

1 Understanding the interactions of cellulose fibres and 2 deep eutectic solvent of choline chloride and urea

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11 Abstract

12 A deep eutectic solvent (DES) composed of choline chloride (ChCl) and urea has been recently introduced as
13 a promising cellulose compatible medium that enables e.g. fibre spinning. This paper clarifies the influence of
14 such a solvent system on the structure and chemical composition of the cellulosic pulp fibres. Special emphasis
15 was placed on the probable alterations of the chemical composition due to the dissolution of the fibre
16 components and/or due to the chemical derivatisation taking place during the DES treatment. Possible changes
17 in fibre morphology were studied with atomic force microscopy (AFM) and scanning electron microscopy
18 (SEM). Chemical compositions of pulp fibres were determined from the carbohydrate content, and by
19 analysing the elemental content. Detailed structural characterisation of the fibres was carried out using
20 spectroscopic methods; namely X-Ray Photoelectron Spectroscopy (XPS), solid state Nuclear Magnetic
21 Resonance (NMR) and Raman Spectroscopy. No changes with respect to fibre morphology were revealed and
22 negligible changes in the carbohydrate composition were noted. The most significant change was related to
23 the nitrogen content of the pulp after the DES treatment. Comprehensive examination using spectroscopic
24 methods revealed that the nitrogen originated from strongly bound ChCl residuals that could not be removed
25 with a mild ethanol washing procedure. According to Raman spectroscopic data and methylene blue adsorption
26 tests, the cationic groups of ChCl seems to be attached to the anionic groups of pulp by electrostatic forces.
27 These findings will facilitate the efficient utilisation of DES as a cellulose compatible medium without
28 significantly affecting the native fibre structure.

29 *Deep eutectic solvent, urea, choline chloride, DES, pulp*

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30 Introduction

31 Interest in deep eutectic solvents (DES) for utilisation as cellulose compatible solvent system has increased in
32 recent years. A number of applications of this solvent system, varying from its use as a fibre spinning medium
33 to a pre-treatment prior to nanofibrillation, have been proposed (Zhang et al. 2012; Sirviö et al. 2015; Tenhunen
34 et al. 2016). The physicochemical properties of DES solvents are comparable to ionic liquids. They are
35 however composed of two or three chemicals that consist of a hydrogen bond donor and a hydrogen bond
36 acceptor. These components form a eutectic mixture with a lower melting point than the individual
37 components. Compared to ionic liquids, DESs are generally considered to be easier to prepare, less expensive
38 and less toxic (Abbott et al. 2006; Zhang et al. 2012; Wen et al. 2015).

39
40 Choline chloride (ChCl) and urea has been the most popular DES system probably due to the availability of
41 these chemicals and their low melting point (~12 °C) (Abbott et al. 2003). Even though this solvent system
42 does not dissolve cellulose, it has been investigated for several applications with promising results (Abbott et
43 al. 2006; Park et al. 2013; Sirviö et al. 2015; Wang et al. 2015; Tenhunen et al. 2016; Xu et al. 2016; Suopajarvi
44 et al. 2017; Willberg-Keyriläinen et al. 2017). Abbott et al. (2006) have utilised a eutectic mixture of a choline
45 chloride derivative (Chlorcholine chloride-based (ClChCl; ClCH₂CH₂N(Me)₃Cl)) and urea to cationise cotton.
46 Successful cationisation was detected via an increased hydrophilicity and by a repulsion of a cationic dye.
47 According to their study, cationic functionalisation occurred when choline chloride reacted with the available
48 OH-groups of cellulose. Sirviö et al. (2015) and Suopajarvi et al. (2017) utilised a DES system composed of
49 ChCl and urea as a pre-treatment to promote nanofibrillation of bleached pulp or secondary fibre sources. They
50 suggested that some of the hemicelluloses might dissolve during the treatment. They also suggested that a
51 small number of cellulose hydroxyl groups are possibly converted to carbamates, leading to the distortion of
52 the hydrogen bonding of the fibres. Carbamate conversion was observed by Willberg-Keyriläinen et al. (2017)
53 when they treated wet pulp with a urea based DES system; this was found to occur most readily at 120 °C. Xu
54 et al. (2016) tested ChCl-urea as a pre-treatment in order to remove hemicelluloses and lignin from corn stover
55 prior to butanol fermentation. However, that particular DES system did not have any significant effect on the
56 removal of these components. Park et al. (2013) used a mixture of 3,3',4,4'-benzophenone tetracarboxylic
57 dianhydride (BPTCD) and ChCl-urea as a treatment medium in order to introduce antibacterial properties to
58 cotton. Wang et al. (2015) used ChCl-urea as a plasticizer in regenerated cellulose films. They concluded that
59 the ChCl-urea DES disrupted the inter- and intra-hydrogen bonds of cellulose, but there was no chemical
60 reaction between these components and the regenerated cellulose.

61
62 Choline chloride itself has been used to cationise cotton. The method was originally developed by Harper Jr.
63 and Stone (1986). Since then there have been several reported studies of this process, where choline-based
64 substances have been used for cationic functionalisation by introducing quaternary ammonium groups to
65 cellulose (Abbott et al. 2006; Ho et al. 2011; Kim and Choi 2014; Samanta et al. 2015). Urea is known to

66 interact with cellulose. Several authors have reported on the formation of cellulose carbamate due to a reaction
67 between the OH-groups of cellulose and urea (Segal and Eggerton 1961; Ekman et al. 1984; Fu et al. 2015).
68 Dissolution becomes possible in solvents such as aqueous NaOH by first converting cellulose to cellulose
69 carbamate. Urea has also been extensively used with alkaline solvents for the direct dissolution of cellulose
70 (Cai and Zhang 2005). Ershova et al. (2012) presented the possibility of decreasing cellulose degradation
71 (peeling) under alkaline conditions by using urea as a co-solvent.

72
73 Previously we have shown that a DES system comprising choline chloride (ChCl) and urea was a suitable
74 medium for pulp fibre yarn manufacturing (Tenhunen et al. 2016). This eutectic mixture was able to disperse
75 pulp fibres and dissolve the crosslinking polymer (polyacrylic acid). Furthermore, this solvent system was
76 shown to form a gel-like suspension, which was then spun into fibre yarns using an extrusion method. Since
77 no dissolution of cellulose took place in the process, and the cellulose I structure remained intact without
78 regeneration to cellulose II, the method could enable the production of wood-based textiles. This was achieved
79 without the use of harsh chemicals or excessive consumption of water, bringing new options to the textile
80 industry.

81
82 Despite several promising new applications and research efforts, the interactions between cellulose fibres and
83 ChCl-urea based DES systems are still mostly unknown. In the present work, the aim was to clarify the
84 interactions between bleached pine pulp and mentioned choline chloride/urea DES system. Since both choline
85 chloride and urea have been used together and separately to functionalise cellulose and also as a reaction
86 medium, it raises a number of questions. Does DES have an influence on fibre morphology or does it act as an
87 inert medium for cellulosic fibres? Does DES chemically modify pulp fibres? Our approach is an extensive
88 and systematic characterisation of wood pulp materials treated with a DES system.

89 **EXPERIMENTAL**

90 **Materials**

91 Never-dried bleached, sodium washed pine pulp from a Finnish pulp mill was used as the starting material for
92 the DES treatment. This pulp was ion-exchanged to a sodium form based on a slightly modified version of a
93 method originally described by Swerin et al. (1990); modifications to this method have been described by
94 Lahtinen et al. (2014). In brief, the method includes washing the metal counter ions from the pulp at low pH
95 (0.01M HCl, pH <3). After filtration and washing with deionized water, conversion of the carboxyl groups
96 into their sodium form was achieved by mixing the pulp with 0.005M NaHCO₃ solution. The pH was set to
97 slightly alkaline with 1M NaOH and held constant for 15 min while stirring the suspension. Finally, the pulp
98 was rinsed with deionized water until the conductivity of the filtrate was below 20 μS/cm. This sodium washed
99 pulp was diluted and mixed using Diaf's Minibatch Type 20 (Pilvad Diaf A/S, Denmark) for 30 minutes at

100 2000 rpm. The excess water was then removed by filtration using a Buchner funnel and a double filter cloth.
101 Pulp samples were stored at 4 °C before they were used.

102

103 The DES system was prepared using a modified procedure according to Abbott et al. (2003). Choline chloride
104 (Sigma-Aldrich, USA) and urea (Sigma-Aldrich, USA) (used as purchased without further purification) were
105 mixed in a closed system using a molar ratio of 1:2 at 100 °C until a homogenous and transparent liquid was
106 formed. DES was used immediately once prepared.

107

108 Ethanol and acetone were both analytical grades and supplied by Sigma-Aldrich, USA. Methylene blue (3,7-
109 Bis(dimethylamino)phenothiazinium chloride, C. I. 52015, Reag. PhEur, Merck) was used as received.

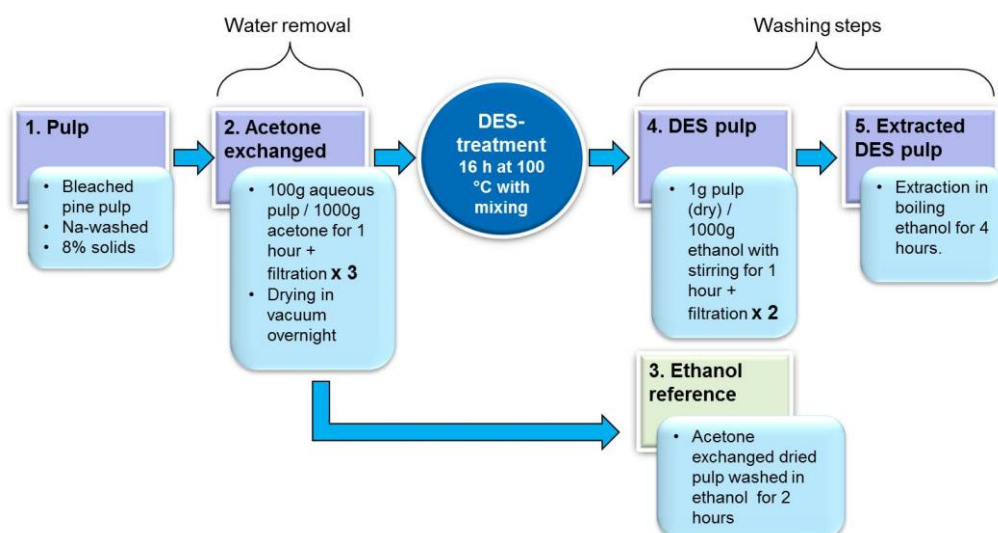
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111 **Methods**

112 *Preparation of samples*

113 Fig. 1 presents the sample preparation protocol. Sample preparation was carried out according to the procedure
114 by Tenhunen et al. (2016), with some modifications. Preparation commenced with water removal by acetone
115 exchange. 100 g of wet (8 wt-%) sodium washed pine pulp (1. Pulp) was mixed with 1 kg of acetone with
116 constant stirring for 1 hour. The mixture was then filtered using a Buchner funnel and a filter cloth. This
117 acetone exchange procedure was repeated 3 times. Final filtering was conducted using a Buchner funnel and
118 filter paper (mesh size 0.45 µm). Finally, the pulp was dried in a vacuum oven (at 40 °C) overnight (2. Acetone
119 exchanged). Part of the dried pulp was washed in an excess of ethanol for 2 hours, vacuum filtrated and dried.
120 The resultant sample was an ethanol treatment reference for DES pulp (3. Ethanol reference). For the DES
121 treatment, dried pulp was placed in a closed glass reactor (Radleys, UK) with the clear DES solution and mixed
122 for 16 hours at 100 °C with constant stirring. The pulp consistency was 1 %. Subsequently, the mixture was
123 washed twice with an excess of ethanol for 1 hour and vacuum filtrated in between each washing step. After
124 the final filtration using filter paper (mesh 0.45 µm) the sample was dried (4. DES pulp). However, due to a
125 rather high variation in nitrogen content after conventional washing, an extra washing step was added to the
126 procedure. Extensive washing was done for dried DES treated pulp using an extraction method in boiling
127 ethanol (80 °C) in a soxhlet for 4 hours (5. Extracted DES pulp). Prior to analysis, all the pulp samples were
128 dried between pulp blotting board sheets at room temperature and stored in desiccator until further use.

129



130

131 **Fig. 1** Scheme of the sample preparation protocol including the water removal phase, treatment with deep eutectic
 132 solvent and the mild washing step with ethanol as well as a more efficient washing step including extraction with
 133 boiling ethanol. Ethanol reference pulp is a reference test point only for the mild ethanol-washing step.

134

135 *Fibre morphology studied by SEM and AFM*

136 *Scanning electron microscopy (SEM)* (Merlin® FE-SEM, Carl Zeiss NTS GmbH, Germany) was used to
 137 investigate the changes in pulp morphology taking place during water removal, DES treatment and the washing
 138 steps. Pulp samples were attached on double-sided carbon adhesive discs on aluminium specimen stubs and
 139 sputter coated with platinum to improve the sample conductivity using an Agar Automatic Sputter Coater
 140 (Agar Scientific Ltd, UK). Imaging with the magnifications of $\times 100$, $\times 500$ and $\times 5000$ was done using an
 141 electron beam energy of 3.0 keV and a 30 pA probe current with a pixel resolution of 2048×1536 .

142

143 *Atomic force microscopy (AFM)* (Nanoscope IIIa multimode AFM, Digital Instrument, Santa Barbara, CA)
 144 was used to characterise the changes in the morphology of the surface of the pulp. Images were scanned in
 145 tapping mode in air using a 10279EVL scanner and silicon cantilevers (NCHV-A, Bruker, Camarillo, CA)
 146 with a spring constant of 42 N/m and a resonant frequency of 320 kHz. Three different areas were scanned and
 147 no image processing, other than flattening, was performed.

148

149 *Overall chemical composition of fibres*

150 *Carbohydrate composition* (rhamnose, arabinose, galactose, glucose, xylose, and mannose) of the pulps was
 151 determined by hydrolysis. The resulting monosaccharides' contents were determined by HPAEC with pulse
 152 amperometric detection (Dionex ICS-5000 equipped with a CarboPac PA20 column) according to an NREL
 153 method (Willför et al. 2009; Sluiter et al. 2012)

154

155 *Elemental analysis* (C, H, N, S) of the pulp samples was carried out by using a FLASH 2000 series analyser
156 (Thermo Scientific, USA). The samples were dried at 105 °C overnight to remove excess moisture. The
157 elemental compositions of the pulp samples were calculated based on the carbon, hydrogen, and oxygen
158 composition of an anhydroglucose unit (C₆H₁₀O₆).

159

160 *Structural characteristics of fibres by spectroscopy*

161 *X-Ray photoelectron spectroscopy (XPS)* was used to analyse the surface elemental compositions and chemical
162 states. The equipment used was an AXIS Ultra electron spectrometer (Kratos Analytical Ltd, UK.) with
163 monochromatic Al K α irradiation at 100 W and effective charge neutralisation with slow thermal electrons.
164 The set-up and acquisition parameters have been previously reported by Johansson & Campbell (2004). Prior
165 to the measurements, the samples were evacuated in a pre-chamber overnight. Low-resolution wide spectra in
166 addition to high resolution spectra of the carbon (C 1s) region were collected. Three measurements from each
167 sample were recorded. A sample of ash free 100% cellulose filter paper, stored under dust free ambient
168 conditions, was analysed as an *in situ* reference (Johansson and Campbell 2004). No degradation of the samples
169 due to ultrahigh vacuum or X-rays was observed during the measurements.

170

171 *Liquid state ¹³C NMR spectroscopy* was carried out using a Bruker Avance III 500 NMR spectrometer with a
172 magnetic flux density of 11.7 T. 30 mg of ChCl or urea was dissolved in DMSO-d₆, and transferred into a
173 regular 5 mm NMR tube. A ¹³C spectrum was acquired with a BB(F)O double resonance probe head at 22 °C,
174 using a 30-degree pulse and a waltz 16 proton decoupling sequence. A total of 1200 scans were collected with
175 a 1.5 s relaxation delay between successive scans. Referencing was carried out using the lock frequency, and
176 the spectrum was processed using Bruker TopSpin 3.5 software.

177

178 *Solid state ¹³C cross polarisation (CP) magic angle spinning (MAS) NMR* measurements were taken in order
179 to detect DES system residuals from dried pulp samples. The measurements were performed using an Agilent
180 600 NMR spectrometer with a magnetic flux density of 14.1 T, using a 3.2 mm triple-resonance MAS NMR
181 probe in a double resonance mode. 20000 scans were accumulated using a 1.1 ms contact time and a 3.0 s
182 relaxation delay between successive scans, with a MAS rate of 10 kHz. In all experiments a SPINAL-64 proton
183 decoupling of 80 kHz was used. 90-degree pulse durations and Hartmann-Hahn matches for cross polarisation
184 were calibrated using α -glycine. The chemical shifts were externally referenced via adamantane by setting the
185 low field signal to ~38.5 ppm.

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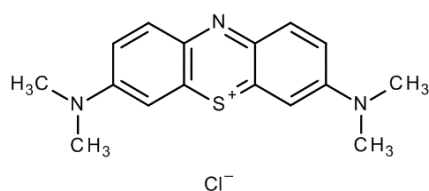
187 *Raman spectroscopy* was used to study the structural properties of pulp fibres. The measurements were
188 performed using a Renishaw RM-1000 System equipped with a thermoelectrically cooled CCD detector. The
189 laser was focused on the samples using a 50 \times objective lens attached to a Leica microscope. A 785 nm
190 wavelength laser was used to record spectra using an exposure time of 10 s and ten accumulations. The power

191 of the laser was kept at 100 % of the source power. The pulp fibres were oriented parallel and perpendicular
192 to the polarisation configuration of the laser used to excite and record the Raman scattering. Raman spectra of
193 pulp fibres were normalised with respect to the intensity of a band located at $\sim 897\text{ cm}^{-1}$ (Agarwal et al. 2010).

194 *Fibre charge determination by methylene blue adsorption*

195 *Methylene blue adsorption* was used to study the changes in the pulp charge due to the DES treatment. The
196 method is based on the adsorption of the cationic dye to the anionic sites of cellulose via electrostatic
197 interactions, and the changes in the intensity level of the supernatant is monitored (Palit and Moulik 2000).
198 The dye adsorptions were carried out according to a protocol described by Ho et al. (2011) with some
199 modifications. Briefly, cationic methylene blue dye solution was prepared by mixing 0.0161 g of methylene
200 blue (Fig. 2) with 100 ml MilliQ-water at room temperature. 0.016 g of dry pulp was mixed with 1 ml of dye
201 solution and 39 g of MilliQ-water. This mixture was continuously shaken for 24 hours at room temperature
202 (speed 160 rpm) using a Stuart orbital shaker (SSL1, UK). The dispersion was then centrifuged for 30 minutes
203 at 10000 rpm and a few millilitres of supernatant was collected and the absorbance was measured using a UV-
204 vis spectrophotometer (UV/VIS/NIR Lambda 900, Perkin Elmer, USA) with a 1 cm polystyrene cuvette. The
205 position of the maximum absorbance (λ_{max}) for methylene blue was 664 nm.

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207

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Fig. 2 Chemical structure of methylene blue

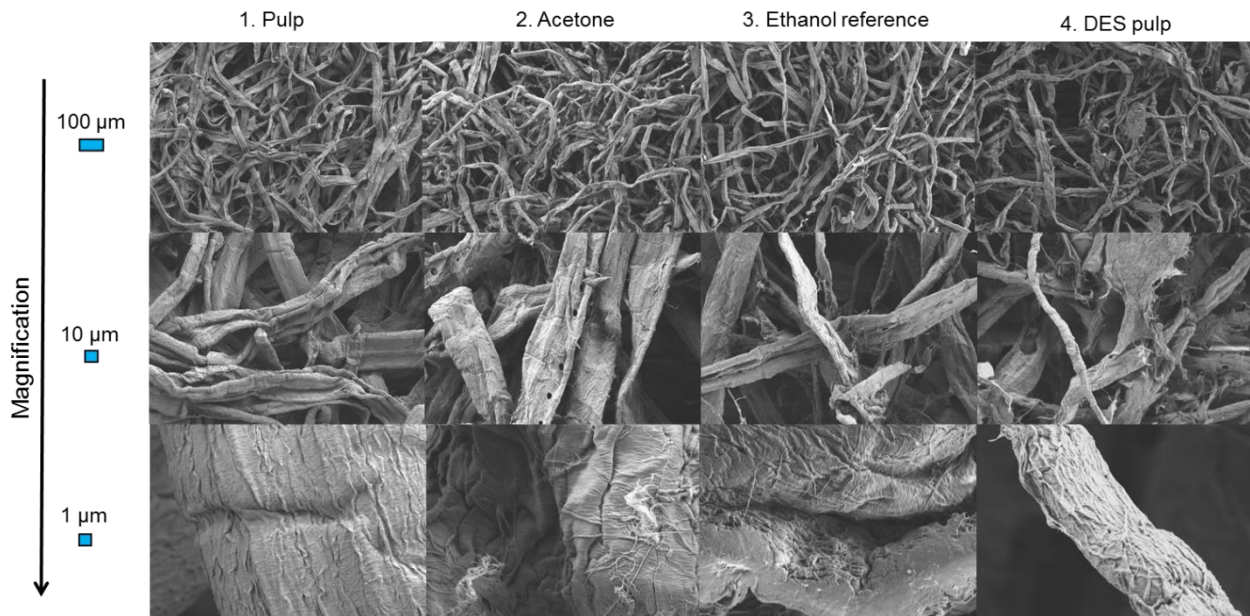
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210 **RESULTS AND DISCUSSION**

211 **Changes in fibre morphology**

212 SEM imaging was used to visually assess any possible changes that may have occurred to the morphology of
213 the pulp fibre during the DES treatment protocol (see Fig. 1). Representative SEM images of samples
214 undergoing this treatment, at different levels of magnification, are shown in Fig 3. Similar structural details
215 are seen for samples that underwent a solvent exchange step in acetone or ethanol, compared to pulp fibres
216 after the DES treatment step. Minor increases in fibrillation and possible cracking of the fibres can be attributed
217 to pulp drying, as previously demonstrated by Suchy et al. (2009), rather than just the DES treatment.

218



220

221

Fig. 3 SEM images with the magnifications of $\times 100$, $\times 500$ and $\times 5000$ for bleached pine pulp samples when exposed to different treatment stages. Scale bars are given on the left hand side of the images.

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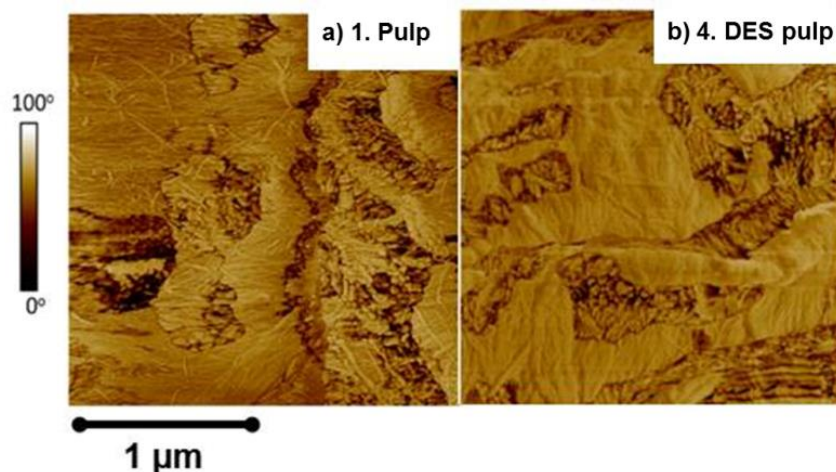
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AFM was used to more closely analyse possible changes in the surface morphology of the fibres, and to also determine if mercerisation of cellulose was taking place. In Fig. 4 the 4. DES pulp sample is compared to the reference 1. Pulp sample. The surfaces of both pulp fibres appear to be identical, and additionally did not indicate that mercerisation had taken place. Eronen et al. (2009) showed that during mercerisation, the pulp fibre surface morphology clearly changes resulting in a formation of an irregular layer on the fibre surface. In the present sample, the microfibrils and cell wall layers are still visible indicating that the cell wall structure remains unchanged. This result is in accordance with the finding that the crystalline structure of cellulose I remains intact during a DES treatment (Sirviö et al. 2015; Tenhunen et al. 2016).

233

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233

234

Fig. 4 Typical phase contrast AFM images of a) a 1. Pulp and b) a 4. DES pulp sample.

235

236 **Overall changes in chemical composition**237 *Carbohydrate composition*

238 Carbohydrate analysis was used to determine the possible dissolution of hemicelluloses. The monosaccharide
 239 compositions of the pulp samples are presented in Table 1. The changes in carbohydrate contents are
 240 negligible, and they fall below the measuring accuracy of the method (internal standard), which varies within
 241 the range 6-8%. In addition, it has been shown that the degree of polymerisation (DP) does not change with
 242 the DES system treatment; this would have been expected to be affected by the dissolution of hemicelluloses
 243 (Sirviö et al. 2015; Tenhunen et al. 2016). The DES treatment does not appear to dissolve glucose or galactose,
 244 but minor dissolution of xylose, mannose and arabinose cannot be completely excluded.

245 **Table 1** The composition of neutral sugars of pulp samples after treatment with a DES system. Values are quoted
 246 standard deviations from the mean as errors.

| Pulp sample | Monosaccharides (mg/100 mg) | | | | | |
|----------------------|-----------------------------|-------------|-------------|--------------|-------------|-------------|
| | Rhamnose | Arabinose | Galactose | Glucose | Xylose | Mannose |
| 1. Pulp | <0.1 ± 0.00 | 0.63 ± 0.03 | 0.22 ± 0.01 | 85.07 ± 0.73 | 7.50 ± 0.12 | 6.23 ± 0.15 |
| 2. Acetone | <0.1 ± 0.00 | 0.63 ± 0.02 | 0.22 ± 0.01 | 83.68 ± 0.41 | 7.40 ± 0.13 | 6.21 ± 0.12 |
| 3. Ethanol reference | <0.1 ± 0.00 | 0.63 ± 0.02 | 0.22 ± 0.01 | 85.28 ± 0.91 | 7.47 ± 0.07 | 6.28 ± 0.09 |
| 4. DES pulp | <0.1 ± 0.00 | 0.54 ± 0.04 | 0.20 ± 0.01 | 83.19 ± 0.11 | 6.96 ± 0.12 | 5.86 ± 0.13 |

247

248 *Elemental analysis*

249 Elemental analysis was carried out to determine changes in chemical composition during the pulp sample
 250 processing steps (Table 2). There was no change in carbon, hydrogen or sulphur contents (no sulphur was
 251 detected); the analysis however revealed changes in nitrogen content of the DES treated pulp sample. The
 252 nitrogen content was also found to vary substantially between two different batches, despite using similar
 253 washing procedures.

254

255 The elemental nitrogen content varied in the range 0.5 % - 1.6 % which indicates that the mild ethanol washing
 256 procedure was not efficient enough to remove the DES derived nitrogen. Therefore, the washing procedure
 257 was improved by implementing an ethanol extraction step. Pulp was extensively washed in boiling ethanol at
 258 80 °C for 4 hours. As a result of this treatment the elemental nitrogen content was decreased to 0.2 %. This
 259 final nitrogen fraction is thought to be relatively tightly bound to the pulp fibres. In order to further clarify the
 260 binding mechanism, spectroscopic methods were employed.

261

262 **Table 2** Elemental composition of pulp samples. Errors are shown as standard deviations (SD) from the mean. If the
 263 error is less than 0.1 then it is given in brackets.

| Pulp sample | Carbon (%) \pm SD | Hydrogen (%) \pm SD | Nitrogen (%) \pm SD |
|-----------------------|---------------------|-----------------------|-----------------------|
| 1. Pulp | 43.6 \pm 0.1 | 6.3 (0.01) | 0.0 (0.0) |
| 2. Acetone | 44.0 (0.0) | 6.3 (0.0) | 0.0 (0.0) |
| 3. Ethanol reference | 43.5 (0.0) | 6.3 (0.0) | 0.0 (0.0) |
| 4. DES pulp | 42.9 \pm 0.1 | 6.4 \pm 0.1 | 1.6 \pm 0.1 |
| | 43.1 \pm 0.2 | 6.2 (0.03) | 0.5 (0.0) |
| 5. Extracted DES pulp | 43.2 (0.0) | 6.3 \pm 0.1 | 0.2 (0.0) |

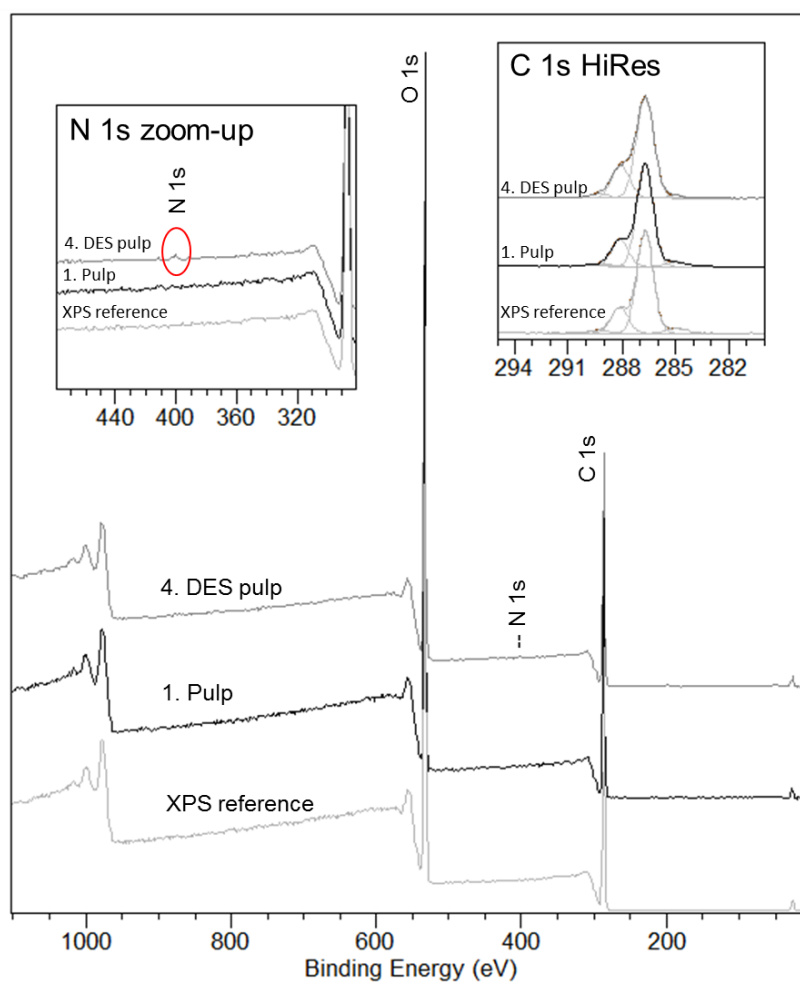
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265 **Revealing structural characteristics by spectroscopy**

266

267 *XPS – Chemical composition of the fibre surface*

268 XPS was used to study the elemental composition of the fibre surface before and after DES-treatment. Fig. 5
 269 presents XPS spectra of samples 1. Pulp and 4. DES pulp, as well as, the XPS reference sample of pure
 270 cellulose (Johansson and Campbell 2004), with high resolution carbon C 1s. Both samples were remarkably
 271 similar to the reference sample, with a typical cellulose C 1s signature consisting of carbons with one or two
 272 bonds to oxygen; namely peaks located at 286.7 eV and 288.1 eV (Beamson and Briggs 1993). Apart from
 273 the presence of these peaks, a non-cellulosic component originating from carbon atoms without oxygen
 274 neighbors was located at 285.0 eV, as is typically the case for all experimental XPS data from cellulose
 275 (Johansson et al. 2011). However, this signal is not more intense than what it is found for the pure cellulose
 276 reference sample. Therefore, the XPS data confirmed that the DES treatment process did not contaminate or
 277 chemically change the sample surfaces. The only difference observed was a barely detectable amount of
 278 nitrogen (0.3 at%) in the DES modified pulp sample (sample no 4). Data are presented in Table 4, and the
 279 nitrogen N 1s peak located at ~400 eV is shown in the inset of Fig. 5. Nitrogen seems to originate from ChCl
 280 since further examination of the chloride region (Cl 2p at 199 eV) revealed minor traces of this substance;
 281 however, the signal was below the quantification limit (not shown, less than 0.1 at% for Cl 2p with the
 282 instrumental setup used).



283

284

285 **Fig. 5** Typical low resolution wide spectra of *in situ* XPS reference for cellulose, 1. Pulp and 4. DES pulp showing signals

286 due to emission of O 1s, N 1s and C 1s. Insets show the magnification of N 1s region and the C 1s HiRes regions.

287

288

Table 3 Elemental surface concentrations and relative abundance of carbon bonds for the fibre samples.

| Sample | Elemental surface concentration (at%) | | | Relative abundance of carbon bonds (at%) | | | |
|----------------------|---------------------------------------|------|------|------------------------------------------|------|-------|-----|
| | C 1s | O 1s | N 1s | C-C | C-O | O-C-O | C=O |
| 1. Pulp | 59.3 | 40.7 | 0.0 | 3.9 | 72.6 | 20.7 | 2.7 |
| 2. Acetone | 60.0 | 39.9 | 0.0 | 3.6 | 66.8 | 24.3 | 5.2 |
| 3. Ethanol reference | 58.6 | 41.4 | 0.0 | 3.0 | 69.1 | 23.7 | 4.2 |
| 4. DES pulp | 58.7 | 41.0 | 0.3 | 2.7 | 70.4 | 23.2 | 3.7 |
| XPS reference | 59.1 | 40.9 | 0.0 | 4.1 | 75.2 | 18.9 | 1.9 |

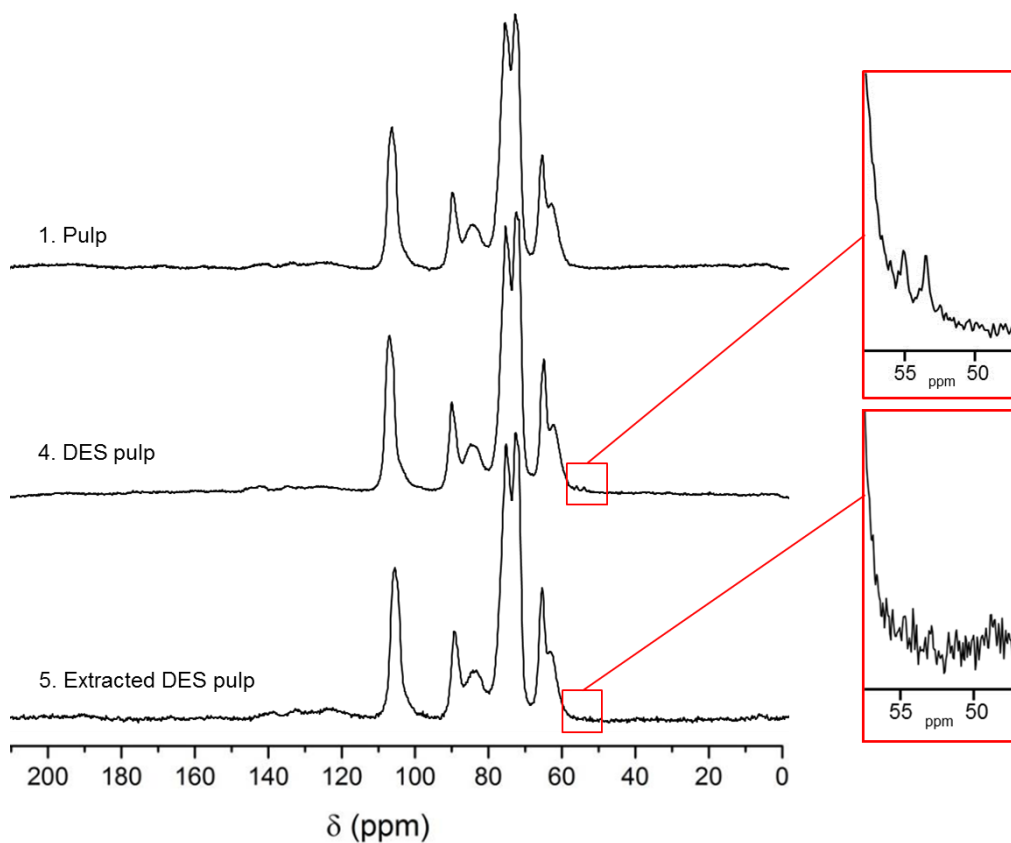
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291 *NMR - Chemical composition in bulk*

292 Both solid state and liquid state NMR techniques were used to determine the origin of the nitrogen observed
293 using XPS and elemental analyses, and to reveal the possible derivatisation of the DES treated pulp. Fig. 6
294 reports solid-state NMR spectra of the reference pine pulp sample. Also reported in this figure are samples of
295 DES treated pulp with a high nitrogen content (4. DES pulp with 1.6 % nitrogen) after mild washing, and DES
296 treated pulp with a low nitrogen content (5. Extracted DES pulp with 0.2 % nitrogen) after extensive washing
297 with boiling ethanol. Liquid state NMR was used for the assignment of signals for pure ChCl and urea (Online
298 Resource Fig. S1). The signal for urea was observed to be located at 161.1 ppm, and resonances for ChCl were
299 determined from signals corresponding to HO-CH₂-CH₂-N- (67.2 ppm) (triplet), HO-CH₂- (55.2 ppm) (singlet)
300 and -CH₂-N-(CH₃)₃ (53.5 ppm) (triplet) moieties. The signals are comparable to previously published data
301 (Ardenkjaer-Larsen et al. 2003; Lobo et al. 2012). Spectra acquired for the reference sample 1. Pulp are typical
302 for cellulose I obtained from softwood pulp (Larsson et al. 1999), without the presence of any additional
303 signals. Spectra of sample 4. DES pulp and 5. Extracted DES pulp were also similar to the spectra obtained
304 from the reference sample. Careful examination of these spectra identified two additional signals located at
305 55.0 and 53.0 ppm. This region of the spectra is comparable to the ChCl moieties containing nitrogen.
306 Additional signals in the region of urea (161.1 ppm) were not detected, and therefore, the formation of
307 carbamate bonds discussed by Sirviö et al. (2015) were thought to not occur. Spectra measured after extensive
308 washing steps (5. Extracted DES pulp) were identical to the reference spectra without any additional signals.
309 This result was expected due to the lesser amount of nitrogen observed from XPS data. These results also
310 agree with the findings of Yin et al. (2007), who showed that it is difficult for urea to impregnate into cellulose
311 without a solvent such as water.

312



313

314 **Fig. 6** Typical solid-state NMR spectra of 1. Pulp (bleached pine pulp reference), 4. DES pulp (after DES treatment and
 315 conventional washing) and 5. Extracted DES pulp (after extensive washing). Insets in the figure show details of peaks
 316 close to the shoulder of peak located in the range 60-70 ppm.

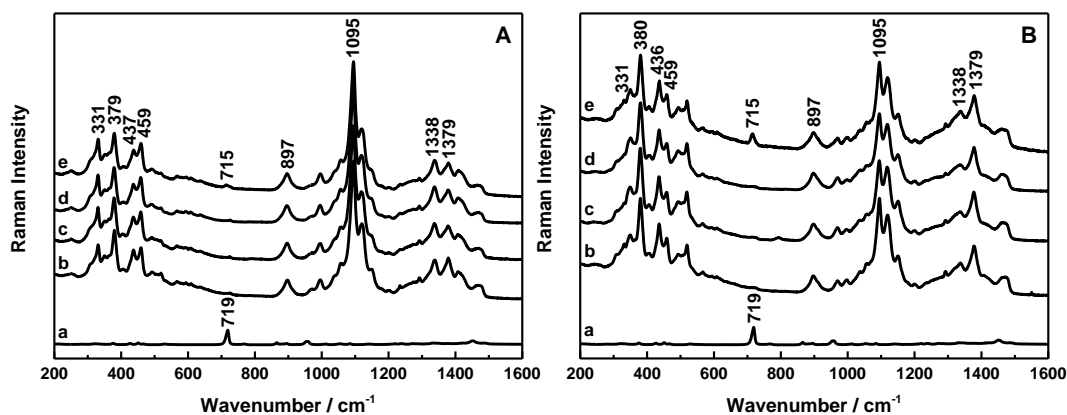
317

318 *Raman – structural properties of bulk materials*

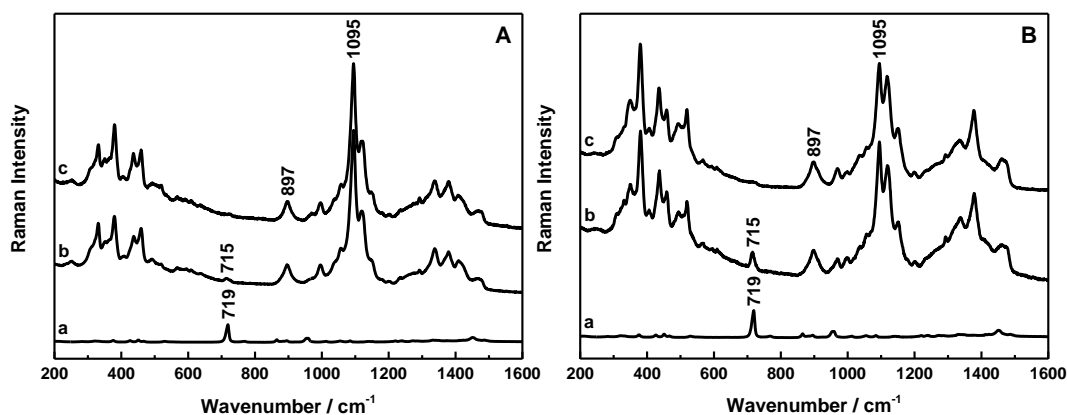
319 Raman spectroscopy was used to study the structural properties of the pulp fibres. Fig. 7 shows typical Raman
 320 spectra of pulp fibres after different stages of treatment. Raman bands emanating from the vibrational modes
 321 of atoms in cellulose chains are sensitive to the orientation of the fibres with respect to the polarisation
 322 configuration of the laser light (Wiley and Atalla 1987; Lewandowska et al. 2015). Typical Raman spectra of
 323 pulp fibres illustrate the changes in the intensity of the Raman bands as a function of the orientation of the
 324 fibres; namely parallel (Fig. 7A) and perpendicular (Fig. 7B), to the polarisation configuration of the laser
 325 light. The bands found in the region 250-600 cm^{-1} are assigned to skeletal-bending modes involving the CCC,
 326 COC, OCC and OCO internal coordinates (Wiley and Atalla 1987). Additionally, the bending (CCH and OCH)
 327 and skeletal stretching modes (CC and CO) are thought to also contribute to peaks within this region (Wiley
 328 and Atalla 1987). The well-resolved Raman bands located at $\sim 897 \text{ cm}^{-1}$ and $\sim 1095 \text{ cm}^{-1}$ are assigned to the
 329 main chain segmental stretching modes (Wiley and Atalla 1987). The band located at $\sim 897 \text{ cm}^{-1}$ is assigned to
 330 the C-O-C in plane stretching (Edwards et al. 1997), while the band centred at $\sim 1095 \text{ cm}^{-1}$ corresponds to C-
 331 O ring stretching modes and the β -1,4 glycosidic linkage (C-O-C) stretching modes between the glucose rings

332 of the cellulose chains (Edwards et al. 1997; Gierlinger et al. 2006). Finally, heavy atom stretching (CC, CO)
333 and the HCC, HCO, HOC and HCH bending modes contribute to the bands shown in the range 1200-1500 cm⁻¹
334 (Wiley and Atalla 1987). Raman spectra of pulp fibres washed with acetone (2. Acetone) and ethanol (3.
335 Ethanol reference) solvents are similar to those obtained from the initial 1. Pulp material (curves b, c and d in
336 Fig. 7). The absence of differences between the Raman spectra of 1. Pulp, 2. Acetone and 3. Ethanol reference
337 materials suggests that the pulp fibres maintain their chemical and structural properties after washing with the
338 solvents. Additionally, a Raman band located at ~715 cm⁻¹ appears in the spectrum obtained from 4. DES pulp
339 fibres treated with the DES solvent (curve e in Fig. 7). The origin of this band seems to result from the moieties
340 of DES in the fibre structure, since the region of 700-850 cm⁻¹ is devoid of any significant features
341 corresponding to cellulose structures. Fig. S2 in Online Resource reports the Raman spectra of pure choline
342 chloride (ChCl) and urea, two principal components of the DES system. The most intense Raman band from
343 ChCl is centred at ~719 cm⁻¹, and is assigned to the “totally” symmetric stretching vibration of four C-N bonds
344 (ν_1) in the choline group (Fig. S2 A, Online Resource (Akutsu 1981)). The medium intensity Raman bands
345 located at ~865 cm⁻¹ and ~954 cm⁻¹ are attributed to the symmetric (ν_2) and asymmetric (ν_3 and ν_4) stretching
346 vibrations of the C-N bonds (Akutsu 1981). The position of Raman bands corresponding to the symmetric
347 stretching vibrations (ν_1 and ν_2) of the C-N bonds indicates that most of the O-C-C-N⁺ backbones in the choline
348 group are in the gauche conformation (Akutsu 1981). A weak Raman band centred at ~768 cm⁻¹ is assigned to
349 the “totally” symmetric stretching vibration of four C-N bonds (ν_1) in the trans conformation of the O-C-C-N⁺
350 backbone in the choline group (Akutsu 1981). The strongest Raman band of urea located at ~1010 cm⁻¹ is
351 assigned to the symmetric stretching vibration of the C-N bonds (Fig. S2 B, Online Resource). The asymmetric
352 stretching vibration of the C-N bonds in the solid state urea appears at ~1463 cm⁻¹ (Keuleers et al. 1999). This
353 suggests that the Raman band located at ~715 cm⁻¹ in the spectrum of 4. DES pulp corresponds to the initial
354 choline group, but excluding the possibility of a chemical reaction between the -OH groups of cellulose and
355 the components of DES during processing. Furthermore, the intensity of this band is sensitive to the orientation
356 of the fibre with respect to the polarisation configuration of the laser, showing a higher intensity when the 4.
357 DES pulp fibre is oriented perpendicular to the polarisation direction (curve b in Fig. 7). This suggests that the
358 choline groups (positive charge) interact electrostatically with the anionic groups of cellulose (negative charge)
359 and their ⁺N-C-C-O backbones ‘poke out’ perpendicularly from the cellulose chain. A shift of Raman band of
360 4. DES pulp (715 cm⁻¹) to a lower wavelength compared to ChCl (~719 cm⁻¹) indicates a slight decrease in the
361 symmetry of the choline group. The relative intensity of the Raman band located at ~715 cm⁻¹ varies between
362 the studied fibres in the perpendicular orientation (Fig. S3 B, Online Resource). The choline groups remain in
363 the 4. DES pulp fibres after mild washing of the material with an excess of ethanol. Fig. 8 shows the changes
364 in the Raman spectra of 4. DES pulp before and after the extraction of the fibres in boiling ethanol (5. Extracted
365 DES pulp). The intensity of the Raman bands assigned to the bond vibrations corresponding to the main chain
366 segmental stretching and bending modes are similar for 4. DES pulp and 5. Extracted DES pulp spectra. This
367 similarity suggests the preservation of chemical and structural properties of cellulose chains. Whereas, the
368 process of boiling the 4. DES pulp in ethanol leads to the substantial removal of the choline groups from the

369 pulp fibres. This is confirmed by the disappearance of the Raman band located at $\sim 715\text{ cm}^{-1}$ in the 5. Extracted
370 DES pulp spectrum (curve c in Fig 8).
371



372
373 **Fig. 7** Typical Raman spectra of (a) ChCl, (b) 1. Pulp, (c) 2. Acetone, (d) 3. Ethanol reference and (e) 4. DES pulp
374 recorded in (A) parallel and (B) perpendicular orientation of the fibres to the polarisation configuration of the laser
375 light.
376



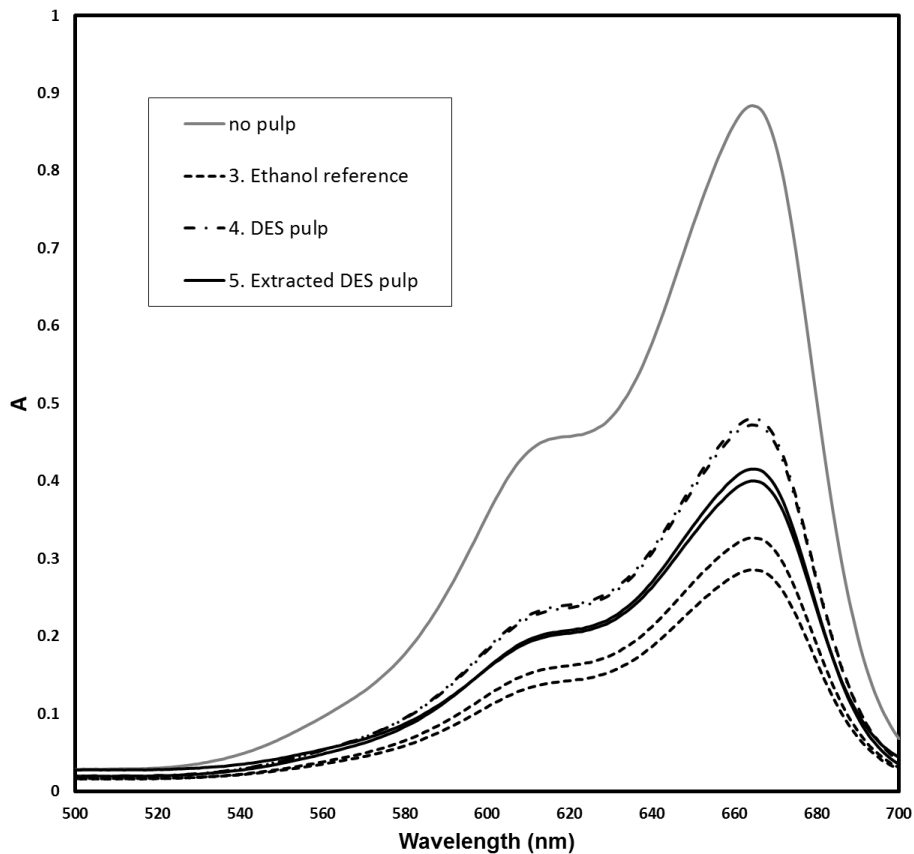
377
378 **Fig. 8** Typical Raman spectra of (a) ChCl, (b) 4. DES pulp and (c) 5. Extracted DES pulp recorded in (A) parallel and
379 (B) perpendicular orientation of the fibres to the polarisation configuration of the laser light.
380

381 **Assessment of the binding of nitrogen**

382 Methylene blue adsorption experiments on the pulp fibres were carried out to clarify the binding mechanism
383 of choline chloride groups to cellulose fibres. Changes in the anionic charge of the DES treated pulp fibres
384 were studied after the mild washing step with ethanol, and after the extensive washing step with boiling ethanol
385 (4. DES pulp and 5. Extracted DES pulp) (see Fig. 9). The results were compared to the ethanol reference pulp
386 (3. Ethanol reference), and furthermore a sample without pulp was measured as an internal reference of the
387 method.
388

389 Significant differences in absorbance of visible light of wavelength of $\lambda_{\max} = 664$ nm can be observed between
390 the pulp samples. The higher the absorbance, the higher the dye concentration is in the supernatant indicating
391 that the anionic sites of pulp are no longer available for the cationic dye particles to adsorb. This also suggests
392 that the sites are occupied with other cationic substances, in this case choline ions. Therefore, the increase in
393 the intensity of supernatant can be considered to be proportional to the decrease in the negative charge of the
394 pulp, which is related to adsorption taking place via electrostatic interactions. The absorbance of visible light
395 for the ethanol reference sample (no DES treatment) with a nitrogen content of 0 % was lower compared to
396 both the DES treated pulp samples. After DES treatment, a higher amount of methylene blue was found in the
397 supernatant as observed from the higher intensity recorded. Extensive washing with boiling ethanol again
398 lowered the intensity indicating the partial removal of the choline groups from the pulp surface. These results
399 support the Raman spectroscopy results that a small amount of choline groups are tightly bound to the pulp
400 fibres, and they seem to be attached via electrostatic forces which directly affects the charge state of the fibres.
401 The strength of the interactions is thought to be relatively strong since choline residuals could not be
402 completely removed even by extensive washing.

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409 **Fig. 9** Typical absorption spectra for 3. Ethanol reference pulp, 4. DES pulp and 5. Extracted DES pulp sample, and a
410 measurement without pulp. Two parallel measurements of each pulp sample are shown.
411

412 **CONCLUSIONS**

413 The influence of a cellulose compatible DES system based on choline chloride and urea on bleached pine pulp
414 fibres was revealed using a systematic approach with complementary research methods. DES treatment carried
415 out for 16 hours at 100 °C has been found to have no influence on pulp fibre morphology. In addition, no
416 evidence on the derivatisation of cellulose was observed during the DES treatment. Negligible changes were
417 observed in the xylose, mannose and arabinose content and thus, minor dissolution of some of the
418 hemicelluloses cannot be excluded. Elemental analysis and XPS surface elemental analysis suggested that
419 nitrogen containing residuals remained even after the extensive pulp washing stage. Thorough examination by
420 NMR and Raman spectroscopy revealed that the nitrogen residuals originate from tightly bound choline
421 chloride. In addition, Raman spectroscopy data suggest that cationic choline ions are interacting with the
422 anionic groups of cellulose fibres via electrostatic interactions. This result was also supported by the cationic
423 methylene blue adsorption results. These findings should facilitate the efficient utilisation of a green and non-
424 toxic solvent system when developing advanced materials ~~solutions~~ from lignocellulosic-based sources.

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431 **References**

- 432 Abbott AP, Bell TJ, Handa S, Stoddart B (2006) Cationic functionalisation of cellulose using a choline based
433 ionic liquid analogue. *Green Chem* 8:784–786. doi: 10.1039/B605258D
- 434 Abbott AP, Capper G, Davies DL, et al (2003) Novel solvent properties of choline chloride/urea mixtures.
435 *Chem Commun* 99:70–71. doi: 10.1039/b210714g
- 436 Agarwal UP, Reiner RS, Ralph SA (2010) Cellulose I crystallinity determination using FT-Raman
437 spectroscopy: Univariate and multivariate methods. *Cellulose* 17:721–733. doi: 10.1007/s10570-010-
438 9420-z
- 439 Akutsu H (1981) Direct determination by Raman scattering of the conformation of the choline group in
440 phospholipid bilayers. *Biochemistry* 20:7359–7366. doi: 10.1021/bi00529a006
- 441 Ardenkjaer-Larsen JH, Fridlund B, Gram A, et al (2003) Increase in signal-to-noise ratio of > 10,000 times
442 in liquid-state NMR. *Proc Natl Acad Sci U S A* 100:10158–63. doi: 10.1073/pnas.1733835100

443 Beamson G, Briggs D (1993) Cellulose Spectra in High Resolution XPS of Organic Polymers: The Scienta
444 ESCA300 database. *J Chem Educ* 70:A25. doi: 10.1021/ed070pA25.5

445 Cai J, Zhang L (2005) Rapid dissolution of cellulose in LiOH/urea and NaOH/urea aqueous solutions.
446 *Macromol Biosci* 5:539–548. doi: 10.1002/mabi.200400222

447 Edwards HG, Farwell DW, Webster D (1997) FT Raman microscopy of untreated natural plant fibres.
448 *Spectrochim Acta A Mol Biomol Spectrosc* 53A:2383–2392. doi: 10.1016/S1386-1425(97)00178-9

449 Ekman K, Eklund V, Fors J, et al (1984) Regenerated Cellulose Fibers From Cellu- lose Carbamate
450 Solutions. 38–40.

451 Ershova O, da Costa E V., Fernandes AJS, et al (2012) Effect of urea on cellulose degradation under
452 conditions of alkaline pulping. *Cellulose* 19:2195–2204. doi: 10.1007/s10570-012-9791-4

453 Fu F, Xu M, Wang H, et al (2015) Improved Synthesis of Cellulose Carbamates with Minimum Urea Based
454 on an Easy Scale-up Method. *ACS Sustain Chem Eng* 3:1510–1517. doi:
455 10.1021/acssuschemeng.5b00219

456 Gierlinger N, Schwanninger M, Reinecke A, Burgert I (2006) Molecular changes during tensile deformation
457 of single wood fibers followed by Raman microscopy. *Biomacromolecules* 7:2077–2081. doi:
458 10.1021/bm060236g

459 Harper Jr. RJ, Stone RL (1986) Cationic Cotton Plus Easy Care. *Text Chem Color* 18:33–35.

460 Ho TTT, Zimmermann T, Hauert R, Caseri W (2011) Preparation and characterization of cationic
461 nanofibrillated cellulose from etherification and high-shear disintegration processes. *Cellulose*
462 18:1391–1406. doi: 10.1007/s10570-011-9591-2

463 Johansson L, Tammelin T, Campbell JM, et al (2011) Experimental evidence on medium driven cellulose
464 surface adaptation demonstrated using nanofibrillated cellulose. *Soft Matter* 7:10917. doi:
465 10.1039/c1sm06073b

466 Johansson LS, Campbell JM (2004) Reproducible XPS on biopolymers: Cellulose studies. *Surf Interface*
467 *Anal* 36:1018–1022. doi: 10.1002/sia.1827

468 Keuleers R, Desseyn HO, Rousseau B, Van Alsenoy C (1999) Vibrational analysis of urea. *J Phys Chem A*
469 103:4621. doi: 10.1021/jp984180z

470 Kim JY, Choi H-M (2014) Cationization of periodate-oxidized cotton cellulose with choline chloride. *Cellul*
471 *Chem Technol* 48:25–32.

472 Lahtinen P, Liukkonen S, Pere J, et al (2014) A Comparative study of fibrillated fibers from different
473 mechanical and chemical pulps. *BioResources* 9:2115–2127.

474 Larsson PT, Hult E, Wickholm K, et al (1999) CPrMAS 13 C-NMR spectroscopy applied to structure and
475 interaction studies on cellulose I. *Solid State Nucl Magn Reson* 15:31–40. doi: 10.1016/S0926-
476 2040(99)00044-2

477 Lewandowska AE, Soutis C, Savage L, Eichhorn SJ (2015) Carbon fibres with ordered graphitic-like
478 aggregate structures from a regenerated cellulose fibre precursor. *Compos Sci Technol* 116:50–57. doi:
479 10.1016/j.compscitech.2015.05.009

480 Lobo HR, Singh BS, Shankarling GS (2012) Deep eutectic solvents and glycerol: a simple, environmentally
481 benign and efficient catalyst/reaction media for synthesis of N- aryl phthalimide derivatives. *Green*
482 *Chem Lett Rev* 5:487–533. doi: 10.1080/17518253.2012.669500

483 Palit D, Moulik SP (2000) Adsorption of methylene blue on cellulose from its own solution and its mixture
484 with methyl orange. *Indian J Chem - Sect A Inorganic, Phys Theor Anal Chem* 39:611–617.

485 Park JH, Oh KW, Choi HM (2013) Preparation and characterization of cotton fabrics with antibacterial
486 properties treated by crosslinkable benzophenone derivative in choline chloride-based deep eutectic
487 solvents. *Cellulose* 20:2101–2114. doi: 10.1007/s10570-013-9957-8

488 Samanta AK, Kar TR, Mukhopadhyay A, et al (2015) Studies on Dyeing Process Variables for Salt Free
489 Reactive Dyeing of Glycine Modified Cationized Cotton Muslin Fabric. *J Inst Eng Ser E* 96:31–44.
490 doi: 10.1007/s40034-015-0062-4

491 Segal L, Eggerton FV (1961) Some aspects of the reaction between urea and cellulose. *Text Res J* 31:460–
492 471.

493 Sirviö JA, Visanko M, Liimatainen H (2015) Deep eutectic solvent system based on choline chloride-urea as
494 a pre-treatment for nanofibrillation of wood cellulose. *Green Chem* 17:3401–3406. doi:
495 10.1039/C5GC00398A

496 Sluiter A, Hames B, Ruiz R, et al (2012) NREL/TP-510-42618 analytical procedure - Determination of
497 structural carbohydrates and lignin in Biomass. *Lab Anal Proced* 17. doi: NREL/TP-510-42618

498 Suchy M, Hakala T, Kangas H, et al (2009) Effects of commercial cellobiohydrolase treatment on fiber
499 strength and morphology of bleached hardwood pulp. *Holzforschung* 63:731–736. doi:
500 10.1515/HF.2009.104

501 Suopajarvi T, Sirviö JA, Liimatainen H (2017) Nanofibrillation of deep eutectic solvent-treated paper and
502 board cellulose pulps. *Carbohydr Polym* 169:167–175. doi: 10.1016/j.carbpol.2017.04.009

503 Swerin A, Odberg L, Lindström T, Pulp S (1990) Deswelling of hardwood kraft pulp fibers by cationic
504 polymers. *Nord Pulp Pap Res J* 5:188–196.

505 Tenhunen T, Hakalahti M, Kouko J, et al (2016) Method for Forming Pulp Fibre Yarns Developed by a.
506 *BioResources* 11:2492–2503.

507 Wang S, Peng X, Zhong L, et al (2015) Choline chloride/urea as an effective plasticizer for production of
508 cellulose films. *Carbohydr Polym* 117:133–139. doi: 10.1016/j.carbpol.2014.08.113

509 Wen Q, Chen JX, Tang YL, et al (2015) Assessing the toxicity and biodegradability of deep eutectic
510 solvents. *Chemosphere* 132:63–69. doi: 10.1016/j.chemosphere.2015.02.061

511 Wiley JH, Atalla R (1987) Band assignments in the Raman-spectra of celluloses. *Carbohydr Res* 160:113–
512 129. doi: dx.doi.org/10.1016/0008-6215(87)80306-3

513 Willberg-Keyriläinen P, Hiltunen J, Ropponen J (2017) Production of cellulose carbamate using urea-based
514 deep eutectic solvents. *Cellulose*. doi: 10.1007/s10570-017-1465-9

515 Willför S, Pranovich A, Tamminen T, et al (2009) Carbohydrate analysis of plant materials with uronic acid-
516 containing polysaccharides-A comparison between different hydrolysis and subsequent

517 chromatographic analytical techniques. *Ind Crops Prod* 29:571–580. doi:
518 10.1016/j.indcrop.2008.11.003

519 Xu GC, Ding JC, Han RZ, et al (2016) Enhancing cellulose accessibility of corn stover by deep eutectic
520 solvent pretreatment for butanol fermentation. *Bioresour Technol* 203:364–369. doi:
521 10.1016/j.biortech.2015.11.002

522 Yin C, Li J, Xu Q, et al (2007) Chemical modification of cotton cellulose in supercritical carbon dioxide:
523 Synthesis and characterization of cellulose carbamate. *Carbohydr Polym* 67:147–154. doi:
524 10.1016/j.carbpol.2006.05.010

525 Zhang Q, De Oliveira Vigier K, Royer S, Jerome F (2012) Deep eutectic solvents : syntheses, properties and
526 applications. *Chem Soc Rev* 41:7108–7146. doi: 10.1039/c2cs35178a

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