anionic polysaccharide, it is very stable in solution preferring to be located in the aqueous phase as demonstrated by the low number of interactions and contacts with the CNC. Also, Alg units interact poorly with each other but significantly with water molecules. All these observations corroborate the experimental findings regarding Alg.

Visual inspection of the simulation trajectories provides the final information about the studied systems. Fig. 7 shows the overall distribution of the polysaccharides around the CNC. CMC, ChM and XG are the ones located closer to the fiber, while Alg is highly dispersed in the aqueous phase. The interaction between CMC-CMC units and XG-XG is also evident, as indicated by the hydrogen bond analysis. It is noteworthy that the visual examination of the trajectories shows that CMC often adsorb to the CNC surface in an almost perpendicular manner (Fig. 8A) and ChM in a combination of perpendicular and lateral approach (Fig. 8B), while the others in a more unspecific way. The stable and linear conformation of CMC and its net charge may influence the approach to cellulose fibers on this 'perpendicular' adsorption, affecting the aggregation and thus favoring the dispersion, as observed experimentally. In fact, if interacting many times through its edges, less hydrogen bonds are counted as well as a less pronounced RDF peak is observed, since some saccharides are placed far from the CNC surface, in comparison with a lateral/longitudinal approach. Finally, when CMC, ChM and XG reach the CNC surface, they remain adsorbed for some time before adopting another point of interaction, consisting in a very dynamic interaction in all cases, but with Alg much less adsorbed than the

others.

With more computing power, larger polysaccharide sizes could have been considered, since specific conformations and structure transitions have been documented for larger XG, for example (Ong et al., 2018). Also, a larger system with more than one nanocrystal, to evaluate the dynamics and aggregation processes would be important to address. Nonetheless, important molecular information has been drawn from the systems studied here, which are in fact already more complex than analogous systems reported in the literature (Aguilera-Segura et al., 2020; Gurina et al., 2020).

4. Conclusion

This work investigated the potential of various polysaccharides as dispersing agents for dried BNC formulations, aiming to achieve homogeneous and stable redispersions. Only CMC and ChM effectively dispersed BNC, while other tested polysaccharides failed to achieve satisfactory redispersion.

QCM-D analysis revealed that CMC and ChM interact with cellulose surfaces through a combination of hydrogen bonding and electrostatic interactions. These interactions lead to a more stable adsorption of CMC and ChM onto cellulose, resulting in a lower detachment percentage after rinsing. In contrast, xanthan gum (XG) and alginate (Alg) showed a higher detachment percentage and lower (stable) adsorption onto cellulose. Molecular dynamics (MD) simulations further supported our experimental findings, indicating that CMC and ChM interact more strongly with cellulose surfaces through hydrogen bonding. The simulations also revealed that CMC and ChM tend to adsorb to the cellulose surface in an almost perpendicular manner, which may affect the approach of cellulose fibers and favor dispersion.

Furthermore, this work introduces chia seed mucilage (ChM) as a

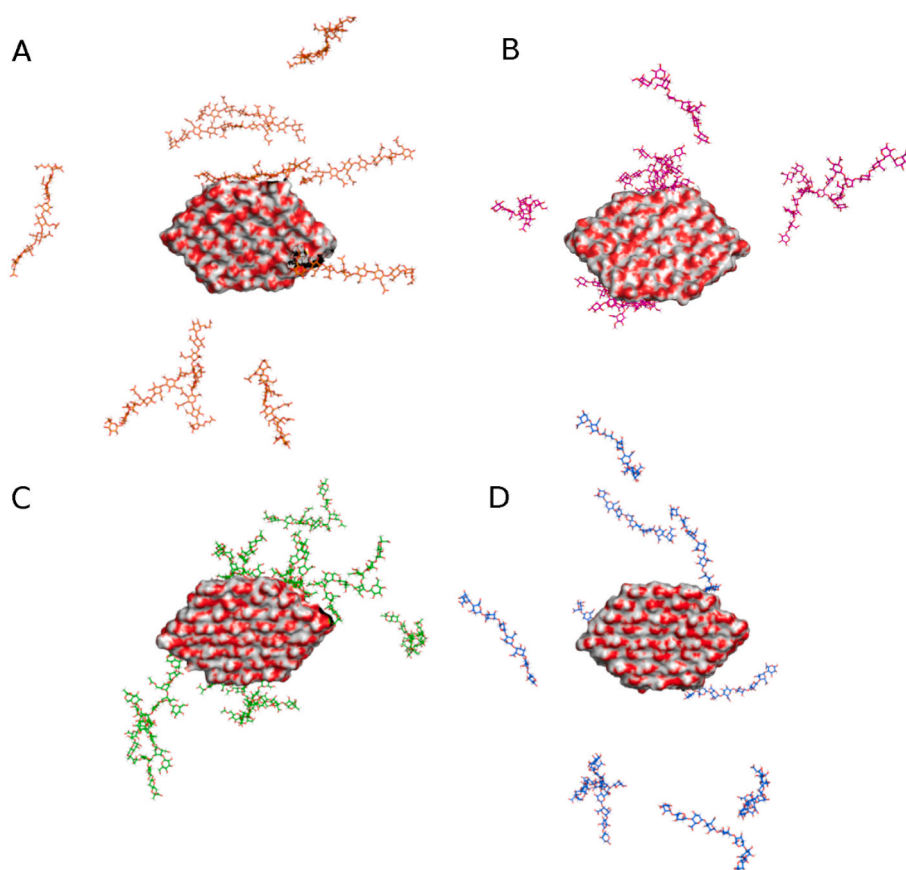


Fig. 7. Final snapshot from the 60 ns of simulation time, of the systems polysaccharide-CNC, where (A) is orange CMC, (B) magenta ChM, (C) green XG, and (D) blue Alg. The CNC surface is represented, with grey carbon and red oxygens.

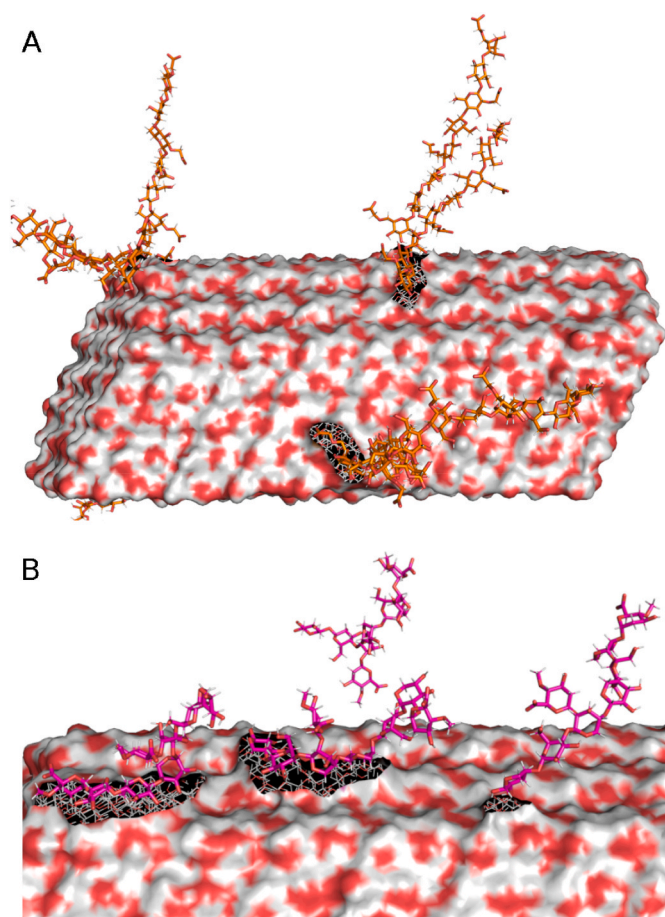


Fig. 8. Random snapshots highlighting interactions between (A) CMC-CNC, that displays ‘perpendicular’ interactions towards the crystal and (B) ChM-CNC, that shows intermolecular interactions between polysaccharide units and the cellulose crystal in varied orientations. The CNC is represented in surface style, with grey carbons and red oxygens.

promising and previously unexplored bio-based dispersing agent for BNC, exhibiting comparable performance to the widely used CMC.

CRedit authorship contribution statement

Ricardo Silva-Carvalho: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Daniela Martins:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Tarsila G. Castro:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ana Cristina Rodrigues:** Writing – review & editing. **Bruna Costa:** Methodology, Investigation. **Paula Parreira:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Artur Cavaco-Paulo:** Resources. **Fernando Dourado:** Writing – review & editing, Conceptualization. **M. Cristina L. Martins:** Writing – review & editing, Resources. **Miguel Gama:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2025.112234>.

Data availability

Data will be made available on request.

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