Accepted Manuscript

Title: A facile route to prepare cellulose-based films

Author: Qin Xu Chen Chen Katelyn Rosswurm Tianming Yao Srinivas Janaswamy



PII:	S0144-8617(16)30491-X
DOI:	http://dx.doi.org/doi:10.1016/j.carbpol.2016.04.114
Reference:	CARP 11054

To appear in:

 Received date:
 21-12-2015

 Revised date:
 14-4-2016

 Accepted date:
 27-4-2016

Please cite this article as: Xu, Qin., Chen, Chen., Rosswurm, Katelyn., Yao, Tianming., & Janaswamy, Srinivas., A facile route to prepare cellulose-based films. *Carbohydrate Polymers* http://dx.doi.org/10.1016/j.carbpol.2016.04.114

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

A facile route to prepare cellulose-based films

Qin Xu^{1*}, Chen Chen¹, Katelyn Rosswurm¹, Tianming Yao^{1,2}, Srinivas Janaswamy^{1,2*}

¹Department of Food Science, Purdue University, West Lafayette, IN 47907-2009, USA ²Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University, West Lafayette, IN 47907-2009, USA

> * Corresponding authors Tel: (765) 494-4183; (765) 494-4914 Fax: (765) 494-7953 E-mail: xuq@purdue.edu; janaswam@purdue.edu

> > December 21, 2015 Submitted to Carbohydrate Polymers Revision: April 14, 2016

Graphical abstract

 Zn^{2+} ions are effective in solubilizing cellulose and crosslink through Ca^{2+} ions leading to stronger cellulose films.



Highlights

- Cellulose is solubilized in the presence of Zn^{2+} ions.
- Zn²⁺ ions hydrogen bond to the atom O3H leading to flexibility in the cellulose chains.
- Presence of Ca²⁺ ions promotes nano fibrils and induces gelation in the Zn-cellulose solution.
- Tensile strength of the Zn-cellulose films is enhanced in the presence of Ca^{2+} ions.
- These cellulose-based novel biodegradable films are potential for food, pharmaceutical and medicinal applications.

Abstract

Cellulose is the most abundant renewable and biodegradable material available in nature. Its insoluble character in water as well as common organic and inorganic liquids, however, curtails the wholesome utility. The continuous rise for biodegradable products based on cellulose coupled with its intrinsic ability to form a viable substitute for the petroleum-based materials necessitates the critical need for solubilizing the cellulose. Herein, we demonstrate the feasibility of ZnCl₂ solutions, especially the 64 - 72% concentrations, to dissolve cellulose. FTIR results suggest that Zn^{2+} ions promote $Zn \cdots O3H$ interactions, which in-turn weaken the intrinsic O3H…O5 hydrogen bonds that are responsible for strengthening the cellulose chains. Interestingly, Ca²⁺ ions promote interactions among the Zn-cellulose chains leading to the formation of nano fibrils and yield gelling solutions. The tensile strength of the Ca²⁺ added Zn-cellulose films increases by around 250% compared to the Zncellulose films. Overall, utilization of inorganic salt solutions to solubilize and crosslink cellulose is cost-effective, recyclable and certainly stands out tall among the other available systems. More importantly, the proposed protocol is simple and is a "green" process, and thus its large-scale adaptability is quite feasible. We strongly believe that the outcome opens up a new window of opportunities for cellulose in the biomedical, pharmaceutical, food and non-food applications.

Keywords: Cellulose solubility; Zinc chloride; Calcium chloride; Cellulose gels; Cellulose film, Nano fibrils

1. Introduction

Cellulose is the most abundant telluric material. It is renewable, inexpensive and can be extracted from wood, cotton, hemp and plant-based materials as well as synthesized by tunicates and microorganisms. On an average, around 700 billion tons of cellulose are

produced every year. Cellulose manifests good biocompatibility, biodegradability and low toxicity, and hence is one of the promising substituents for petroleum materials (Edgar *et al.*, 2001; Klemm, Heublein, Fink & Bohn, 2005; Osullivan, 1997). However, only a small fraction is being exploited mainly because of its insolubility in water as well as common organic and inorganic solvents. Cellulose is a linear polysaccharide composed of β -1–4- linked D-glucopyranosyl units. The chains form a tight network structure stabilized through strong intra- and inter-chain hydrogen bonds (Figueiredo, Ismael, Anjo & Duarte, 2010; Nishiyama, Langan & Chanzy, 2002; Nishiyama, Sugiyama, Chanzy & Langan, 2003; Osullivan, 1997). Such sturdy structural arrangement bestows water insoluble characteristics and thus limits the wholesome utility.

Several solvent systems are being explored as possible agents to circumvent cellulose insolubility. For example, cuoxam, cadoxen, cuen, ammonium, calcium and sodium thiocyanate, lithium chloride/1,3-dimethyl-2-imidazolidinone (LiCl/DMI), lithium chloride/*N*,*N*-dimethylacetamide *N*-methyl morpholine *N*-oxide (NMMO), ammonia/ammonium salt and phosphoric acid are proven to be effective for solubilizing and molding cellulose to tailored modification (Gu & Huang, 2013; Heinze & Koschella, 2005; Heinze & Liebert, 2001; Henniges, Schiehser, Rosenau & Potthast, 2009; Lindman, Karlstrom & Stigsson, 2010; Liu, Sun, Zhang & Li, 2009; Lu, Xu & Wang, 2014; Medronho & Lindman, 2015; Olsson & Westman, 2013; Perepelkin, 2007; Zhang, Xu, Lu, Li & Wang, 2015). However, in these cases issues on toxicity, environmental threat, limited solvency and need of elevated temperatures to dissolve as well as high

power consumption during solvent recycle are unfavorable to large scale utilization. Similarly, ionic liquids such as 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) are effective (El Seoud, Koschella, Fidale, Dorn & Heinze, 2007; Heinze & Liebert, 2001; Isik, Sardon & Mecerreyes, 2014; Lindman, Karlstrom & Stigsson, 2010; Lu, Xu & Wang, 2014; Swatloski, Spear, Holbrey & Rogers, 2002; Xu, Wang & Wang, 2010) but higher production costs, viscosity and sensitivity to moisture hinder their complete utilization. Solubilizing through NaOH, urea and thiourea has also invoked research interest (Heinze & Koschella, 2005; Olsson & Westman, 2013; Xiong, Zhao, Hu, Zhang & Cheng, 2014; Zhang, Li, Yu & Hsieh, 2010); however, difficult solvent recovery and side effects are the impediments. In addition, currently on the industrial scale, there is a growing demand for developing processes that are nonpolluting and effective for environmental conservation. In this regard, great efforts are being focused on inorganic salts. They are simple to use and easily recyclable, non-toxic, environmental friendly and inexpensive (Fischer & Thummler, 2009; Fischer, Voigt & Fischer, 1999; Heinze & Liebert, 2001; Leipner, Fischer, Brendler & Voigt, 2000; Medronho & Lindman, 2014; Olsson & Westman, 2013; Richards & Williams, 1970; Sen, Losey, Gordon, Argyropoulos & Martin, 2016; Sen, Martin & Argyropoulos, 2013; Wang, Lu & Zhang, 2016; Xu & Chen, 1994). We have embarked on a systematic study of solubilizing cellulose through common inorganic salts. This article highlights the important role of zinc chloride as the effective solubilizer and even more as a gelling agent in the presence of calcium chloride. The novelty of our research is the creation of nano fibrils by crosslinking the cellulose chains through zinc and calcium ions, which certainly stands out as a simple and viable protocol, leading to strong and biodegradable films.

5

2. Materials and Methods

2.1 Materials

Microcrystalline cellulose Avicel PH 101 was a gift from FMC Corporation, USA. NH₄Cl, KCl, CaCl₂, FeCl₂, MgCl₂ and ZnCl₂ were purchased from Fisher Scientific.

2.2 Preparation of Zn-cellulose solutions without and with added salts

Initially cellulose paste was prepared by adding 0.8 g (3% w/w) of microcrystalline cellulose (0.05 mole of anhydrous glucose unit, molecular weight = 162) to 1.6 mL of distilled water. In a separate beaker, predetermined weights of ZnCl₂ were dissolved in 6.0 mL of distilled water to make solutions with weight percentage of 50 - 78% and equilibrated at 65 °C (\pm 1) in a water bath for about 10 mins. Later, ZnCl₂ solution was added to the cellulose paste and mixed thoroughly for 30 min.

2.3 Viscosity of Zn-cellulose solutions in the presence of cations

For the study, 3% cellulose dissolved in 68% ZnCl₂ (w/w) was used. The salts NH₄Cl, KCl, CaCl₂, FeCl₂ and MgCl₂ were added in the molar ratio of 0.03 to ZnCl₂. The viscosity of each solution was measured at 65 °C with the Cannon-Fenske Routine viscometer (Cannon Instrument Co., State College PA, USA). The sizes of the capillary tube were 100, 200, 500 and 600 series (Model No. S769, 2918, L318 and 554). Average values from duplicate measurements are reported.

2.4 Rheology

Viscoelastic measurements were conducted using an ARG2 mechanical spectrometer from the TA Instruments, New Castle, DE. The instrument was equipped with cone and plate geometry (40 mm steel, 2° cone) along with a solvent trap to minimize water evaporation from the sample during experiment. Linear viscoelastic region was identified from the strain sweep measurements in the range 0.01 to 20% strain at 1Hz and 5 °C. Change in the storage modulus (*G*') and loss modulus (*G*'') as a function of frequency from 0.1 to 100 Hz at 5% strain (within the linear viscoelastic range) were obtained at two selected temperatures 25 and 65 °C. The measurements were done in duplicate and average values are reported.

2.5 FTIR characterization

Spectra of Avicel powder and films made from the Zn-cellulose complex solution without and with calcium ions were recorded using a Nexus 670 FTIR Spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with a liquid nitrogen cooled mercury cadmium telluride detector and KBr beam splitter. The scan range was set from 500 - 4000 cm⁻¹ with a total of 256 scans at 4 cm⁻¹ resolution. Film measurements were obtained by applying a uniform pressure to the film on a Smart Multibounce HATR flat plate accessory (ZnSe crystal with a 45° angle). The powders were dispersed at a ratio of 0.1g in 0.5g spectroscopic grade KBr, mixed using a steel ball pestle and vial in a Wig-L-Bug[®] for 15 s, and spectra measured using a Smart Diffuse Reflectance FT-IR accessory.

2.6 Zn- & Ca-Zn-cellulose film preparation

The bubbles were vacuumed out, if any, from the Zn-cellulose and Ca-Zn- cellulose solutions. The films were hand casted on a glass plate (21 x 30 cm) using a thin-layer chromatographic plate applicator. The clearance between the applicator and glass plate was set to 1 mm. Later, the glass plate along with the film was immersed in 500 mL ethanol (200 proof). The film was coagulated for 30 min and immersed in fresh ethanol bath (500 mL) for another 30 min. The film was then fixed on a frame and air dried at the room temperature. It was placed in a water bath for 30 min to remove any excess salt and subsequently soaked in glycerol (5% v/v) for another 30 min. Finally, the film was air dried for further characterization.

2.7 Scanning Electron Microscopy (SEM)

A FEI NOVA NanoSEM from the Purdue Life Science Microscopy Facility was used to image the cellulose films. The instrument was operated at 5 kV. Samples were coated with platinum using a Cressington turbo-pumped supper coater.

2.8 Tensile strength of the films

The films were cut into rectangular strips of roughly 8 cm long and 1 cm wide. The tensile strengths were measured using the MTS EM Tensile Testing System by adapting the ISO1924-2:2008 protocol with minor modifications. The 100N MTS Advantage Pneumatic Grip was used and the force measurement recorded using the 50N sensor that has the accuracy of 0.01N. The gap between the grips was set to be 6 cm and the speed of elongation as 15mm/s. The width and thickness of the strip were measured accurately

using the 6" steel precision dial caliper. Average values from triplicate measurements are reported.

3. Results

3.1 Effect of zinc chloride on cellulose solubility

A range of cellulose concentrations up to 3% has been added to 50 - 78% ZnCl₂ solutions at 65 °C. **Fig. 1** highlights 1 and 3% cellulose in the 50, 68 and 78% ZnCl₂ solutions mixed at 65 °C. Transparent and homogeneous cellulose solutions could be obtained from 68% ZnCl₂. On the other hand, in the 50% solution cellulose dissolves partially with sedimented particles leading to a white turbid mixture and at 78% a thick opaque paste is noticed. These observations are intact at 25 °C mixing too but increasing the temperature to 80 °C hydrolyzes cellulose especially in the 78% salt solutions (results are not shown). Further analysis suggests that 64 – 72% of ZnCl₂ is the optimal range for dissolving cellulose; therefore, rest of the investigation has focused on this salt range using 3% cellulose and 65 °C as the mixing temperature. The rationale behind choosing these parameters is to obtain homogeneous solutions as well for easy film casting.

Fig.2 highlights the viscosity change of 3% cellulose solution measured at 65 °C. The viscosity gradually decreases from 4000 to 1500 cps as the Zn salt concentration increases from 64 to 68% and later raises to 9000 cps at 72%. As noted in the **Fig. 2** inset, the viscosity of pure ZnCl₂ solution monotonously increases with salt, which could be attributed to the formation of polymeric Zn-Cl-Zn bridges through a series of electrostatic

9

interactions (Richards & Williams, 1970). These Zn-Cl-Zn bridges seem to be broken by the presence of cellulose presumably due to the preferential interactions of Zn^{2+} ions with the cellulose chains (**Fig. 8b**). It is a well-established fact that the insoluble characteristics of cellulose are mainly derived from its strong intra- and inter-sheet hydrogen bonding network arrangements. The structure analysis of cellulose suggests that intra-chain O3H····O5 hydrogen bonds bestow the stiff molecular stability and interchains interactions O3H····O6 and O2H····O6 promote the network association (Langan, Nishiyama & Chanzy, 1999, 2001; Nishiyama, Langan & Chanzy, 2002; Nishiyama, Sugiyama, Chanzy & Langan, 2003; Wada, Nishiyama & Langan, 2006). Thus, it appears that upon the addition of cellulose to the ZnCl₂ solution, the Zn²⁺ ions disperse through the cellulose matrix and permeate between the sheets, and compete for strong interactions with the hydroxyl groups O2H, O3H and O6H. Consequently, the intra- and inter-sheet hydrogen bonds are broken leading to cellulose solubility.

3.2 Effect of salts on Zn-cellulose viscosity

In the presence of other cations the viscosity of Zn-cellulose solution could further change due to specific cation-cation and cation-cellulose interactions. In this regard, influence of monovalent (NH_4^+ , K^+) and divalent (Ca^{2+} , Mg^{2+} and Fe^{2+}) ions, at a molar ratio (cation vs. Zn) of 0.03, has been examined. **Fig. 3** highlights the cation induced viscosity change in the 3% cellulose dissolved in 68% ZnCl₂ solution (as control). The control solution has a viscosity around 1300 cps and remains similarly constant across all the salts. However, among the cations added to Zn-cellulose solution, only Ca^{2+} ions appear to promote favorable interactions and viscosity rises almost 2.5 times to 3100 cps.

10

On the other hand, addition of salts to pure $ZnCl_2$ solutions does not change the viscosity (inset **Fig. 3**). Thus, there appears to be some preferential interactions among the Zn-cellulose chains in the presence of Ca^{2+} ions. In order to gain better insights about the solution behavior of Ca-Zn-cellulose, its viscoelastic properties are measured.

3.3 Effect of calcium on the Zn-cellulose viscoelastic properties

The elastic (*G*') and loss (*G*'') moduli variations of the solutions in the frequency range 0.1 - 100 Hz at 25 and 65 °C are shown in **Fig. 4**. In the Zn-cellulose solution, *G*' and *G*'' at 25 °C and 1 Hz are very low at 0.03 and 6.58 Pa, respectively (**Fig. 4a**). This trend is preserved at 65 °C too with values of 0.02 and 1.09 Pa (**Fig. 4b**), in the same order. On the other hand, with the Ca²⁺ ions addition (molar ratio of 0.03) both *G*' and *G*'' increase to 1783 and 886 Pa, respectively, at 25 °C and 1 Hz (**Fig. 4c**), indicating new gelation behavior. However, at 65 °C this trend reverses *i.e. G*'' (21.7 Pa) is greater than *G*' (7.4 Pa) as a result of the viscous nature of the sample (**Fig. 4d**). Interestingly, increasing the Ca²⁺ ions (molar ratio of 0.06) boosts *G*' and *G*'' to 3320 and 1626 Pa at 25 °C (**Fig. 4e**), and 946 and 528 Pa at 65 °C (**Fig. 4f**), respectively, suggesting the stronger gelling behavior of the solution. Thus, it appears that Ca²⁺ ions are able to crosslink adjacent Zn-cellulose chains and promote junction zones leading to gelation.

3.4 Spectroscopic characterization of cellulose films

The FTIR spectra from Avicel (in powder form), Zn-cellulose film and Ca-Zn-cellulose film in the range 660 -1480 cm⁻¹ are compared in **Fig. 5a.** In the case of Avicel,

absorption bands at 1431, 1373, 1338, 1321, 1284, 1234, 1207, 1165, 1134, 1072, 1047, 933, 889, 820, 712, 677 and 667 cm⁻¹ are observed. Among them, the band at 1047 cm⁻¹ corresponds to the C-O valence vibration from C3-O3H (Marechal & Chanzy, 2000), while the rest signify HOC in-plane bending, OH plane deformation and vibration, CH vibration and deformation, COC, CCO and CCH deformation and stretching vibrations, and C-OH out-of-plane bending, to name a few (Fan, Dai & Huang, 2012; Fengel & Strobel, 1994; Kondo & Sawatari, 1996; Oh, Yoo, Shin & Seo, 2005; Schwanninger, Rodrigues, Pereira & Hinterstoisser, 2004). These bands are more or less maintained in the Zn-cellulose film but without the 1047 cm⁻¹ peak. In addition, new peaks at 1442, 1311, 1263, 1117, 1099, 1020, 995, 901 and 652 cm⁻¹ are observed, which could be attributed to the presence of Zn²⁺ in the cellulose matrix and possible OH…Zn interactions. In the Ca-Zn-cellulose film, peaks are similar to those of the Zn-cellulose but with enhanced intensities suggesting similar interaction mechanism as in Zncellulose.

In general, vibrations of the intramolecular hydrogen bonds for O2H····O6, O3H····O5 and intermolecular hydrogen bonds for O6H····O3 as well as the valence vibrations of hydrogen bonded OH groups appear at 3455-3410, 3375-3340, 3310-3230 and 3570-3450 cm⁻¹, respectively (Kondo & Sawatari, 1996; Oh, Yoo, Shin & Seo, 2005; Schwanninger, Rodrigues, Pereira & Hinterstoisser, 2004). In order to further realize the changes in the hydrogen-bonded OH stretching due to the presence of Zn²⁺ and Ca²⁺ ions the FTIR spectra in the range 3000 - 3700 cm⁻¹ have been compared (**Fig. 5b**). Furthermore, assuming that all the vibration modes follow a Gaussian distribution, the mixed modes of hydrogen bonded OH stretching have been resolved into a minimum of

three individual bands using the OriginPro 9.1.0 (Academic). The fitting procedure involves a Gaussian distribution function at the above four mentioned bands with the maximum absorbance and bandwidth at half-maximum. The analysis reveals that only three bands could be fitted in each spectrum. In the Avicel, bands are observed at 3514, 3354 and 3230 cm⁻¹ (Fig. 5c) and correspond to the valence vibrations of hydrogen bonded OH groups (Oh, Yoo, Shin & Seo, 2005; Schwanninger, Rodrigues, Pereira & Hinterstoisser, 2004), intramolecular hydrogen bonds of O3H····O5 and intermolecular hydrogen bonds of O6H…O3, respectively (Fengel & Strobel, 1994; Kondo & Sawatari, 1996; Oh, Yoo, Shin & Seo, 2005). On the other hand, in the presence of Zn^{2+} ions, the bands are observed at 3446, 3328 and 3193 cm^{-1} (Fig. 5d). The band at 3328 cm^{-1} could be attributed to the intramolecular hydrogen bonds of O3H····O5 and that at 3446 cm⁻¹ to the Zn…OH stretching (Kloprogge, Hickey & Frost, 2004) as observed in the hydrotalcites, the layered double hydroxides also known as anionic clays. Similarly, 3193 cm⁻¹ to the strongly bound water to the OH groups of the cellulose as well as hydrogen bonded interlayer water surrounding the interlayer anion (Kalutskaya & Gusev, 1980; Olsson & Salmen, 2004; Walrafen & Klein, 1987). Interestingly, with the addition of Ca²⁺ ions, the bands observed in Zn-cellulose are more or less maintained but with little changes at 3440, 3370 and 3240 cm⁻¹ (Fig. 5e).

The disappearance of the 1047 cm⁻¹ peak in Zn-cellulose films suggests the waning of C3-O3H vibrations presumably due to the formation of O3H····Zn interactions (**Fig 8b**), which in-turn weakens the existing intra-chain O3H····O5 hydrogen bonds (**Fig. 8a**) that are responsible for strengthening the cellulose molecular structure. In the cellulose

network, adjacent layers are separated by around 3.9 Å (Nishiyama, Langan & Chanzy, 2002; Nishiyama, Sugiyama, Chanzy & Langan, 2003; Wada, Nishiyama & Langan, 2006) and such tight arrangement along with the stiff and sheet like molecular structure bestows insolubility. However, in the presence of Zn^{2+} ions the adjacent cellulose layers might be pushed apart (**Fig. 8b**). The culminating effect of flexible cellulose chains due to O3H…Zn interactions coupled with increase in the adjacent cellulose layer spacing appear to be key in solubilizing cellulose.

3.5 Electron micrographs of cellulose films

As the Zn^{2+} ions are able disintegrate the cellulose network, Zn-cellulose complex could be solubilized and in-turn Ca^{2+} ions could be incorporated leading to cellulose-based biodegradable films. SEM micrographs captured with a magnification of 40,000 on the cross-section of the films are shown in **Fig. 6**. The Zn-cellulose displays a smooth structure (**Fig. 6a**) while addition of Ca^{2+} ions (Ca:Zn = 0.03 Molar ratio) results in undulating surface (**Fig. 6b**) presumably due to fibrils formation. These fibrils become more pronounced (**Fig. 6c**) by increasing the Ca^{2+} ions (Ca:Zn = 0.06 Molar ratio) amount. Interestingly, the size of the fibrils is in the range 50-150 nm. In recent times, cellulose nano fibrils have gained attraction due to their intrinsic properties of excellent mechanical strength, optical transparency and high surface area, especially for tailoring materials properties (Lin & Dufresne, 2014; Moon, Martini, Nairn, Simonsen & Youngblood, 2011). Several protocols based on mechanical treatment (Lavoine, Desloges, Dufresne & Bras, 2012), chemical hydrolysis (Dufresne, 2013; Leung et al., 2011), high pressure homogenization coupled with acid hydrolysis (Tian, Yi, Wu, Wu,

Qing & Wang, 2016) and utilization of subcritical water (Novo, Bras, Garcia, Belgacem & Curvelo, 2015), to name a few, are being exploited successfully. On the other hand, our research highlights that cellulose nano fibrils could be formed through ion crosslinking and certainly appears to be a simple and viable protocol. However, an indepth study is necessary to take advantage of the procedure and produce large scale cellulose nano fibrils.

3.6 Tensile strengths of cellulose films

Zn-cellulose films are white in color with a tensile strength of 21.7 KN/m² (**Fig. 7**). The addition of Ca²⁺ ions (Ca:Zn = 0.03 Molar ratio) yields transparent films and the tensile strength surges to 69.4 KN/m² (~ 250% increase). The strength further augments to 88.2 KN/m² (~ 350% increase) by adding more Ca²⁺ ions (Ca:Zn = 0.06 Molar ratio). Overall, it appears that addition of Ca²⁺ ions increases the gel strength in the Zn-cellulose solutions (**Fig. 4c**, **4e** and **4f**) with shifts in the FTIR wavenumbers (**Fig. 5d** and **5e**) and the films tensile strengths (**Fig. 7**) due to the formation of nano fibrils (**Fig. 6**) through crosslinking the Zn-cellulose chains. The formation of cellulose nano fibrils through ion crosslinking could be one of the reasons in obtaining stronger cellulose films.

4. Discussion

Three-dimensional structure analysis (Langan, Nishiyama & Chanzy, 1999, 2001; Nishiyama, Langan & Chanzy, 2002; Nishiyama, Sugiyama, Chanzy & Langan, 2003;

Wada, Nishiyama & Langan, 2006) reveals that cellulose adapts a rigid 2-fold helical structure with strong intra-chain O3H…O5 hydrogen bonds (Fig. 8a). On the other hand, a heuristic survey on the cellulosic polysaccharides suggests that substitution at the atom O3H leads to coiled structures. Bacterial polysaccharides such as welan, RMDP17, xanthan and acetan, to name a few, are the best-known examples. With a tetrasaccharide chemical repeat of \rightarrow 3)- β -D-Glc-(1 \rightarrow 4)- β -D-GlcA-(1 \rightarrow 4)- β -D-Glc-(1 \rightarrow 4)- α -L-Rha- $(1 \rightarrow both welan and RMDP17 have the substitution of a monosaccharide (\alpha-L-$ Rhamnose or α -L-Manose) and disaccharide (β -D-Glucose-1 \rightarrow 4- β -D-Glucose), respectively, on one of the glucose units that results in breaking the canonical $O3H \cdots O5$ hydrogen bonds leading to 3-fold helical structures (Bian, Chandrasekaran & Rinaudo, 2002; Chandrasekaran, Radha & Lee, 1994). Similarly, with overpowering side groups of trisaccharide (β -D-Glucose-1 \rightarrow 4- β -D-Glucose1 \rightarrow 4- β -D-Glucose) and pentasaccharide $(\beta$ -D-Glucose-1 \rightarrow 4- β -D-Glucose1 \rightarrow 4- β -D-Glucose1 \rightarrow 4- β -D-Glucose1 \rightarrow 4- β -D-Glucose) even in the xanthan and acetan $O3H \cdots O5$ hydrogen bonds are broken leading to 5-fold molecular structures (Chandrasekaran, Janaswamy & Morris, 2003; Chandrasekaran & Radha, 1997). Thus, abandoning of one or more of the O3H…O5 hydrogen bonds in the cellulose stems the chain flexibility and looks like bacteria adopts this elegant approach in producing water-soluble cellulosic polysaccharides. It appears that similar phenomenon is occurring in the Zn-cellulose complexes too, especially Zn^{2+} ions pairing with the O3H atoms in forming the Zn···O3H interactions and in-turn tampering the intra-chain hydrogen bonds (**Fig. 8b**) leading to flexibility. The resulting non-rigid molecular structures are amenable for water penetration and hence cellulose gets solubilized. It appears that these non-rigid Zn-cellulose chains could be crosslinked

effectively through Ca^{2+} ions (**Fig. 8c**) toward forming nano fibrils. More experiments are needed to establish the mode of Ca^{2+} interactions within the Zn-cellulose network, however.

5. Conclusions

The demand for cellulose-based biodegradable products is on continuous rise and in order to meet these growing requirements, solubilizing cellulose is critical. Over all these years, the quest for new solvent systems has led to the design and development of several toxic and non-toxic, expensive and inexpensive as well as stable and less-stable systems. Our research reports the favorable effect of Zn^{2+} ions on solubilizing cellulose via breaking or weaning O3H…O5 hydrogen bonds that are responsible for the tight and insoluble cellulose network structure. The presence of Ca^{2+} ions readily crosslinks the Zn-cellulose chains towards nano fibrils formation and yields sturdy and transparent films. We strongly believe that this stimulating result opens up a new window of opportunities for cellulose in the biomedical, pharmaceutical, food and non-food applications.

Cellulose is environmental friendly and forms a viable substitute for the petroleum-based materials. Hence, its successful solubilization could aid in the design and development of novel packaging materials, control delivery carriers, water purification systems, sensors, to name a few. Utilization of inorganic salt solutions to solubilize and crosslink cellulose certainly stands out tall due to their intrinsic cost-effective and recyclable nature, and more importantly the method is a "green" process. Further investigation is planned on the

17

preparation of cellulose particles, hydrogels, nanofibers, bioactive membranes, and loading and control release of proteins and other bioactive compounds.

Acknowledgements

We thank Mattews Allen and Lohit Myneedu for help with the FTIR and Rheology measurements, and Dr. Gudrun Schmidt for critical review of the manuscript. Financial support is from the John & Emma Tse through Li-Fu Chen Memorial Laboratory fund, Martin Undergraduate Research Fund Scholarship from Agricultural Research at Purdue (ARP) and Whistler Center for Carbohydrate Research.

References

- Bian, W., Chandrasekaran, R., & Rinaudo, M. (2002). Molecular structure of the rhamsan-like exocellular polysaccharide RMDP17 from Sphingomonas paucimobilis. *Carbohydrate Research*, 337(1), 45-56.
- Chandrasekaran, R., Janaswamy, S., & Morris, V. J. (2003). Acetan: glucomannan interactions a molecular modeling study. *Carbohydrate Research*, *338*(24), 2889-2898.
- Chandrasekaran, R., & Radha, A. (1997). Molecular modeling of xanthan:galactomannan interactions. *Carbohydrate Polymers*, *32*(3-4), 201-208.
- Chandrasekaran, R., Radha, A., & Lee, E. J. (1994). Structural roles of calcium-ions and side-chains in welan an X-ray study. *Carbohydrate Research*, 252, 183-207.
- Dufresne, A. (2013). Nanocellulose: a new ageless bionanomaterial. *Materials Today*, *16*(6), 220-227.
- Edgar, K. J., Buchanan, C. M., Debenham, J. S., Rundquist, P. A., Seiler, B. D., Shelton, M. C., & Tindall, D. (2001). Advances in cellulose ester performance and application. *Progress in Polymer Science*, 26(9), 1605-1688.
- El Seoud, O. A., Koschella, A., Fidale, L. C., Dorn, S., & Heinze, T. (2007). Applications of ionic liquids in carbohydrate chemistry: A window of opportunities. *Biomacromolecules*, 8(9), 2629-2647.
- Fan, M., Dai, D., & Huang, B. (2012). Fourier transform infrared spectroscopy for natural fibers. In S. M. Salih (Ed.). *Fourier Transform - Materials Analysis* (pp. 45-68): In Tech.
- Fengel, D., & Strobel, C. (1994). FTIR spectroscopic studies on the heterogeneous transformation of cellulose-I into cellulose-II. *Acta Polymerica*, 45(4), 319-324.
- Figueiredo, J. A., Ismael, M. I., Anjo, C. M. S., & Duarte, A. P. (2010). Cellulose and derivatives from wood and fibers as renewable sources of raw-materials. In A. P. Rauter, P. Vogel & Y. Queneau (Eds.). *Carbohydrates in Sustainable Development I: Renewable Resources for Chemistry and Biotechnology* (Vol. 294, pp. 117-128). Berlin: Springer-Verlag Berlin.
- Fischer, S., & Thummler, K. (2009). Molten inorganic salts as reaction medium for cellulose. *Cellulose Solvents: for Analysis, Shaping and Chemical Modification*, 1033, 91-101.

- Fischer, S., Voigt, W., & Fischer, K. (1999). The behaviour of cellulose in hydrated melts of the composition LiX center dot $nH(2)O(X = I^-, NO_3^-, CH_3COO^-, ClO_4^-)$. *Cellulose*, 6(3), 213-219.
- Gu. Y., & Huang, J. (2013). Ultrathin cellulose film coating of porous alumina membranes for adsorption of superoxide dismutase. *Journal of Materials Chemistry B*, 1, 5636-5643.
- Heinze, T., & Koschella, A. (2005). Solvents applied in the field of cellulose chemistry: A mini review. *Polímeros: Ciência e Tecnologia*, 15(2), 84-90.
- Heinze, T., & Liebert, T. (2001). Unconventional methods in cellulose functionalization. *Progress in Polymer Science*, *26*(9), 1689-1762.
- Henniges, U., Schiehser, S., Rosenau, T., & Potthast, A. (2009). Cellulose solubility: Dissolution and analysis of "Problematic" cellulose pulps in the solvent system DMAc/LiCl. Cellulose Solvents: for Analysis, Shaping and Chemical Modification, 1033, 165-177.
- Isik, M., Sardon, H., & Mecerreyes, D. (2014). Ionic liquids and cellulose: Dissolution, chemical modification and preparation of new cellulosic materials. *International Journal of Molecular Sciences*, 15(7), 11922-11940.
- Kalutskaya, E. P., & Gusev, S. S. (1980). An infrared spectroscopic investigation of the hydration of cellulose. *Polymer Science U.S.S.R.*, 22(3), 550-556.
- Klemm, D., Heublein, B., Fink, H. P., & Bohn, A. (2005). Cellulose: Fascinating biopolymer and sustainable raw material. *Angewandte Chemie-International Edition*, 44(22), 3358-3393.
- Kloprogge, J. T., Hickey, L., & Frost, R. L. (2004). FT-Raman and FT-IR spectroscopic study of synthetic Mg/Zn/Al-hydrotalcites. *Journal of Raman Spectroscopy*, 35(11), 967-974.
- Kondo, T., & Sawatari, C. (1996). A Fourier transform infra-red spectroscopic analysis of the character of hydrogen bonds in amorphous cellulose. *Polymer*, *37*(3), 393-399.
- Langan, P., Nishiyama, Y., & Chanzy, H. (1999). A revised structure and hydrogenbonding system in cellulose II from a neutron fiber diffraction analysis. *Journal of the American Chemical Society*, 121(43), 9940-9946.
- Langan, P., Nishiyama, Y., & Chanzy, H. (2001). X-ray structure of mercerized cellulose II at 1 angstrom resolution. *Biomacromolecules*, *2*(2), 410-416.

- Lavoine, N., Desloges, I., Dufresne, A., & Bras, J. (2012). Microfibrillated cellulose Its barrier properties and applications in cellulosic materials: A review. *Carbohydrate Polymers*, 90(2), 735-764.
- Leipner, H., Fischer, S., Brendler, E., & Voigt, W. (2000). Structural changes of cellulose dissolved in molten salt hydrates. *Macromolecular Chemistry and Physics*, 201(15), 2041-2049.
- Leung, A. C. W., Hrapovic, S., Lam, E., Liu, Y. L., Male, K. B., Mahmoud, K. A., & Luong, J. H. T. (2011). Characteristics and properties of carboxylated cellulose nanocrystals prepared from a novel one-step procedure. *Small*, 7(3), 302-305.
- Lin, N., & Dufresne, A. (2014). Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal*, 59, 302-325.
- Lindman, B., Karlstrom, G., & Stigsson, L. (2010). On the mechanism of dissolution of cellulose. *Journal of Molecular Liquids*, 156(1), 76-81.
- Liu, C., Sun, R., Zhang, A., & Li, W. (2009). Dissolution of cellulose in ionic liquids and its application for cellulose processing and modification. *Cellulose Solvents: for Analysis, Shaping and Chemical Modification, 1033*, 287-297.
- Lu, B., Xu, A., & Wang, J. (2014). Cation does matter: how cationic structure affects the dissolution of cellulose in ionic liquids. *Green Chemistry*, *16*(3), 1326-1335.
- Marechal, Y., & Chanzy, H. (2000). The hydrogen bond network in Iβ cellulose as observed by infrared spectrometry. *Journal of Molecular Structure*, *523*, 183-196.
- Medronho, B., & Lindman, B. (2014). Competing forces during cellulose dissolution: From solvents to mechanisms. *Current Opinion in Colloid & Interface Science*, 19(1), 32-40.
- Medronho, B., & Lindman, B. (2015). Brief overview on cellulose dissolution/regeneration interactions and mechanisms. Advances in Colloid and Interface Science, 222, 502-508.
- Moon, R. J., Martini, A., Nairn, J., Simonsen, J., & Youngblood, J. (2011). Cellulose nanomaterials review: structure, properties and nanocomposites. *Chemical Society Reviews*, 40(7), 3941-3994.
- Nishiyama, Y., Langan, P., & Chanzy, H. (2002). Crystal structure and hydrogenbonding system in cellulose 1β from synchrotron X-ray and neutron fiber diffraction. *Journal of the American Chemical Society*, *124*(31), 9074-9082.

- Nishiyama, Y., Sugiyama, J., Chanzy, H., & Langan, P. (2003). Crystal structure and hydrogen bonding system in cellulose 1α, from synchrotron X-ray and neutron fiber diffraction. *Journal of the American Chemical Society*, *125*(47), 14300-14306.
- Novo, L. P., Bras, J., Garcia, A., Belgacem, N., & Curvelo, A. A. S. (2015). Subcritical water: A method for green production of cellulose nanocrystals. ACS Sustainable Chemistry & Engineering, 3(11), 2839-2846.
- Oh, S. Y., Yoo, D. I., Shin, Y., & Seo, G. (2005). FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. *Carbohydrate Research*, *340*(3), 417-428.
- Olsson, A. M., & Salmen, L. (2004). The association of water to cellulose and hemicellulose in paper examined by FTIR spectroscopy. *Carbohydrate Research*, *339*(4), 813-818.
- Olsson, C., & Westman, G. (2013). *Direct dissolution of cellulose: Background, means and applications*. InTech.
- Osullivan, A. C. (1997). Cellulose: the structure slowly unravels. *Cellulose*, 4(3), 173-207.
- Perepelkin, K. E. (2007). Lyocell fibres based on direct dissolution of cellulose in N-methylmorpholine N-oxide: Development and prospects. *Fibre Chemistry*, 39(2), 163-172.
- Richards, N. J., & Williams, D. G. (1970). Complex formation between aqueous zinc chloride and cellulose-related D-glucopyranosides. *Carbohydrate Research*, 12(3), 409-420.
- Schwanninger, M., Rodrigues, J. C., Pereira, H., & Hinterstoisser, B. (2004). Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose. *Vibrational Spectroscopy*, 36(1), 23-40.
- Sen, S., Martin, J. D., & Argyropoulos, D. S. (2013). Review of cellulose nonderivatizing solvent interactions with emphasis on activity in inorganic molten salt hydrates. Acs Sustainable Chemistry & Engineering, 1(8), 858-870.
- Sen, S., Losey, B. P., Gordon, E. E., Argyropoulos, D. S., & Martin, J. D. (2016). Ionic Liquid Character of Zinc Chloride Hydrates Define Solvent Characteristics that Afford the Solubility of Cellulose. *Journal of Physical Chemistry B*, 120(6), 1134-1141.
- Swatloski, R. P., Spear, S. K., Holbrey, J. D., & Rogers, R. D. (2002). Dissolution of cellose with ionic liquids. *Journal of the American Chemical Society*, 124(18), 4974-4975.

- Tian, C. H., Yi, J. A., Wu, Y. Q., Wu, Q. L., Qing, Y., & Wang, L. J. (2016). Preparation of highly charged cellulose nanofibrils using high-pressure homogenization coupled with strong acid hydrolysis pretreatments. *Carbohydrate Polymers*, 136, 485-492.
- Wada, M., Nishiyama, Y., & Langan, P. (2006). X-ray structure of ammonia-cellulose I: New insights into the conversion of cellulose I to cellulose III. *Macromolecules*, 39(8), 2947-2952.
- Walrafen, G. E., & Klein, P. H. (1987). Infrared investigation of hydrogen-bonding on the surface of beryllium fluoride glass. *Journal of Chemical Physics*, 86(11), 6515-6522.
- Wang, S., Lu, A. & Zhang, L. (2016). Recent advances in regenerated cellulose materials. *Progress in Polymer Science*, *53*, 169-206.
- Xiong, B., Zhao, P., Hu, K., Zhang, L., & Cheng, G. (2014). Dissolution of cellulose in aqueous NaOH/urea solution: Role of urea. *Cellulose*, *21*(3), 1183-1192.
- Xu, A., Wang, J., & Wang, H. (2010). Effects of anionic structure and lithium salts addition on the dissolution of cellulose in 1-butyl-3-methylimidazolium-based ionic liquid solvent systems. *Green Chemistry*, 12(2), 268-275.
- Xu, Q., & Chen, L. F. (1994). Characterization of cellulose film prepared from zinc cellulose complexes. *Biomass & Bioenergy*, 6(5), 415-417.
- Zhang, S., Li, F.-X., Yu, J.-y., & Hsieh, Y.-L. (2010). Dissolution behaviour and solubility of cellulose in NaOH complex solution. *Carbohydrate Polymers*, 81(3), 668-674.
- Zhang, Y., Xu, A., Lu, B., Li, Z., & Wang, J. (2015). Dissolution of cellulose in 1-allyl-3-methylimizodalium carboxylates at room temperature: A structure property relationship study. *Carbohydrate Polymers*, 117, 666-672.

Figure captions

Fig. 1. Comparison of ZnCl₂ solutions containing 1% (top panel) and 3% (bottom panel) cellulose mixed at 65 °C. In the 50% salt solution, cellulose dissolves partially with sedimented particles and at 78% a thick paste is noticed. A transparent and homogeneous cellulose solutions could only be obtained at 68% ZnCl₂.

Fig. 2. The viscosity of 3% cellulose solution as a function of $ZnCl_2$ measured at 65 °C. At 68% the viscosity deceases substantially. The drop in the cellulose viscosity is clearly due to favorable interactions among the Zn^{2+} ions and cellulose chains towards destabilizing the tight network structure and dissolving cellulose. The inset highlights the viscosity change in the pure $ZnCl_2$ solutions.

Fig. 3. The inset highlights that viscosity of 68% $ZnCl_2$ solution does not change with the added salts. However, viscosity of 3% cellulose in 68% $ZnCl_2$ solution (control) in the presence Ca^{2+} ions increases significantly compared to that of NH4⁺, K⁺, Fe²⁺ and Mg²⁺ ions suggesting cooperative interactions among the Zn-cellulose chains in the presence of Ca^{2+} ions.

Fig. 4. The viscoelastic properties *G*' (solid line) and *G*" (dashed line) of 3% cellulose in 68% ZnCl₂ solutions as a function of frequency at (a) 25 and (b) 65 °C highlight the dissolution nature of cellulose. The addition of CaCl₂ (Ca/Zn molar ratio of 0.03) to ZnCl₂ solution induces gelation at (c) 25 °C but (d) at higher temperature (65 °C) still viscous solutions are observed. Further addition of CaCl₂ (Ca/Zn molar ratio 0.06) yields strong gels at (e) 25 and (f) 65 °C.

Fig. 5. Comparison of FTIR spectra of Avicel, Zn-cellulose film and Ca-Zn-cellulose film (from bottom to top) in the region (a) 700-1500 cm⁻¹ and (b) 3000-3700 cm⁻¹. The peak shifts and/or absence and/or appearance of new profiles indicate the Zn···cellulose and or Ca/Zn···cellulose interactions. Resolution of hydrogen-bonded OH stretching in

(c) Avicel, (d) Zn-cellulose film and (e) Ca-Zn-cellulose film in the region 3000-3700 cm⁻¹.

Fig. 6. SEM micrographs on the film cross-section of (a) Zn-cellulose and Ca-Zncellulose (b) Ca/Zn molar ratio of 0.03 and (c) Ca/Zn molar ratio of 0.06. The presence of Ca^{2+} in the Zn-cellulose network appear to be responsible for the formation fibrils of the size 50-150 nm.

Fig. 7. Tensile strength of Zn-cellulose and Ca-Zn-cellulose films suggests that the addition of CaCl₂ (Ca/Zn molar ratio of 0.03) to Zn-cellulose increases the tensile strength by about 250% and further boosts to around 350% in the presence of higher CaCl₂ (Ca/Zn molar ratio of 0.06). Inset highlights the opaque and transparent nature of the Zn-cellulose film (left side) and Ca-Zn-cellulose film (right side), respectively. The Ca-Zn-cellulose film appear to withstand the weight of 25 lb presumably due to the formation of nano fibrils.

Fig. 8. A cartoon highlighting the interactions between a pair of cellulose chains, viewed normal to the helix axis. (a) In the native cellulose, chains held together through strong hydrogen bonding (broken lines) interactions. (b) Zn^{2+} ions bound to O3H break the canonical O3H····O5 hydrogen bonds and thus the cellulose chain gains flexibility and consequently water molecules (H₂O) are able to penetrate in the network leading to cellulose dissolution. (c) Subsequent addition of Ca²⁺ ions appears to crosslink the free Zn-cellulose chains through cooperative interactions between the hydroxyl groups, Zn²⁺ ions and water molecules resulting in the formation of nano fibrils and subsequent gelation. This regenerated cellulose structure is responsible for the higher tensile strength films.



Fig. 1





Fig. 3



Fig. 4



Fig. 5



Fig. 6

•



Fig. 7



Fig. 8