



CHALMERS
UNIVERSITY OF TECHNOLOGY

Influence of urea on methyl β -D-glucopyranoside in alkali at different temperatures

Downloaded from: <https://research.chalmers.se>, 2020-01-17 16:10 UTC

Citation for the original published paper (version of record):

Gunnarsson, M., Hasani, M., Bernin, D. (2019)

Influence of urea on methyl β -D-glucopyranoside in alkali at different temperatures

Cellulose, 26(18): 9413-9422

<http://dx.doi.org/10.1007/s10570-019-02730-4>

N.B. When citing this work, cite the original published paper.



Influence of urea on methyl β -D-glucopyranoside in alkali at different temperatures

Maria Gunnarsson · Merima Hasani · Diana Bernin 

Received: 14 June 2019 / Accepted: 3 September 2019 / Published online: 14 September 2019
© The Author(s) 2019

Abstract The dissolution efficiency plays an important role on the properties of regenerated cellulose-based products. Urea is known to be one of the additives aiding to improve cellulose dissolution in the NaOH(aq) system. The acting mechanism caused by urea has been debated and one of the hypothesis is that urea could induce a conformational change on cellulose, which promotes dissolution. Here we used NMR spectroscopy on a model system for cellulose, namely, methyl β -D-glucopyranoside (β -MeO-Glcp) and compared chemical shifts and *J* couplings, which both are indicators for conformational changes, as a function of temperature and upon the addition of urea. We found that in NaOH(aq), the hydroxymethyl group changes its conformation in favour of the population of the gt

rotamer, while the presence of urea induced temperature dependent conformational changes. Heteronuclear Overhauser effect experiments showed that urea associates with cellulose but in a non-specific manner. This suggests that urea rather than binding to the carbohydrate, changes the chemical environment inducing a change in conformation of β -MeO-Glcp and likely also for cellulose when dissolved in NaOH(aq) with urea.

Keywords Methyl β -D-glucopyranoside · Cellulose · NaOH · Urea · Dissolution · NMR

Introduction

Dissolution of cellulose is an important process in the production of several products used in daily life such as textile fibers, barriers and absorptive material. This process demands a solvent able to break the present intermolecular forces such as hydrogen bonds and hydrophobic interactions, which are present between the cellulose chains and between the sheets formed by the associating chains, respectively. One of the most attractive solvents from an environmental perspective is NaOH(aq). This solvent system was patented already in 1924 by Lilienfeld (1924) and reported on in more detail by Davidson during the 1930s (Davidson 1934). Later, Sobue et al. construed a phase

M. Gunnarsson (✉) · M. Hasani
Division of Forest Products and Chemical Engineering,
Department of Chemistry and Chemical Engineering,
Chalmers University of Technology, 412 96 Gothenburg,
Sweden
e-mail: maria.gunnarsson@chalmers.se

M. Hasani
Wallenberg Wood Science Center, The Royal Institute of
Technology, Chalmers University of Technology,
100 44 Stockholm, Sweden

D. Bernin (✉)
Division of Chemical Reaction Engineering, Department
of Chemistry and Chemical Engineering, Chalmers
University of Technology, 412 96 Gothenburg, Sweden
e-mail: diana.bernin@chalmers.se

diagram where it was shown that NaOH(aq) only dissolves cellulose within a narrow concentration and temperature range, more precisely around 2.0 M and -5°C (Sobue et al. 1939). This phenomenon has been studied for many years, leading to several hypotheses, but the true mechanism or a combination of mechanisms are still unknown (Budtova and Navard 2016).

However, it is evident that at a low temperature, NaOH(aq) behaves in a distinct manner making cellulose willing to dissolve. Considering the high concentration of NaOH(aq) required for a successful dissolution of cellulose, a certain degree of deprotonation of the hydroxyl groups will occur, which provides a charging up of the cellulose chain and introduces the appearance of electrostatic interactions (Bialik et al. 2016).

With the focus set on reaching a sufficient separation of the chains to assist dissolution, it is rational to look into additives that are able to promote this. In the beginning of the 1990s Laszkiewicz et al. discovered that urea aids dissolution of cellulose in NaOH(aq) (Laszkiewicz and Wcislo 1990) and that the dissolution rate of bacterial cellulose could be improved from 17 to 48.6% by the addition of 1 wt% urea (Laszkiewicz 1998). Later, the role of urea in the dissolution process of cellulose in NaOH(aq) has been intensively studied by other research groups and reviewed by Budtova and Navard (2016). In summary, two types of hypotheses have been developed through both experimental and theoretical studies to explain the mechanism of urea in NaOH(aq), namely, that urea impacts the quality of the solvent or that urea interacts with the cellulose through some type of binding or solvation.

Urea is stated to impact the entropy (Zhao et al. 2013) and shift the dissolution equilibrium by interacting favourably with cellulose in solution (Wernersson et al. 2015). In addition to this, it has been shown, using DSC, that urea does not interact with neither NaOH nor with cellulose (Egal et al. 2007), while Jiang et al. (2014) reported on a direct interaction between OH^- and amino groups of urea through hydrogen bonds, but no direct interaction between urea and cellulose. The lack of interaction between cellulose and urea was also concluded with NMR spectroscopy by Cai et al. (2008). However, although Cai et al. (2008) reported on the lack of an interaction they suggested that temperatures close to freezing promote the formation of hydrogen-bonded networks of NaOH, urea and water.

In terms of binding of urea to cellulose, it has also been suggested that urea accumulates on the hydrophobic surfaces of the cellulose chains and, thus, weakens the hydrophobic interactions, similar to protein denaturation, and hence facilitates dissolution in NaOH(aq) (Xiong et al. 2014). The association of urea with cellulose has been studied by MD simulations, which were conducted in the absence of NaOH but indicated an accumulation of urea on hydrophobic surfaces of cellulose (Bergensträhle-Wohlert et al. 2012; Wernersson et al. 2015). Experimental results on the cellulose/NaOH(aq) or LiOH(aq)-system with urea suggested the accumulation of urea to prevent agglomeration of the chains (Xiong et al. 2014; Isobe et al. 2012).

The proposed lack of a specific interaction between cellulose and urea has been concluded from the absence of chemical shift changes of both cellulose and urea when dissolved together (Cai et al. 2008). The breakage of hydrophobic interactions could, however, result in conformational changes of the cellulose chains, which can be monitored through investigation of coupling constants between neighbouring ^1H 's ($^3J_{\text{HH}}$ coupling), and ^1H 's and ^{13}C 's ($^1J_{\text{CH}}$ coupling). The J couplings as a function of temperature for cellotetraose in water were recently investigated by Angles d'Ortoli et al. (2015) and Bergensträhle-Wohlert et al. (2016) and found to be temperature independent. The conformational changes that occur in carbohydrates such as pyranosides are ring conformation, hydroxymethyl conformation and C–O rotation of the hydroxyl groups. Hydroxymethyl group conformation modulates the hydrogen bonding characteristics and the dipole moment of the molecule, which both affect the overall physical and chemical properties. (Stenutz et al. 2002) The hydroxymethyl group adopts conformation of three staggered rotamers, namely, gauche-gauche (*gg*, $\omega = -60^{\circ}$), gauche-trans (*gt*, $\omega = 60^{\circ}$) and trans-gauche (*tg*, $\omega = 180^{\circ}$) where the first letter refers to the torsional relationship between O6 and O5, while the second letter refers to the relationship between O6 and C4 (Fig. 1). In solution, all three rotamers coexist and the ratio varies with different parameters such as solvent, temperature and interactions with other molecules.

To our knowledge, the possible conformations of cellulose or cellulose model compounds dissolved in NaOH(aq) have not been investigated yet. Hence, we

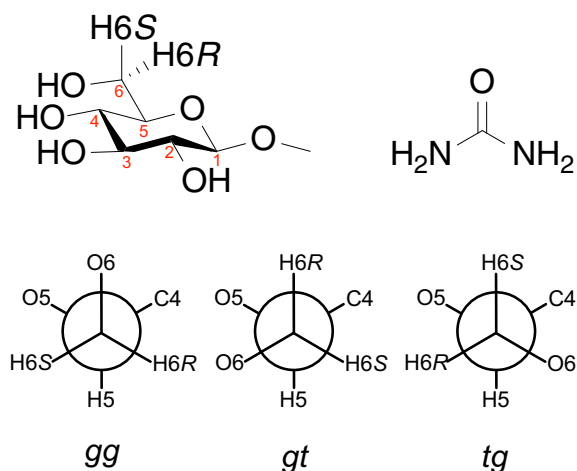


Fig. 1 The molecular structure of β -MeO-Glcp (with numbering of the positions) and urea at the top and the three staggered rotamers of the hydroxymethyl group at the bottom

herein mapped conformational changes for a model compound, namely, methyl β -glucopyranoside (β -MeO-Glcp), dissolved in NaOH(aq), which is induced by temperature changes and/or by the addition of urea. Equimolar NaCl(aq) as a reference solvent enables an accurate comparison to NaOH(aq) in terms of ionic strength.

Due to the lack of experimental techniques to study conformational changes of cellulose upon the addition of urea with atomic resolution, we chose the model compound β -MeO-Glcp (Fig. 1 left) to represent the monomeric unit of cellulose and used microcrystalline cellulose and ^{15}N -labelled urea (Fig. 1 right) to evaluate the hypothesis of a specific association between cellulose and urea, i.e. the accumulation of urea on cellulose's hydrophobic surfaces.

Experimental

Sample preparation

Methyl β -D-glucopyranoside (< 99%), NaOH (< 98%), NaCl ($\geq 99.5\%$), D₂O (99.9%) and urea- ^{15}N (98 atom% ^{15}N) were purchased from Sigma Aldrich and used as received. Urea (99–100.5%) was purchased from VWR and used as received. Microcrystalline cellulose (MCC) Avicel PH-101, with a degree of polymerisation of 260, was purchased from FMC BioPolymer and used without further treatment.

For the J coupling estimation, solutions were prepared by dissolving NaCl (2.0 M) or NaOH (2.0 M) in D₂O with or without the addition of urea (2.5 M). The solutions were then cooled down to -5°C . The cold solutions were added to pre-weighed β -MeO-Glcp (0.4 M), let to dissolve and stored at $+5^\circ\text{C}$.

For the steady-state heteronuclear Overhauser effect (HOE) measurements, NaOH (2.0 M) and urea- ^{15}N (2.5 M) were dissolved in D₂O at room temperature. MCC (0.4 M) was dispersed in the NaOH/urea- ^{15}N solution by shaking the suspension intensely and instantly transfer it into an NMR tube. The NMR tube was then placed in a freezer at -20°C for the MCC suspension to completely freeze. The frozen suspension was put into the magnet, which was pre-cooled to $+5^\circ\text{C}$. The sample was held at this temperature for at least 15 min prior to the NMR measurements.

Characterisation

All NMR experiments were run on an 800 MHz magnet equipped with a Bruker Avance HDIII console and a TXO cryoprobe. ^1H NMR spectra were recorded with the relaxation delay and number of scans set to 5 s and 8, respectively. ^{13}C NMR spectra were recorded with a low angle radio frequency (RF) pulse to minimise relaxation-weighting using a single pulse experiment with ^1H decoupling during acquisition. A capillary containing D₂O with 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) was placed inside the tube as an internal reference.

The $^1J_{\text{CH}}$ couplings were estimated from ^{13}C NMR spectra recorded without decoupling.

The $^{2,3}J_{\text{HH}}$ couplings were estimated from homonuclear 2D J correlation experiments using 128 points and a spectral width of 100 Hz in the second dimension.

The presence of any specific interaction between urea and β -MeO-Glcp, was accessed with 1D steady-state heteronuclear Overhauser effect (HOE) experiments using urea- ^{15}N to transfer the magnetisation from the ^{15}N to the ^1H , where we are able to observe all bonded H (H1–H6) of β -MeO-Glcp in a ^1H NMR spectrum. A low power 90° RF pulse was applied on resonance of the urea peak (^{15}N) 100 times with a delay of 10 ms in between to saturate the urea signal. After a delay of 13 s, the ^1H signal was excited with a strong short 90° RF pulse and recorded. The difference between the two experiments, one with and

one without saturation, indicates which sites that interact with urea. 1600 accumulations of the signal were recorded for both experiments at +5°C.

Results and discussion

Impact of solvent and urea on vicinal ^1H - ^1H (J_{HH}) coupling constants

Variations in conformation, induced by a change of the chemical environment at the ^1H nucleus, might be observed as both changes in chemical shift and coupling constant. The assessment of the chemical shift is often straightforward in contrast to the vicinal couplings also denoted J couplings, which might be small in magnitude and/or reveal a complex pattern according to the Pascal's triangle depending on the number of neighbouring bonded H atoms. The $^3J_{\text{HH}}$ coupling is the coupling that senses the ^1H nucleus, which is bonded to the next carbon while the $^3J_{\text{HH}}$ coupling is found within a CH_2 group. The $^3J_{\text{HH}}$ couplings are on the order of 2–12 Hz, which is obviously challenging to measure with an accuracy of 1 Hz. This is also true for the ^1H chemical shift i.e. a change in the chemical shift of 20 Hz corresponds to 0.025 ppm at a magnetic field strength of 18.8 T. Chemical shifts and J couplings recorded on a molecule in solution always represent a mean value of an assemble of different molecular orientations, which might have a preferred orientation but reorient on a nanoscale timescale.

Here, ^1H chemical shift values for β -MeO-Glcp dissolved in NaCl(aq) compared to NaOH(aq) decreased in NaOH(aq), which is attributed to the high pH that facilitates partial deprotonation of the hydroxyl groups and is in agreement with earlier work (Gunnarsson et al. 2019). Hence, it is not surprising that an increase in temperature from -10 to $+5^\circ\text{C}$ impacted the ^1H chemical shift values insignificantly in both solvents (data not shown). Moreover, the addition of urea to the NaOH(aq) or NaCl(aq) system did not influence the ^1H chemical shift values for β -MeO-Glcp in neither of the solvents.

In order to account for the ionic strength, which likely affects the chemical environment, we subtracted the J_{HH} observed for the β -MeO-Glcp in NaOH(aq) from the ones estimated for the β -MeO-Glcp in NaCl(aq). The comparison of the $^3J_{\text{HH}}$ couplings of the β -MeO-Glcp revealed a change for the ^1H 's of

the hydroxymethyl group at position C6 (Fig. 1), namely the $^3J_{\text{H5,H6R}}$, when dissolved in NaOH(aq) compared to NaCl(aq) (Table 1, marked in bold). The same change was also observed when comparing the β -MeO-Glcp dissolved in NaOH(aq) with urea or NaCl(aq) with urea, which suggests that this observation is rather triggered by the change in pH than the addition of urea.

An advantage with short-range couplings such as $^3J_{\text{HH}}$ coupling is the estimation of the torsion angles (Fig. 1) when other constants have been determined. Hence, the hydroxymethyl torsion might be extracted by inserting the estimated coupling constants of the hydroxymethyl group in NaCl(aq) and NaOH(aq) into a Karplus equation.

$$^3J_{\text{H5,H6R}} = 5.08 + 0.47\cos(\omega) + 0.90\sin(\omega) - 0.12\cos(2\omega) + 4.86\sin(2\omega) \quad (1)$$

This Karplus equation (Eq. 1) developed by Stenutz et al. (2002) describes the relationship between the $^3J_{\text{H5,H6R}}$ coupling constant and the torsion angle of the C5 and C6 carbons. The estimated $^3J_{\text{H5,H6R}}$ coupling constants corresponds to a decrease in torsion angle from 91° to 85° when changing from NaCl(aq) to NaOH(aq) as solvent (Fig. 2). Here, and generally valid for molecules in solution, the calculated torsion angle presents an average of the populations of three

Table 1 Difference in $^3J_{\text{HH}}$ couplings (Hz) of β -MeO-Glcp when dissolved in NaOH(aq) or NaOH(aq) with urea in comparison to NaCl(aq) or NaCl(aq) with urea at $+5^\circ\text{C}$

^1H position	δ (Hz) NaOH(aq)	δ (Hz) NaOH(aq) with urea
H1	- 0.2	- 0.1
H2	- 0.5	- 0.5
H2	- 0.3	0.2
H3	- 0.4	- 0.4
H3	- 0.4	- 0.4
H4	- 0.4	- 0.2
H4	- 0.2	- 0.2
H5	0.2	0.5
H5	1.1	1.0
H5	- 0.6	- 0.3
H6 _R	1.0	0.8
^a H6 _R	- 0.2	- 0.3
H6 _S	0.2	0.2
^a H6 _S	- 0.2	- 0.2

^a $^2J_{\text{HH}}$ couplings

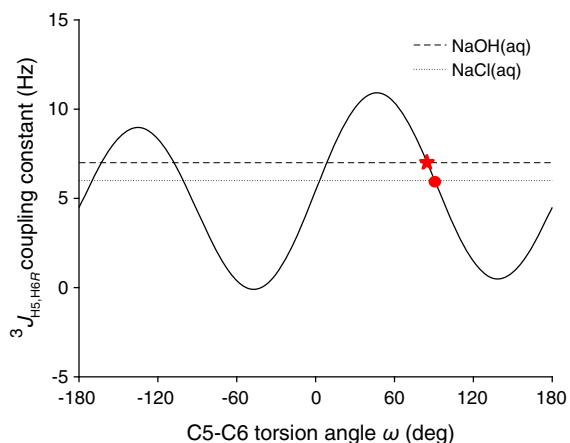


Fig. 2 Plot of the dependency between experimental $^3J_{H5,H6R}$ coupling constants in NaCl(aq) or NaOH(aq) and torsion angles calculated from Eq. 1. Torsion angle in NaCl(aq) and NaOH(aq) marked with a circle and a star, respectively

staggered rotamers gauche-gauche (*gg*), gauche-trans (*gt*) and trans-gauche (*tg*) (Fig. 1).

In this case, the change in the torsion angle suggests an increased population of the *gt* rotamer, which is not surprising since this is the most stable rotamer due to hydrogen bonding between the hydroxyl and the ring oxygen (Rockwell and Grindley 1998). The reason for this could be a difference in solvation shells for

different hydroxymethyl rotamers depending on the solvent (Rockwell and Grindley 1998).

In addition to this, the $^3J_{HH}$ couplings remained unchanged within our temperature window, which is in accord with the results found by others (Bergensträhle-Wohlert et al. 2016). This indicates that the conformational change of the hydroxymethyl group is not induced by a temperature change within the dissolution temperature window.

^{13}C chemical shifts and $^1J_{CH}$ coupling variations as an indicator of conformational change

Although the $^1J_{CH}$ couplings are short-range couplings, i.e. one bond couplings, they are on the order of 130–150 Hz, which makes it possible to observe smaller changes with an increased accuracy. While the addition of urea or variation in temperature left the ^1H chemical shifts or $^3J_{HH}$ couplings unaltered, differences were observed for ^{13}C chemical shifts and $^1J_{CH}$ couplings.

An increase in temperature from -10 to $+5^\circ\text{C}$ gave rise to a decrease in ^{13}C chemical shifts for all carbons in $\beta\text{-MeO-Glcp}$ (Fig. 3) for NaOH(aq) (blue) and NaOH(aq) with urea (red). Interestingly, the decrease appeared to be greater at the positions C1, C3 and C5 (ca. 0.2–0.3 ppm) while the other carbons only

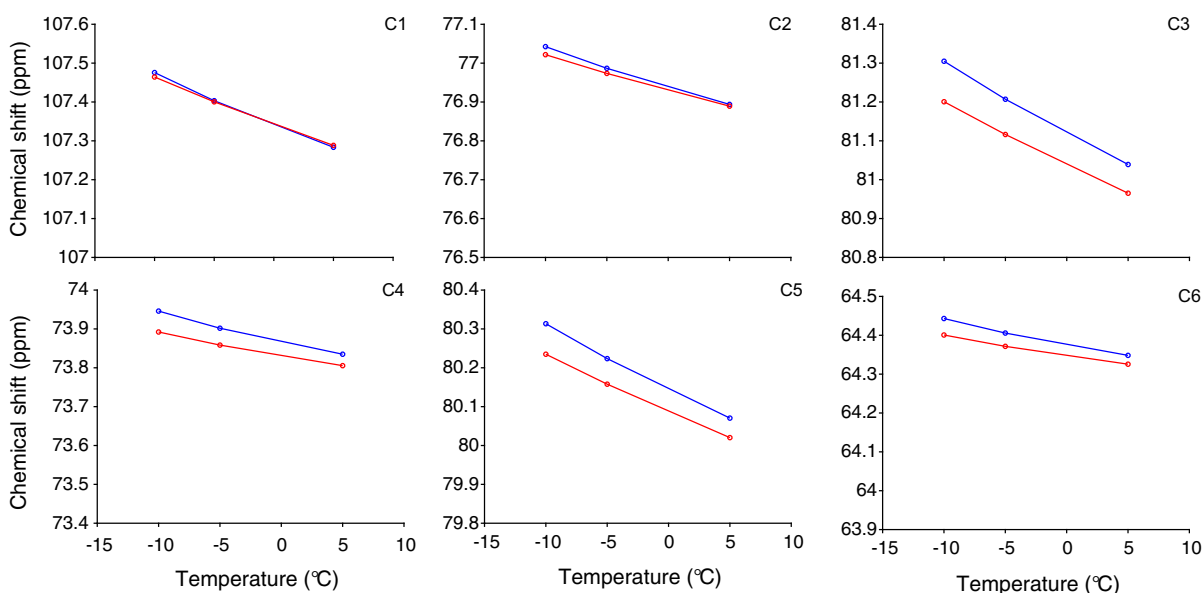


Fig. 3 The change in chemical shift for all carbons in $\beta\text{-MeO-Glcp}$ when dissolved in NaOH(aq) (blue) or NaOH(aq) with urea (red) as a function of temperature. All measurements were recorded in D_2O

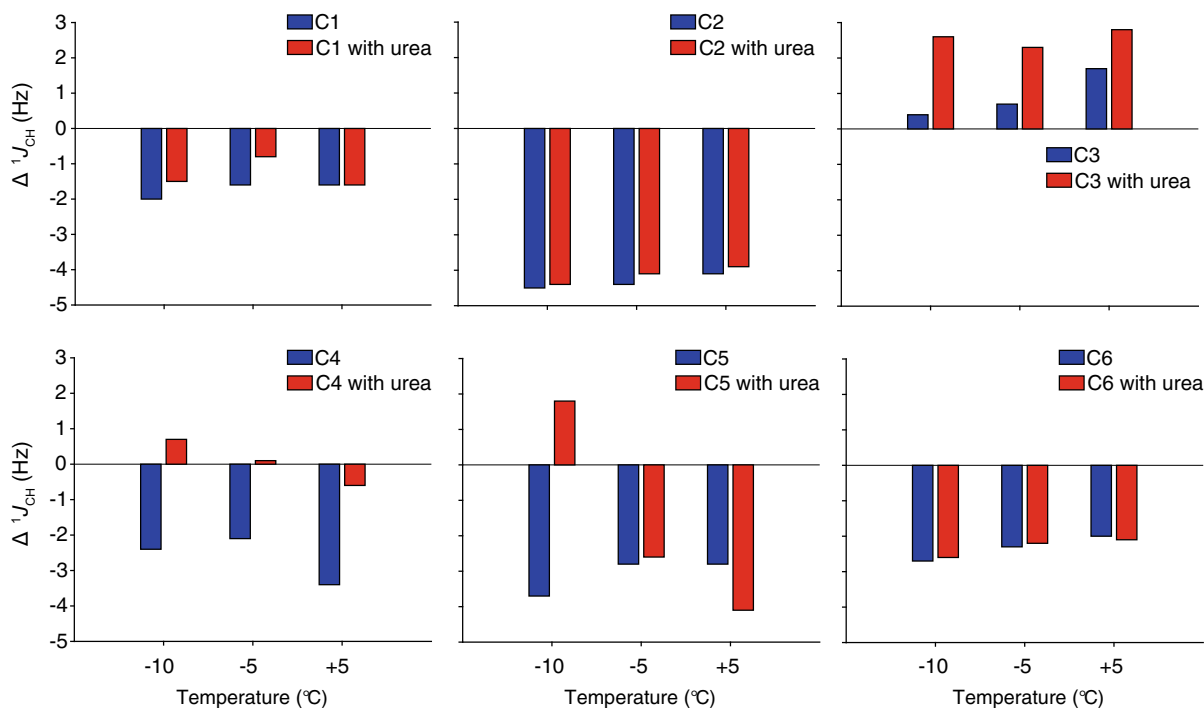


Fig. 4 The difference in $^1J_{CH}$ couplings of β -MeO-Glcp when dissolved in NaOH(aq) (blue) or NaOH(aq) with urea (red) in comparison to NaCl(aq) or NaCl(aq) with urea, respectively, for each temperature. All measurements were recorded in D_2O

exhibited a minor decrease (ca. 0.1 ppm). The change of the chemical shift of the methyl group was less than 0.05 ppm independently of the solvent composition (data not shown).

Upon the addition of urea, again, the C3 and C5 positions were affected the most, which suggest that these positions are sensitive to the solvent i.e. the chemical environment, which could induce conformational changes. Interestingly, the ^{13}C chemical shift was identical for the β -MeO-Glcp in NaCl(aq) within the temperature window and the addition of urea. Hence, the observed changes in ^{13}C chemical shift in NaOH(aq), both upon temperature variation and the addition of urea, is suggested to be attributed to the properties of the solvent, which promotes a partial deprotonation of the hydroxyl groups on the β -MeO-Glcp.

While the $^3J_{HH}$ couplings report on the geometry between the 1H 's bonded to nearby carbons, the $^1J_{CH}$ coupling instead informs on changes of the direct covalent bond between C and H. The $^1J_{CH}$ coupling is field-independent as other J couplings and depends on the bond angle between the C and H, and the bond length. Although the C–H bonds point at different

directions in β -MeO-Glcp, the chemical environment around the carbon atom is similar i.e. all have a bonded oxygen atom. Hence, the magnitude of the $^1J_{CH}$ couplings are of similar size with C3 being the lowest and C1 the highest, 137 and 161 Hz, respectively. The $^1J_{CH}$ coupling might be a better indicator for conformational changes induced by the presence of urea as the carbon is somewhat more protected from the solvent. To determine the exact conformation of the β -MeO-Glcp ring from long-range J_{CH} or J_{CC} couplings, the nearby carbon atoms require ^{13}C labelling, which was not the case here.

The short-range $^1J_{CH}$ couplings in Fig. 4 present the difference between NaOH(aq) and NaCl(aq) as well as NaOH(aq) with urea and NaCl(aq) with urea in order to compensate for the ionic strength. Comparison of the difference in $^1J_{CH}$ couplings for the β -MeO-Glcp when dissolved in NaOH(aq) or NaOH(aq) with urea against NaCl(aq) or NaCl(aq) with urea, respectively, again revealed large variations for the different ^{13}C 's (Fig. 4). For NaOH(aq) in comparison to NaCl(aq), all ^{13}C 's experienced a decrease in $^1J_{CH}$ coupling due to a larger $^1J_{CH}$ coupling for NaCl(aq) except for the C3 position, which showed a positive

difference. In addition to this, the positive trend of C3 continued with increasing temperature (Fig. 4 blue). Interestingly, this phenomenon was even greater with urea present in the NaOH(aq) system.

The largest decrease in $^1J_{CH}$ coupling was observed for the C2 position, which is not surprising since this position is the most acidic in β -MeO-Glcp and manifests the highest degree of deprotonation in NaOH(aq). Moreover, the difference in $^1J_{CH}$ coupling was equal for the positions C1 and C6 at all temperatures and in the presence of urea, which might be induced solely by the swap in solvent from NaCl(aq) to NaOH(aq). There was a slight change of the $^1J_{CH}$ of the methyl group, which, however, remained constant independently of temperature or addition of urea (data not shown). However, for positions C4 and C5, both variation in temperature and the presence of urea impacted the $^1J_{CH}$ couplings. The C4 position experienced a negative change in $^1J_{CH}$ coupling in NaOH(aq) at -10°C , but an increase at the same temperature in NaOH(aq) with urea. At -5°C , the negative trend continued in NaOH(aq) while the opposite occurred in NaOH(aq) with urea and the J coupling went from positive to negative. The previously observed ^{13}C chemical shift changes for the C3 and C5 positions might interrelate with the observed changes in $^1J_{CH}$ coupling at position C4. The C5 position experienced a similar behaviour for NaOH(aq) with urea but turned into a negative $^1J_{CH}$ coupling difference already at -5°C . At $+5^\circ\text{C}$, the negative trend was even more pronounced for $^1J_{CH}$ couplings in NaOH(aq) with urea compared to NaOH(aq).

Taken together, the observed $^1J_{CH}$ couplings appeared to be highly influenced for the β -MeO-Glcp when dissolved in NaOH(aq) without being strongly affected by variation in temperature. This indicates that conformational changes or a possible present exchange phenomenon are not driven by temperature. This is in contrast to the NaOH(aq) system with urea, which clearly revealed conformational changes as a function of temperature for the C4 position and even more pronounced for the C5 position. Relating these results to the cellulose dissolution capacity of NaOH(aq) at low temperature, it is evident that NaOH(aq) affects the conformation of the sugar ring. NaOH(aq) in combination with urea induces temperature dependent conformational changes differently compared to pure NaOH(aq), which could be a

contributing factor to the dissolution mechanism for cellulose in NaOH(aq).

To further examine the impact of temperature, a comparison of the $^1J_{CH}$ coupling values at $+5^\circ\text{C}$ against the values at -10°C for β -MeO-Glcp in NaCl(aq) or NaCl(aq) with urea, and NaOH(aq) or NaOH(aq) with urea was made (Fig. 5). In NaCl(aq), all changes in $^1J_{CH}$ couplings except for the C2 position and the methyl group revealed a significant temperature dependence. Upon addition of urea to NaCl(aq), the trend was reverted for C1, C3 and C5, and once again suggests that urea in itself affects the conformation of the β -MeO-Glcp. Position C5 exhibited the largest difference induced by urea in NaCl(aq), which was -1.2 Hz.

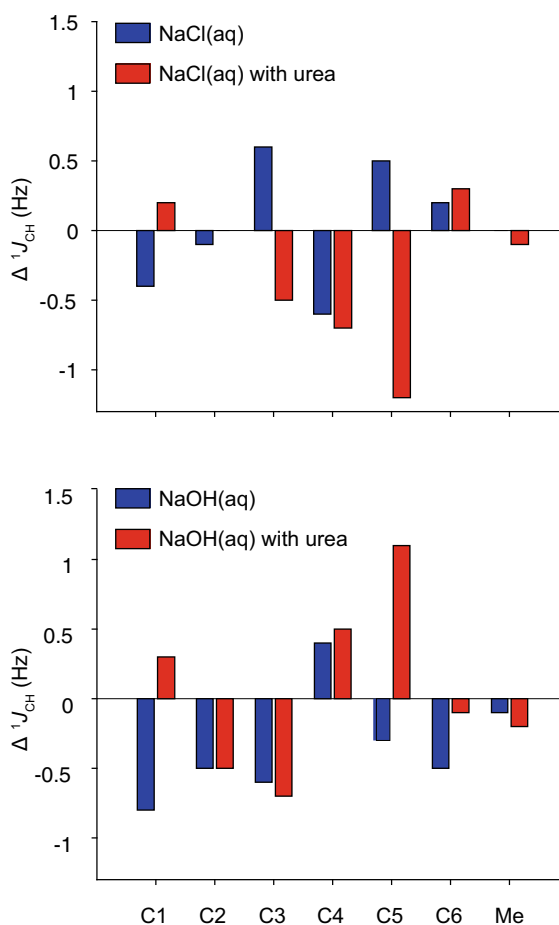


Fig. 5 The change in $^1J_{CH}$ couplings of β -MeO-Glcp dissolved in NaCl(aq) or NaCl(aq) with urea and NaOH(aq) or NaOH(aq) with urea at $+5^\circ\text{C}$ in comparison to -10°C . All measurements were recorded in D_2O . Me stands for methyl group

Moreover, in NaOH(aq), position C1, C2 and C6 revealed a more pronounced temperature dependence. Surprisingly, the temperature trend on position C5 in NaCl(aq) was inverted in NaOH(aq). Even more interesting is that the effect on C5 with temperature in NaOH(aq) with urea was also inverted compared to NaCl(aq) with urea. This observation clearly describes an influence by urea on the chemical environment around position C5 and suggests an interaction with the β -MeO-Glcp. The same phenomenon of an inverted trend for the difference in $^1J_{CH}$ couplings upon dissolution in NaCl(aq) or NaOH(aq) and with the addition of urea was also observed for position C4 and C6 but to a different extent. Intriguingly, C2 seemed uninfluenced in NaCl(aq) in contrast to NaOH(aq).

Is there an affinity between urea and the carbohydrate?

Another reason for the observed chemical shift and J couplings might be a specific interaction between the β -MeO-Glcp and urea. This was investigated for cellulose dissolved in NaOH(aq) through the heteronuclear Overhauser effect between the ^{15}N -labelled urea and the ^1H from cellulose at $+5^\circ\text{C}$. The steady-state HOE spectra are shown in Fig. 6. Indeed, an interaction was visible between cellulose and urea because the signal intensity of H1–H6 of cellulose was intensified upon the irradiation of the ^{15}N -labelled urea before recording the ^1H spectrum in comparison to the spectrum without irradiation. Since we observed a similar increase for all ^1H 's, we attribute this increase to a non-specific interaction.

The effect by urea on the dissolution of cellulose in NaOH(aq) has been suggested either to impact the solvent quality or interact with cellulose, which promotes dissolution. The observed differences in $^1J_{CH}$ couplings for the β -MeO-Glcp when dissolved with or without the addition of urea clearly demonstrates that urea has a temperature-dependent influence on the conformation, which is consistent with Zhao et al. (2013) that reports on a change in entropy. Furthermore, using MD simulations, Wernersson et al. (2015) reported that urea itself, i.e. without NaOH being present, improves the solvent quality, which favours the interaction with cellulose in solution. The addition of urea decreases the polarity of the solvent slightly, which turns it into a more favourable

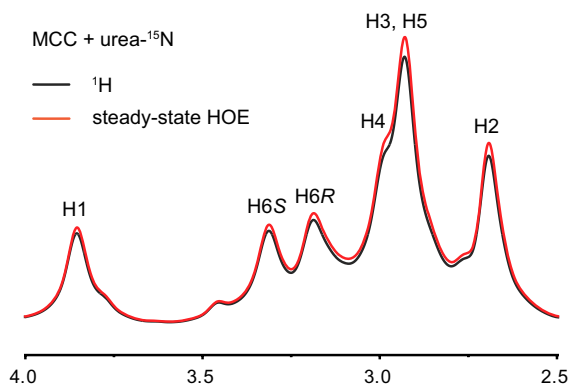


Fig. 6 Steady-state HOE spectra of MCC dissolved in NaOH(aq) with urea- ^{15}N . All measurements were recorded at $+5^\circ\text{C}$ in D_2O

solvent for a hydrophobic molecule to dissolve in. This is in agreement with our results, which indicates that urea creates a beneficial chemical environment for the dissolution of cellulose. Hypothetically, the role of urea in the polar NaOH(aq) solution could be to facilitate a more thermodynamically stable conformation of the amphiphilic polymer and through that aid dissolution. The thermodynamically stable conformation appears to be dependent on the C–O rotation of the hydroxyl groups rather than the conformation of the ring because not all the carbons revealed changes. Chen et al. (2015) studied the impact on dissolution of cellulose polymorphs in the presence of urea, concluding that different conformations impact the solubility, which is in line with our results. In a study by Jiang et al. (2014), a direct interaction between OH^- and amino groups of urea through hydrogen bonds and no direct interaction between urea and cellulose was found.

Concerning the influence of urea on the hydrophobic interactions in cellulose, Bergensträhle-Wohlert et al. (2012) and Wernersson et al. (2015) reported on the accumulation of urea close to the hydrophobic surfaces on cellulose in water without alkali using MD simulations while Xiong et al. (2014) and Isobe et al. (2012) suggested from experimental results that an accumulation of urea in the cellulose/NaOH or LiOH-system on the hydrophobic part of the cellulose occurred to prevent agglomeration of the chains. In the work by Cai et al. (2008), experimental results indicated that NaOH hydrates were in favour to bind to cellulose chains through the formation of a new hydrogen-bonded network at low temperatures in

contrast to urea hydrates. However, urea hydrates might self-assemble at the surface of the NaOH hydrogen-bonded cellulose to form an inclusion complex. Later, Cai et al. (2012) showed with MD simulations that urea binds to cellulose via hydrogen bonds.

Our results indicate an interaction, however a non-specific one, which suggests that urea is not in hydrogen binding distance and does not remain close to the cellulose chain for a longer period of time. Depending on how the urea weakens the hydrophobic interactions, a close association to the hydrophobic patch does not agree with our results. Furthermore, our results do not agree with Egal et al. (2008) who reported on the lack of difference in interaction between cellulose and NaOH(aq) in the presence or absence of urea. Hence, it appears that urea rather than accumulating at a hydrophobic surface instead facilitates a more favourable chemical environment around the carbohydrate inducing conformational changes, which could be the driving force to improved dissolution of cellulose in NaOH(aq).

Conclusions

The role of urea during the dissolution process of cellulose in NaOH(aq) as a function of temperature was investigated using a model compound and evaluated in terms of ^1H and ^{13}C chemical shifts, and J couplings between neighbouring H's and the C–H bond obtained from NMR spectroscopy. We found a conformational change to be driven by NaOH(aq) in comparison to NaCl(aq) but also the presence of urea induced conformational changes, which appeared to be temperature dependent. In NaOH(aq) in comparison to NaCl(aq), the population of the *gt* rotamer is dominated. At last, a steady-state HOE confirmed the lack of any specific interactions of urea with cellulose as expected but proved that urea associates to cellulose, which suggests that urea facilitates a chemical environment that induces a conformational change of the β -MeO-Glcp, and most likely also cellulose, which improves dissolution in NaOH(aq).

Acknowledgments Open access funding provided by Chalmers University of Technology. This work has been carried out as a part of the framework of Avancell - Center for Fiber Engineering, which is a research collaboration between Södra Innovation and Chalmers University of Technology. The

author thanks the Södra Skogsägarnas Foundation for Research, Development and Education for their financial support. The Swedish NMR Center is acknowledged for spectrometer time.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Angles d'Ortoli T, Sjöberg NA, Vasiljeva P, Lindman J, Widmalm G, Bergensträhle-Wohlert M, Wohlert J (2015) Temperature dependence of hydroxymethyl group rotamer populations in cellooligomers. *J Phys Chem B* 119(30):9559–9570. <https://doi.org/10.1021/acs.jpcc.5b02866>
- Bergensträhle-Wohlert M, Berglund LA, Brady JW, Larsson PT, Westlund PO, Wohlert J (2012) Concentration enrichment of urea at cellulose surfaces: results from molecular dynamics simulations and NMR spectroscopy. *Cellulose* 19(1):1–12. <https://doi.org/10.1007/s10570-011-9616-x>
- Bergensträhle-Wohlert M, Angles d'Ortoli T, Sjöberg NA, Widmalm G, Wohlert J (2016) On the anomalous temperature dependence of cellulose aqueous solubility. *Cellulose* 23(4):2375–2387. <https://doi.org/10.1007/s10570-016-0991-1>
- Bialik E, Stenqvist B, Fang Y, Östlund Å, Furó I, Lindman B, Lund M, Bermin D (2016) Ionization of cellobiose in aqueous alkali and the mechanism of cellulose dissolution. *J Phys Chem Lett* 7(24):5044–5048. <https://doi.org/10.1021/acs.jpcclett.6b02346>
- Budtova T, Navard P (2016) Cellulose in NaOH-water based solvents: a review. *Cellulose* 23(1):5–55. <https://doi.org/10.1007/s10570-015-0779-8>
- Cai J, Zhang L, Liu S, Liu Y, Xu X, Chen X, Chu B, Guo X, Xu J, Cheng H, Han CC, Kuga S (2008) Dynamic self-assembly induced rapid dissolution of cellulose at low temperatures. *Macromolecules* 41(23):9345–9351. <https://doi.org/10.1021/ma801110g>
- Cai L, Liu Y, Liang H (2012) Impact of hydrogen bonding on inclusion layer of urea to cellulose: study of molecular dynamics simulation. *Polymer* 53(5):1124–1130. <https://doi.org/10.1016/j.polymer.2012.01.008>
- Chen X, Chen J, You T, Wang K, Xu F (2015) Effects of polymorphs on dissolution of cellulose in NaOH/urea aqueous solution. *Carbohydr Polym* 125:85–91. <https://doi.org/10.1016/j.carbpol.2015.02.054>
- Davidson GF (1934) The dissolution of chemically modified cotton cellulose in alkaline solutions. Part I—In solutions

- of sodium hydroxide, particularly at temperatures below the normal. *J Text Inst Trans* 25(5):T174–T196. <https://doi.org/10.1080/19447023408661621>
- Egal M, Budtova T, Navard P (2007) Structure of aqueous solutions of microcrystalline cellulose/sodium hydroxide below 0 °C and the limit of cellulose dissolution. *Biomacromolecules* 8(7):2282–2287. <https://doi.org/10.1021/bm0702399>
- Egal M, Budtova T, Navard P (2008) The dissolution of microcrystalline cellulose in sodium hydroxide-urea aqueous solutions. *Cellulose* 15(3):361–370. <https://doi.org/10.1007/s10570-007-9185-1>
- Gunnarsson M, Bernin D, Hasani M (2019) The CO₂/CO₃²⁻ chemistry of the NaOH(aq) model system applicable to cellulose solutions. Manuscript submitted for publication
- Isobe N, Kimura S, Wada M, Kuga S (2012) Mechanism of cellulose gelation from aqueous alkali-urea solution. *Carbohydr Polym* 89(4):1298–1300. <https://doi.org/10.1016/j.carbpol.2012.03.023>
- Jiang Z, Fang Y, Xiang J, Ma Y, Lu A, Kang H, Huang Y, Guo H, Liu R, Zhang L (2014) Intermolecular interactions and 3D structure in cellulose-NaOH-urea aqueous system. *J Phys Chem B* 118(34):10250–10257. <https://doi.org/10.1021/jp501408e> PMID: 25111839
- Laskiewicz B (1998) Solubility of bacterial cellulose and its structural properties. *J Appl Polym Sci* 67(11):1871–1876. [https://doi.org/10.1002/\(SICI\)1097-4628\(19980314\)67:11<1871::AID-APP5>3.0.CO;2-I](https://doi.org/10.1002/(SICI)1097-4628(19980314)67:11<1871::AID-APP5>3.0.CO;2-I)
- Laszkiewicz B, Wcislo P (1990) Sodium cellulose formation by activation process. *J Appl Polym Sci* 39(2):415–425. <https://doi.org/10.1002/app.1990.070390217>
- Lilienfeld L (1924) Manufacture of Cellulose solution. British patent no. 212864
- Rockwell GD, Grindley TB (1998) Effect of solvation on the rotation of hydroxymethyl groups in carbohydrates. *J Am Chem Soc* 120(42):10953–10963. <https://doi.org/10.1021/ja9819581>
- Sobue H, Kiessig H, Hess K (1939) The system: cellulose-sodium hydroxide-water in relation to the temperature. *Z Phys Chem B* 43:309–328
- Stenutz R, Carmichael I, Widmalm G, Serianni AS (2002) Hydroxymethyl group conformation in saccharides: structural dependencies of ²J_{HH}, ³J_{HH}, and ¹J_{CH} spin-spin coupling constants. *J Org Chem* 67(3):949–958. <https://doi.org/10.1021/jo010985i>
- Wernersson E, Stenqvist B, Lund M (2015) The mechanism of cellulose solubilization by urea studied by molecular simulation. *Cellulose* 22(2):991–1001. <https://doi.org/10.1007/s10570-015-0548-8>
- 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. <https://doi.org/10.1007/s10570-014-0221-7>
- Zhao Y, Liu X, Wang J, Zhang S (2013) Effects of anionic structure on the dissolution of cellulose in ionic liquids revealed by molecular simulation. *Carbohydr Polym* 94(2):723–730. <https://doi.org/10.1016/j.carbpol.2013.02.011>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.