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

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Abstract

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

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

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Introduction

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

Choline chloride (ChCl) and urea has been the most popular DES system probably due to the availability of these chemicals and their low melting point (~12 °C) (Abbott et al. 2003). Even though this solvent system does not dissolve cellulose, it has been investigated for several applications with promising results (Abbott et al. 2006; Park et al. 2013; Sirviö et al. 2015; Wang et al. 2015; Tenhunen et al. 2016; Xu et al. 2016; Suopajärvi et al. 2017; Willberg-Keyriläinen et al. 2017). Abbott et al. (2006) have utilised a eutectic mixture of a choline chloride derivative (Chlorcholine chloride-based (ClChCl;$ClCH_2CH_2N(Me)_3Cl$)) and urea to cationise cotton. Successful cationisation was detected via an increased hydrophilicity and by a repulsion of a cationic dye. According to their study, cationic functionalisation occurred when choline chloride reacted with the available OH-groups of cellulose. Sirviö et al. (2015) and Suopajärvi et al. (2017) utilised a DES system composed of ChCl and urea as a pre-treatment to promote nanofibrillation of bleached pulp or secondary fibre sources. They suggested that some of the hemicelluloses might dissolve during the treatment. They also suggested that a small number of cellulose hydroxyl groups are possibly converted to carbamates, leading to the distortion of the hydrogen bonding of the fibres. Carbamate conversion was observed by Willberg-Keyriläinen et al. (2017) when they treated wet pulp with a urea based DES system; this was found to occur most readily at 120 °C. Xu et al. (2016) tested ChCl-urea as a pre-treatment in order to remove hemicelluloses and lignin from corn stover prior to butanol fermentation. However, that particular DES system did not have any significant effect on the removal of these components. Park et al. (2013) used a mixture of 3,3′4,4′-benzophenone tetracarboxylic dianhydride (BPTCD) and ChCl-urea as a treatment medium in order to introduce antibacterial properties to cotton. Wang et al. (2015) used ChCl-urea as a plasticizer in regenerated cellulose films. They concluded that the ChCl-urea DES disrupted the inter- and intra-hydrogen bonds of cellulose, but there was no chemical reaction between these components and the regenerated cellulose.

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

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

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

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

EXPERIMENTAL

Materials

Never-dried bleached, sodium washed pine pulp from a Finnish pulp mill was used as the starting material for the DES treatment. This pulp was ion-exchanged to a sodium form based on a slightly modified version of a method originally described by Swerin et al. (1990); modifications to this method have been described by Lahtinen et al. (2014). In brief, the method includes washing the metal counter ions from the pulp at low pH (0.01M HCl, pH <3). After filtration and washing with deionized water, conversion of the carboxyl groups into their sodium form was achieved by mixing the pulp with 0.005M $NaHCO_3$ solution. The pH was set to slightly alkaline with 1M NaOH and held constant for 15 min while stirring the suspension. Finally, the pulp was rinsed with deionized water until the conductivity of the filtrate was below 20 µS/cm. This sodium washed pulp was diluted and mixed using Diaf's Minibatch Type 20 (Pilvad Diaf A/S, Denmark) for 30 minutes at

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

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

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

Methods

Preparation of samples

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

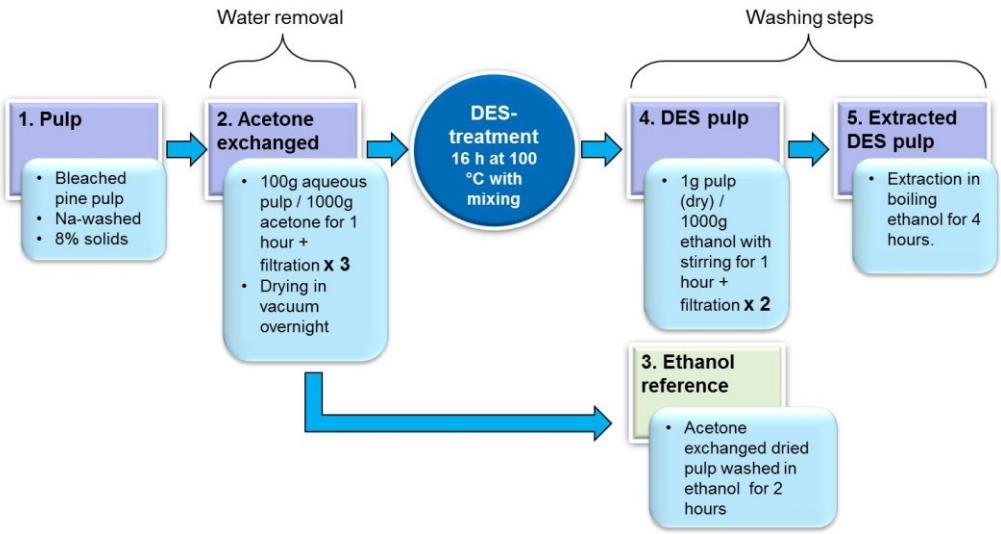

Fig. 1 Scheme of the sample preparation protocol including the water removal phase, treatment with deep eutectic solvent and the mild washing step with ethanol as well as a more efficient washing step including extraction with boiling ethanol. Ethanol reference pulp is a reference test point only for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

Fibre morphology studied by SEM and AFM

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

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

Overall chemical composition of fibres

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

Elemental analysis (C, H, N, S) of the pulp samples was carried out by using a FLASH 2000 series analyser (Thermo Scientific, USA). The samples were dried at 105 °C overnight to remove excess moisture. The elemental compositions of the pulp samples were calculated based on the carbon, hydrogen, and oxygen composition of an anhydroglucose unit ($C_6H_{10}O_6$).

Structural characteristics of fibres by spectroscopy

X-Ray photoelectron spectroscopy (XPS) was used to analyse the surface elemental compositions and chemical states. The equipment used was an AXIS Ultra electron spectrometer (Kratos Analytical Ltd, UK.) with monochromatic A1 Kα irradiation at 100 W and effective charge neutralisation with slow thermal electrons. The set-up and acquisition parameters have been previously reported by Johansson & Campbell (2004). Prior to the measurements, the samples were evacuated in a pre-chamber overnight. Low-resolution wide spectra in addition to high resolution spectra of the carbon (C 1s) region were collected. Three measurements from each sample were recorded. A sample of ash free 100% cellulose filter paper, stored under dust free ambient conditions, was analysed as an *in situ* reference (Johansson and Campbell 2004). No degradation of the samples due to ultrahigh vacuum or X-rays was observed during the measurements.

Liquid state ^{13}C NMR spectroscopy was carried out using a Bruker Avance III 500 NMR spectrometer with a magnetic flux density of 11.7 T. 30 mg of ChCl or urea was dissolved in DMSO-d_6, and transferred into a regular 5 mm NMR tube. A ^{13}C spectrum was acquired with a BB(F)O double resonance probe head at 22 °C, using a 30-degree pulse and a waltz 16 proton decoupling sequence. A total of 1200 scans were collected with a 1.5 s relaxation delay between successive scans. Referencing was carried out using the lock frequency, and the spectrum was processed using Bruker TopSpin 3.5 software.

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

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

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

Fibre charge determination by methylene blue adsorption

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

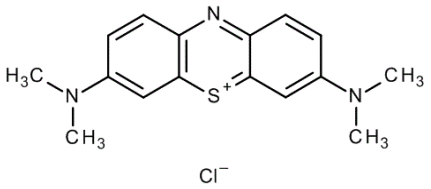

Fig. 2 Chemical structure of methylene blue

RESULTS AND DISCUSSION

Changes in fibre morphology

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

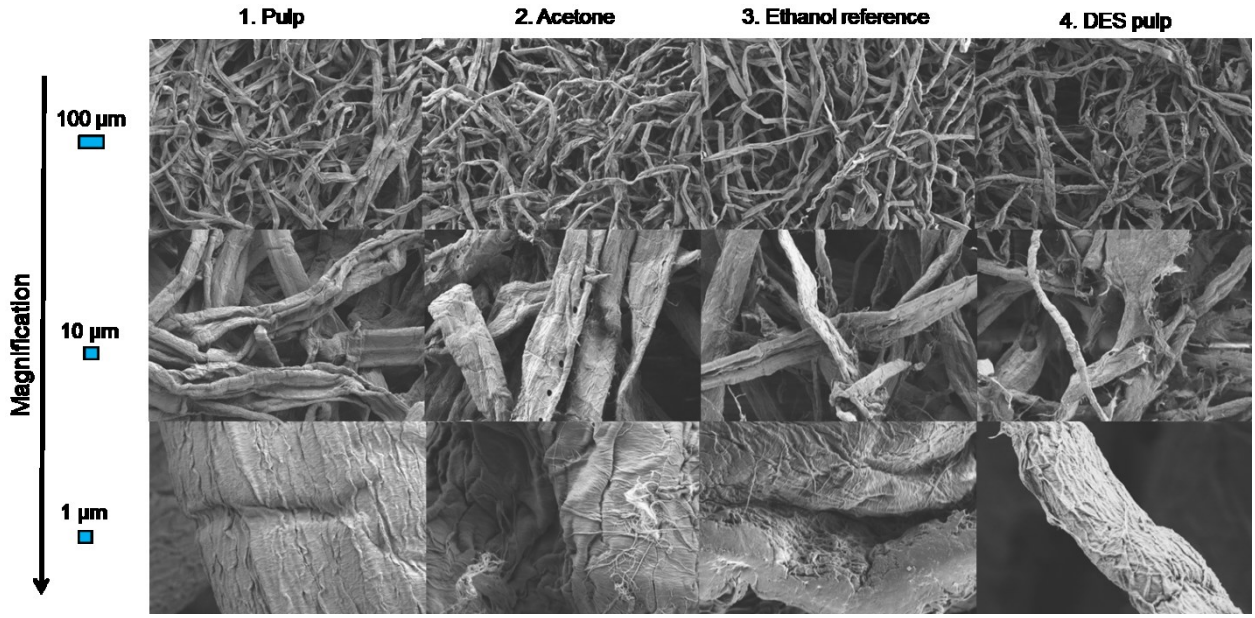

Fig. 3 SEM images with the magnifications of ×100, ×500 and ×5000 for bleached pine pulp samples when exposed to different treatment stages. Scale bars are given on the left hand side of the images.

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).

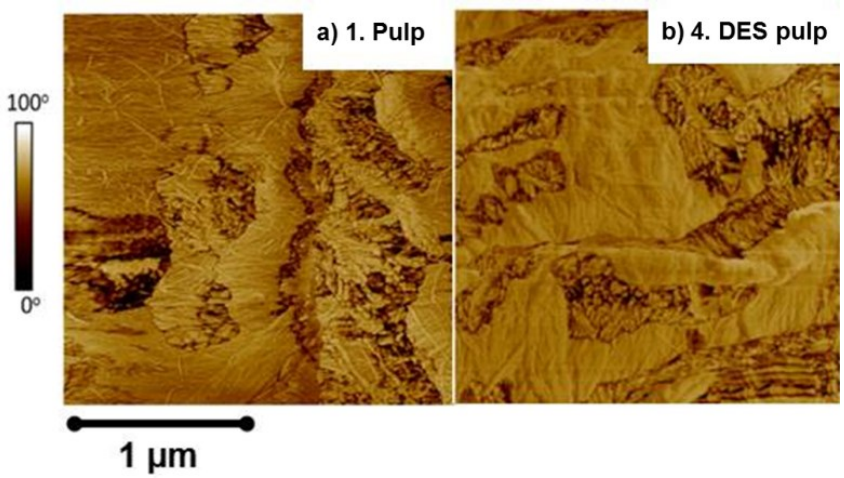

Fig. 4 Typical phase contrast AFM images of a) a 1. Pulp and b) a 4. DES pulp sample.

Overall changes in chemical composition

Carbohydrate composition

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

Table 1 The composition of neutral sugars of pulp samples after treatment with a DES system. Values are quoted 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

Elemental analysis

Elemental analysis was carried out to determine changes in chemical composition during the pulp sample processing steps (Table 2). There was no change in carbon, hydrogen or sulphur contents (no sulphur was detected); the analysis however revealed changes in nitrogen content of the DES treated pulp sample. The

nitrogen content was also found to vary substantially between two different batches, despite using similar washing procedures.

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

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

Pulp sample	Carbon (%) ± SD	Hydrogen (%) ± SD	Nitrogen (%) ± SD
1. Pulp	43.6 ± 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 ± 0.1	6.4 ± 0.1	1.6 ± 0.1
	43.1 ± 0.2	6.2 (0.03)	0.5 (0.0)
5. Extracted DES pulp	43.2 (0.0)	6.3 ± 0.1	0.2 (0.0)

Revealing structural characteristics by spectroscopy

XPS – Chemical composition of the fibre surface

XPS was used to study the elemental composition of the fibre surface before and after DES-treatment. Fig. 5 presents XPS spectra of samples 1. Pulp and 4. DES pulp, as well as, the XPS reference sample of pure cellulose (Johansson and Campbell 2004), with high resolution carbon C 1s. Both samples were remarkably similar to the reference sample, with a typical cellulose C 1s signature consisting of carbons with one or two bonds to oxygen; namely peaks located at 286.7 eV and 288.1 eV (Beamson and Briggs 1993). Apart from the presence of these peaks, a non-cellulosic component originating from carbon atoms without oxygen neighbors was located at 285.0 eV, as is typically the case for all experimental XPS data from cellulose (Johansson et al. 2011). However, this signal is not more intense than what it is found for the pure cellulose reference sample. Therefore, the XPS data confirmed that the DES treatment process did not contaminate or chemically change the sample surfaces. The only difference observed was a barely detectable amount of

nitrogen (0.3 at%) in the DES modified pulp sample (sample no 4). Data are presented in Table 4, and the nitrogen N 1s peak located at ~400 eV is shown in the inset of Fig. 5. Nitrogen seems to originate from ChCl since further examination of the chloride region (Cl 2p at 199 eV) revealed minor traces of this substance; however, the signal was below the quantification limit (not shown, less than 0.1 at% for Cl 2p with the instrumental setup used).

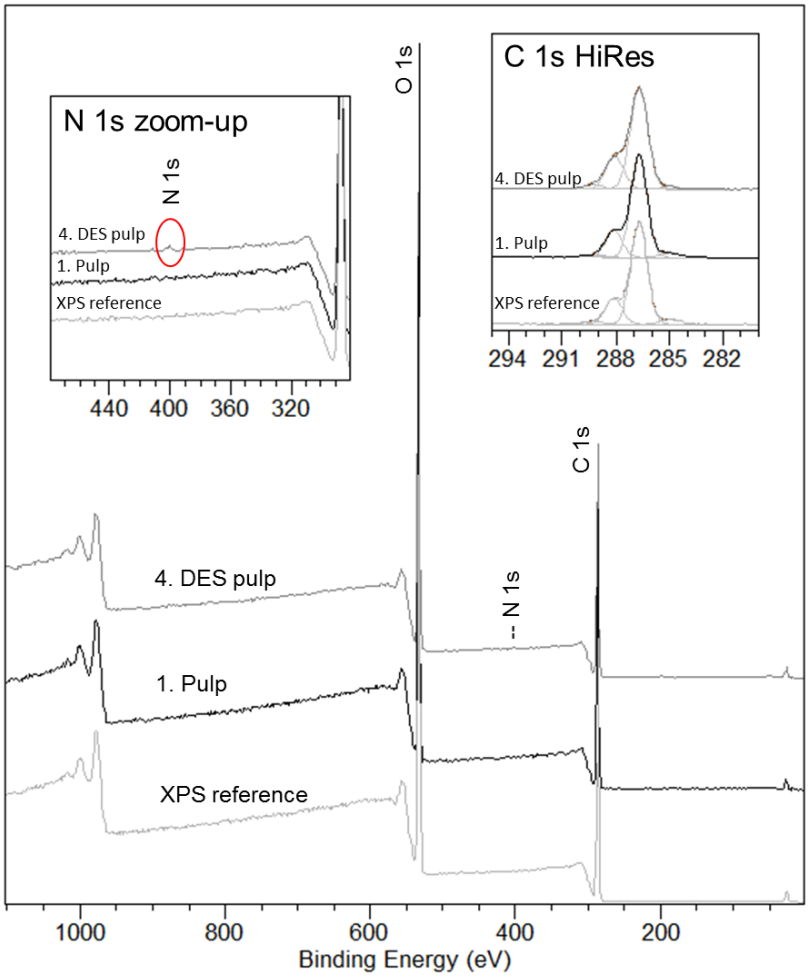

Fig. 5 Typical low resolution wide spectra of *in situ* XPS reference for cellulose, 1. Pulp and 4. DES pulp showing signals due to emission of O 1s, N 1s and C 1s. Insets show the magnification of N 1s region and the C 1s HiRes regions.

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

297
298
299 *NMR - Chemical composition in bulk*
300 Both solid state and liquid state NMR techniques were used to determine the origin of the nitrogen observed
301 using XPS and elemental analyses, and to reveal the possible derivatisation of the DES treated pulp. Fig. 6
302 reports solid-state NMR spectra of the reference pine pulp sample. Also reported in this figure are samples of
303 DES treated pulp with a high nitrogen content (4. DES pulp with 1.6 % nitrogen) after mild washing, and DES
304 treated pulp with a low nitrogen content (5. Extracted DES pulp with 0.2 % nitrogen) after extensive washing
305 with boiling ethanol. Liquid state NMR was used for the assignment of signals for pure ChCl and urea (Online
306 Resource Fig. S1). The signal for urea was observed to be located at 161.1 ppm, and resonances for ChCl were
307 determined from signals corresponding to HO-CH_2-CH_2-N- (67.2 ppm) (triplet), HO-CH_2- (55.2 ppm) (singlet)
308 and -CH_2-N-$(CH_3)_3$ (53.5 ppm) (triplet) moieties. The signals are comparable to previously published data
309 (Ardenkjaer-Larsen et al. 2003; Lobo et al. 2012). Spectra acquired for the reference sample 1. Pulp are typical
310 for cellulose I obtained from softwood pulp (Larsson et al. 1999), without the presence of any additional
311 signals. Spectra of sample 4. DES pulp and 5. Extracted DES pulp were also similar to the spectra obtained
312 from the reference sample. Careful examination of these spectra identified two additional signals located at
313 55.0 and 53.0 ppm. This region of the spectra is comparable to the ChCl moieties containing nitrogen.
314 Additional signals in the region of urea (161.1 ppm) were not detected, and therefore, the formation of
315 carbamate bonds discussed by Sirviö et al. (2015) were thought to not occur. Spectra measured after extensive
316 washing steps (5. Extracted DES pulp) were identical to the reference spectra without any additional signals.
317 This result was expected due to the lesser amount of nitrogen observed from XPS data. These results also
318 agree with the findings of Yin et al. (2007), who showed that it is difficult for urea to impregnate into cellulose
319 without a solvent such as water.
320

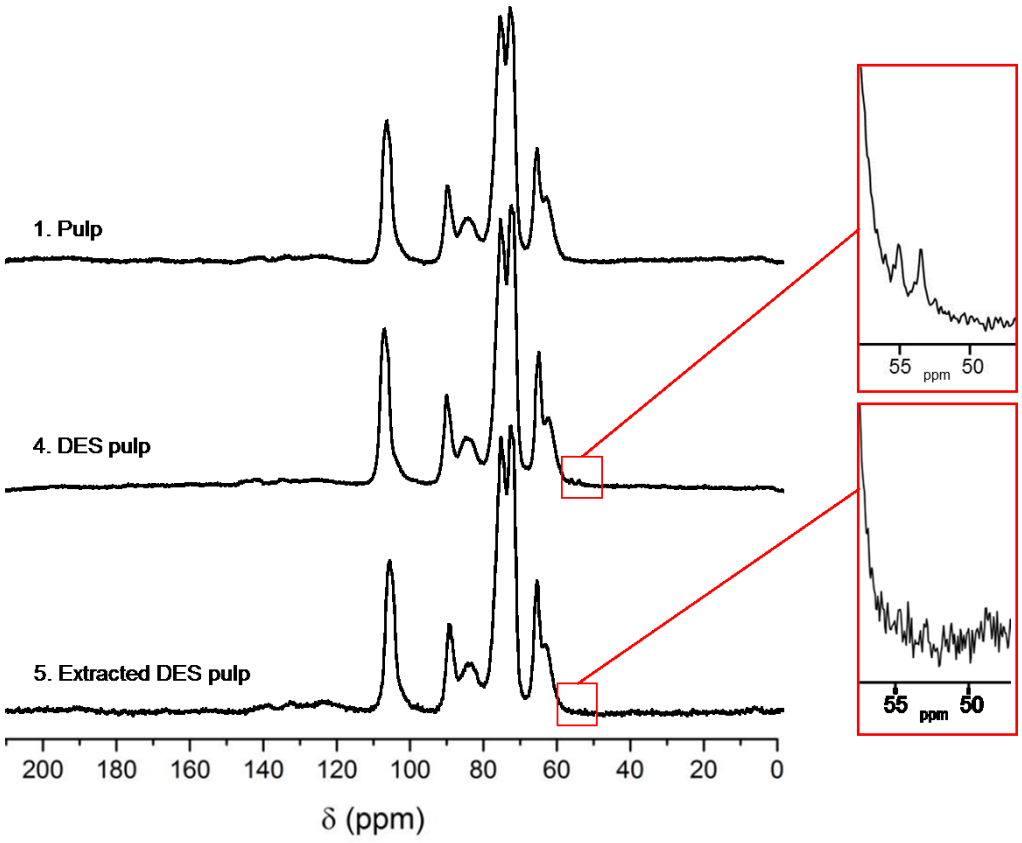

Fig. 6 Typical solid-state NMR spectra of 1. Pulp (bleached pine pulp reference), 4. DES pulp (after DES treatment and conventional washing) and 5. Extracted DES pulp (after extensive washing). Insets in the figure show details of peaks close to the shoulder of peak located in the range 60-70 ppm.

Raman – structural properties of bulk materials

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

of the cellulose chains (Edwards et al. 1997; Gierlinger et al. 2006). Finally, heavy atom stretching (CC, CO) and the HCC, HCO, HOC and HCH bending modes contribute to the bands shown in the range 1200-1500 cm^{-1} (Wiley and Atalla 1987). Raman spectra of pulp fibres washed with acetone (2. Acetone) and ethanol (3. Ethanol reference) solvents are similar to those obtained from the initial 1.Pulp material (curves b, c and d in Fig. 7). The absence of differences between the Raman spectra of 1. Pulp, 2. Acetone and 3. Ethanol reference materials suggests that the pulp fibres maintain their chemical and structural properties after washing with the solvents. Additionally, a Raman band located at ~715 cm^{-1} appears in the spectrum obtained from 4. DES pulp fibres treated with the DES solvent (curve e in Fig. 7). The origin of this band seems to result from the moieties of DES in the fibre structure, since the region of 700-850 cm^{-1} is devoid of any significant features corresponding to cellulose structures. Fig. S2 in Online Resource reports the Raman spectra of pure choline chloride (ChCl) and urea, two principal components of the DES system. The most intense Raman band from ChCl is centred at ~719 cm^{-1}, and is assigned to the "totally" symmetric stretching vibration of four C-N bonds (υ_1) in the choline group (Fig. S2 A, Online Resource (Akutsu 1981). The medium intensity Raman bands located at ~865 cm^{-1} and ~954 cm^{-1} are attributed to the symmetric (υ_2) and asymmetric (υ_3 and υ_4) stretching vibrations of the C-N bonds (Akutsu 1981). The position of Raman bands corresponding to the symmetric stretching vibrations (υ_1 and υ_2) of the C-N bonds indicates that most of the O-C-C-N^+ backbones in the choline group are in the gauche conformation (Akutsu 1981). A weak Raman band centred at ~768 cm^{-1} is assigned to the "totally" symmetric stretching vibration of four C-N bonds (υ_1) in the trans conformation of the O-C-C-N^+ backbone in the choline group (Akutsu 1981). The strongest Raman band of urea located at ~1010 cm^{-1} is assigned to the symmetric stretching vibration of the C-N bonds (Fig. S2 B, Online Resource). The asymmetric stretching vibration of the C-N bonds in the solid state urea appears at ~1463 cm^{-1} (Keuleers et al. 1999). This suggests that the Raman band located at ~715 cm^{-1} in the spectrum of 4. DES pulp corresponds to the initial choline group, but excluding the possibility of a chemical reaction between the –OH groups of cellulose and the components of DES during processing. Furthermore, the intensity of this band is sensitive to the orientation of the fibre with respect to the polarisation configuration of the laser, showing a higher intensity when the 4. DES pulp fibre is oriented perpendicular to the polarisation direction (curve b in Fig. 7). This suggests that the choline groups (positive charge) interact electrostatically with the anionic groups of cellulose (negative charge) and their $^+$N-C-C-O backbones 'poke out' perpendicularly from the cellulose chain. A shift of Raman band of 4. DES pulp (715 cm^{-1}) to a lower wavelength compared to ChCl (~719 cm^{-1}) indicates a slight decrease in the symmetry of the choline group. The relative intensity of the Raman band located at ~715 cm^{-1} varies between the studied fibres in the perpendicular orientation (Fig. S3 B, Online Resource). The choline groups remain in the 4. DES pulp fibres after mild washing of the material with an excess of ethanol. Fig. 8 shows the changes in the Raman spectra of 4. DES pulp before and after the extraction of the fibres in boiling ethanol (5. Extracted DES pulp). The intensity of the Raman bands assigned to the bond vibrations corresponding to the main chain segmental stretching and bending modes are similar for 4. DES pulp and 5. Extracted DES pulp spectra. This similarity suggests the preservation of chemical and structural properties of cellulose chains. Whereas, the process of boiling the 4. DES pulp in ethanol leads to the substantial removal of the choline groups from the

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

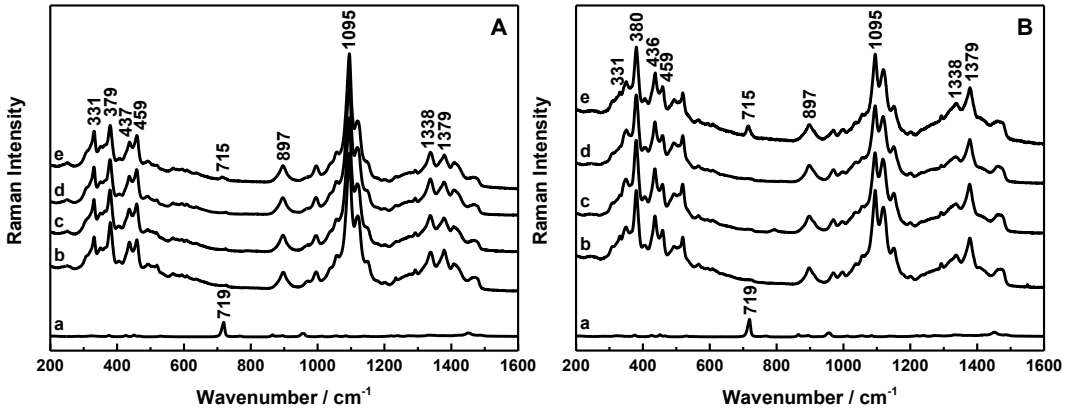

Fig. 7 Typical Raman spectra of (a) ChCl, (b) 1. Pulp, (c) 2. Acetone, (d) 3. Ethanol reference and (e) 4. DES pulp recorded in (A) parallel and (B) perpendicular orientation of the fibres to the polarisation configuration of the laser light.

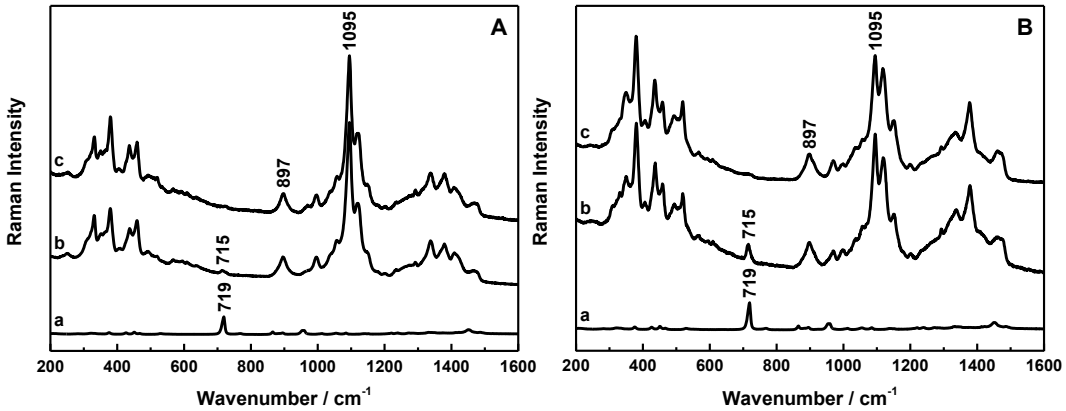

Fig. 8 Typical Raman spectra of (a) ChCl, (b) 4. DES pulp and (c) 5. Extracted DES pulp recorded in (A) parallel and (B) perpendicular orientation of the fibres to the polarisation configuration of the laser light.

Assessment of the binding of nitrogen

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

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

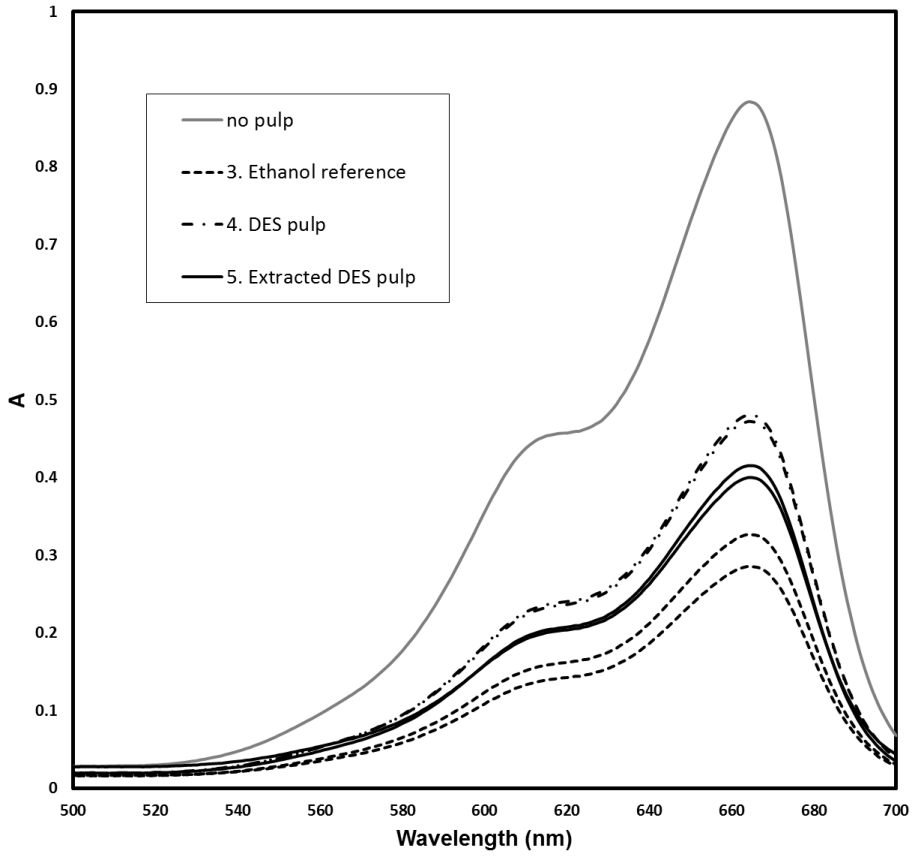

Fig. 9 Typical absorption spectra for 3. Ethanol reference pulp, 4. DES pulp and 5. Extracted DES pulp sample, and a measurement without pulp. Two parallel measurements of each pulp sample are shown.

CONCLUSIONS

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

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